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Modelling of Chemical Reactions in Foods: Possibilities and Pitfalls

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Abstract

This paper describes some problems and solutions concerning kinetic analysis of experimental data. One pitfall is the transformation of data (by taking logarithms or inverses) followed by linear regression. Because the error structure is then transformed as well, it is preferable to perform nonlinear regression of non-transformed data. Another pitfall is to use the correlation coefficient as a tool to discriminate between models or to prove a good fit. It is much better to study residuals, and in addition to use more advanced statistical tests for goodness of fit and discrimination. Yet another pitfall is to use simple kinetics based on the general rate law, resulting in an estimate of the order of a reaction. Because in foods the order is usually reflecting a much more complicated behavior than simple kinetics, multiresponse modelling is presented as an alternative. This means the simultaneous analysis and modelling of products and reactants, resulting in a much more powerful tool to test a proposed model. Once a model appears acceptable the resulting parameter estimates are much more precise than with the conventional uniresponse modelling. The pitfalls and possibilities are illustrated by a case study on the degradation of chlorophyll in heated spinach.

Keywords: kinetics; modeling; multiresponse; statistics; chlorophyll; heat treatment

INTRODUCTION

The quality of food is to a large extent determined by (bio)chemical reactions taking place. Control of food quality thus means control of chemical reactions in foods. Consequently, kinetic information regarding these reactions is needed because reactions in foods are usually kinetically controlled, not thermodynamically. Kinetic modelling appears to be a very powerful tool, on the one hand to predict quality atributes, and on the other hand to unravel mechanisms, the understanding of which ultimately leads to better quality control.

Kinetics tells us how fast a reaction will go. Kinetic information needs to be gathered from experiments. However, experimental measurements are always prone to error, and therefore the kinetic parameters obtained from the experiments will also be error ridden. Fortunately, errors can also be modelled, and hence, the use of statistics becomes an indispensable tool in modelling.

The following steps are necessary for modelling. First of all, one needs some sort of mechanistic insight in the reaction under study; this can be very basic at the start but it should be updated in the modelling process. Based on this insight, kinetic models can be built, kinetic parameters can be estimated and various models can be tested and discriminated. New experiments can be devised to refine the model. Furthermore, once a model is accepted the uncertainty in parameters needs to be estimated. The estimation of uncertainty is really necessary for the predictive part of modelling, and unfortunately, it is often not reported in food science literature. It is stressed however that when we address the issue of food quality that the quality of the prediction itself must not be neglected, otherwise we may end up with a useless prediction. All in all, modelling is an iterative process (Fig. 1).

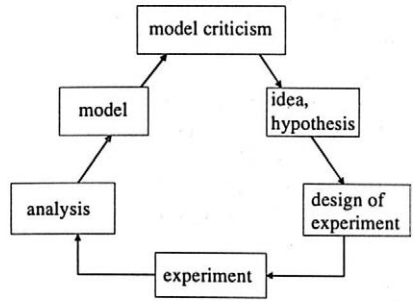

Figure 1. The iterative nature of modelling

When it comes to describing chemical reactions in terms of kinetic models, we can differentiate between empirical models and mechanistic models. Empirical models are the ones in which reactions are described in terms of the order of a reaction; this is based on the general rate law for the change in a concentration c over time t:

$$-\frac{dc}{dt} = kc^n \quad [1]$$

with n the order of the reaction and k the reaction rate constant. Integration of equation [1] gives:

$$c_t^{1-n} = c_0^{1-n} + (n-1)kt \quad (n \neq 1) \quad [2a]$$

$$c = c_0 \exp(-kt) \quad (n=1) \quad [2b]$$

There are in fact two types of orders, the first one is found if one determines *initial* rates –(dc/dt) for various concentrations and then one can determine the order n_c from equation [1], which is called the order with respect to concentration. The second possibility is to use equation [2] by following the change in concentration over time, and finding the best fit for the order n_t, called the order with respect to time. Both orders need not to be the same. If $n_t < n_c$ then the reaction rate increases as the reaction moves on (auto-catalysis), if $n_t > n_c$ the reaction rate decreases as the reaction progresses (auto-inhibition). The value of the order n is usually reported to be between 0 and 3; it needs not to be an integer value. It is important to note that equations [1] and [2] only give a mathematical description, not a mechanistic description of what is going on. In mechanistic terms, a reaction is either monomolecular or bimolecular. An order of 1 or 2 may thus indicate a mono- or a bimolecular reaction, but not necessarily so. An order that is not 1 or 2 indicates a more complex behavior, usually because one observes a combination of several reactions. This overview paper compares empirical and mechanistic modelling, and the possibilities and pitfalls of the several approaches. This will be done by means of a case-study from literature on chlorophyll breakdown in spinach. Preliminary results were already reported elsewhere for chlorophyll a (1). Here a more detailed analysis is given for the behavior of chlorophyll b.

Case study on Chlorophyll Breakdown in Heated Spinach

Chlorophyll breakdown in vegetables results usually in a quality loss because of undesired colour changes. The breakdown of chlorophyll is quite complex; a scheme is presented in Fig. 2, based on literature (2). It is a combination of chemical and enzymatic changes.

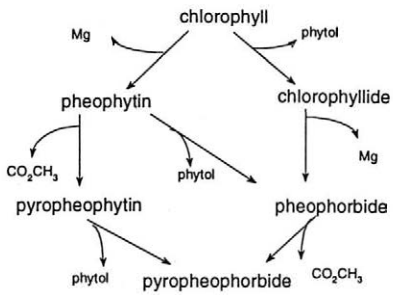

Figure 2. Breakdown pathways of chlorophyll in vegetables

Unireponse modelling: Schwartz & von Elbe (3) analysed the degradation of chlorophyll in heated spinach. The authors started their analysis by assuming a first-order degradation of chlorophyll; they estimated the rate constant via linear regression of the log-transformed data. The result is presented in Fig. 3A. The question is whether this is convincing evidence for a first order reaction. The first problem consists in the logarithmic transformation, as this not only changes the data but also their error structure, and depending on the actual error structure this transformation may violate the conditions for linear regression. We therefore did the analysis again via nonlinear regression without transformation of the data (Fig. 3B), and for comparison the result obtained via linear regression is also given. It can be seen that there is a difference. How large the difference is depends on the error structure of the data, and varies from case to case. In general it is recommended to perform nonlinear regression because that does not require transformation of the data.

Furthermre, the data were also analysed by not fixing the order n =1 but rather searching for a best fit for the

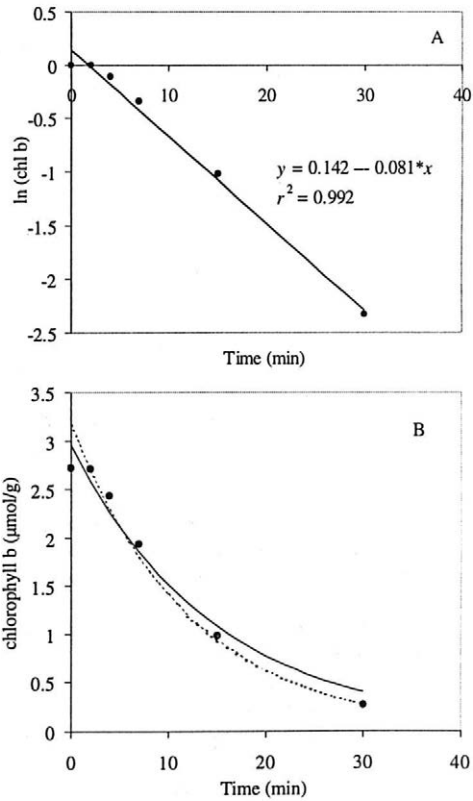

Figure 3. First-order model for degradation of chlorophyll b in heated spinach. Logarithmic plot (the equation indicates the regression line) (A); nonlinear regression; the dotted line gives the result obtained from the analysis in Figure 3A (B)

order n via nonlinear regression. The result is presented in Fig. 4 and it turned out that $n = 0.6 \pm 0.4$ ($\pm 95\%$ confidence interval). The fit seems to be slightly improved for $n = 0.6$. It thus appears that it is not so clear whether this is indeed a first-order equation (interestingly enough, the same result was found for chlorophyll a, results not shown). However, the 95% confidence interval for n is so large that we cannot discriminate between $n = 0.6$ and $n = 1$ based on the present dataset. More experimental data are necessary to be able to discriminate further. Nevertheless, this case indicates another pitfall. Despite the impressive coefficient of determination ($r^2 = 0.992$, Fig. 3A), a first-order reaction is at least doubtful. In contrast to common practice, the r^2 value should not be used to discriminate between models. It only gives the degree of correlation between two variables, not the goodness of fit. A very simple but powerful test is to look at residuals. Other tests are also available as discussed in Stewart et al (4, 5). The residuals in Figs. 3 and 4 do not seem to behave randomly except the fit for $n = 0.6$, and therefore this is another reason to cast some doubt on the applicability of a first-order model.

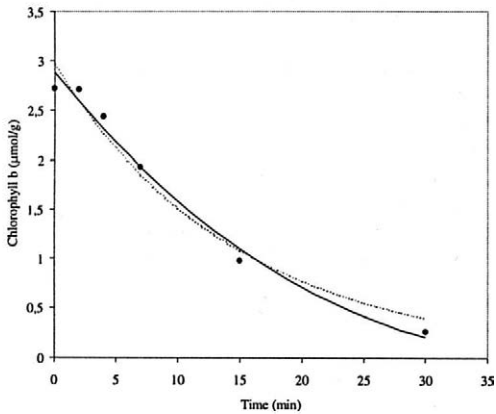

Figure 4. Nonlinear regression of the chlorophyll b data; fit is shown for $n = 0.6$ (solid line) and $n = 1$ (dotted line).

Multiresponse modeling: The results from Schwartz & von Elbe (3) open up the possibility to do multiresponse modelling because they did not only determine chlorophyll but also the degradation products pheophytin and pyropheophytin (cf. Fig. 2). They suggested the following model for chlorophyll breakdown consisting of two consecutive first-order reactions:

$$\text{Chlorophyll} \xrightarrow{k_1} \text{Pheophytin} \xrightarrow{k_2} \text{Pyropheophytin}$$

and they estimated the rate constants k_1 and k_2 by first estimating k_1 from chlorophyll b breakdown as in Fig. 3A (linear regression of log transformed data) and next k_2 by fitting the model to pheophytin data via nonlinear regression using the obtained estimate of k_1 and the initial concentration c_0. Hence they used a 2-step fitting procedure, In contrast, multiresponse parameter estimation estimates all parameters at once, but requires a different approach than the familiar least squares procedure, namely the determinant criterion, as explained elsewhere (6–8). The software used for this was GREGPAK/GREGPLUS (7). The results of the multiresponse estimation for the chlorophyll data are in Fig. 5.

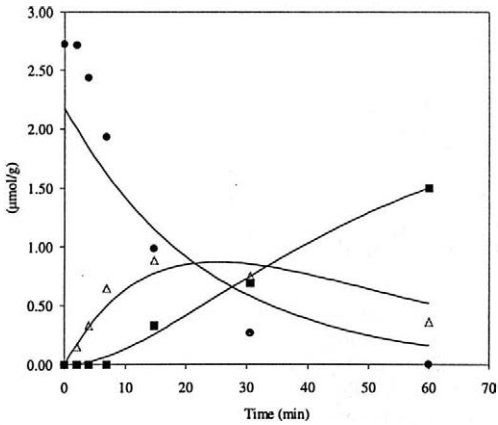

Figure 5. Multiresponse modelling of chlorophyll b degradation in heated spinach. Chlorophyll b (●), pheophytin b (Δ), pyropheophytin b (■)

Whilst the fits for pheophytin and pyropheophytin seem reasonable, the fit is bad for chlorophyll. This signals a problem for the model, and if one takes a closer look at the mass balance (3) it is clear that there must be additional breakdown of components. It is of course not immediately clear which component is subject to further breakdown. Recently, Teng & Chen (9) determined breakdown products of chlorophyll in spinach using various heating methods, and they suggested additional pathways as depicted in Fig. 6.

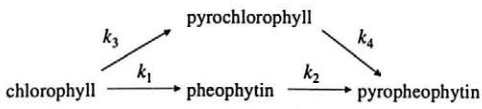

Figure 6. Model for degradation of chlorophyll in heated spinach (9)

Multiresponse analysis of the data given in (9) indicated that the step represented by k_4 was redundant (results not shown). Application of the model displayed in Fig 6 (with $k_4 = 0$) to the data given in (3) lead to the result shown in Fig 7.

This clearly gives a much better fit than the one in Fig. 6. It does of course not imply that now the right model is obtained, we can only state that it is better than the previous one. To test for goodness of fit and discrimination, statistical criteria are now available for multiresponse models (5). It is however necessary to have replicates of the experiments in order to compare pure experimental error

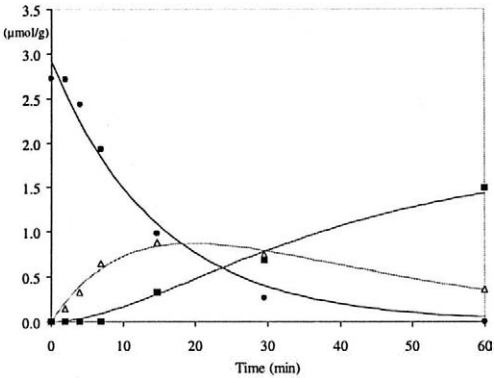

Figure 7. Multiresponse estimation of the model in Fig 6 to degradation of chlorophyll b in heated spinach. Chlorophyll b (l), pheophytin b (D), pyropheophytin b (n)

with residuals of the fit. Unfortunately, this information is not available for the present data set (3).

Another interesting effect can be seen from this modelling exercise; in the fit displayed in Fig. 7 the initial concentration was estimated as a parameter rather than as a fixed value. The estimate (c_0 = 2.92 mmol/g) was considerably higher than the experimentally determined value (c_0 = 2.72 mmol/g). While it is not a direct proof it could indicate that not all chlorophyll is easily determined in untreated spinach, whereas the chlorophyll in thermally treated spinach is more readily extractable (for instance because cell walls/chloroplasts are damaged).

Coming back to the iterative nature of modelling, this modelling result may prompt new experiments to further refine the model. It was suggested before (1) that also additional breakdown of pheophytin into pheophorbide is a possibility, and there is some evidence in literature that this actually occurs (10). The important conclusion here is that multiresponse modelling is very helpful, and in fact essential, to unravel complex reactions in foods. Finally, it is worthwhile looking at the precision obtainable with multiresponse modelling. Table 1 gives a summary and it can be seen that indeed much improved estimates can be obtained. This is due to the fact that much more information enclosed in the data can be utilized than when uniresponse modelling is used.

Table 1. Value and precision of parameters (± 95% confidence interval) for the model in Fig. 6

Parameter	Uniresponse modelling (2 step procedure)	Multiresponse modelling (1 step procedure)
c_0 (μmol/g)	3.15 ± 0.43	2.92 ± 0.14
k_1 ($s^{-1} \times 10^3$)	1.35 ± 0.18	0.7 ± 0.07
k_2 ($s^{-1} \times 10^3$)	1.73 ± 1.1	0.63 ± 0.05
k_3 ($s^{-1} \times 10^3$)	–	0.42 ± 0.10

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Lipid Oxidation in Complex Food Systems. Initiation and Coupling with other Quality Changes

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Abstract

For homogeneous systems, resistance to oxidation depends mainly on the balance between antioxidants and prooxidants. Most food systems are, however, heterogeneous, and in such phase-separated systems mobility of free radicals or oxygen permeation may become rate determining for progression of lipid oxidation. Electron Spin Resonance (ESR) spectroscopy has been used, in combination with Differential Scanning Calorimetry, to identify rate determining steps in such food models and to determine the rate of formation of free radicals and rate of oxygen permeation. Both ESR spin trapping and ESR spin probing techniques hold the potential for further development for the study of food systems undergoing oxidation in conjugation with other quality changes such as crystallisation, structural collapse or light induced discoloration. New developments in methods will be reviewed.

Keywords: lipid oxidation; photooxidation; ESR-spectroscopy; radicals; glass transition; storage stability

INTRODUCTION

Food industry faces an increasing demand for minimally processed "fresh" food with long shelf-life and at the same time an increasing degree of convenience. Protection of sensitive flavour components and nutrients is thus important for the overall wholesomeness and quality of such "fresh" food products with increased shelf life. Important nutritional compounds are destroyed by browning reactions or by oxidation, or the freshness is compromised by water migration between different components during storage which may induce crystallisation or collapse of glassy food matrices (1–3). The conflicting demands of freshness and long shelf-life require an increasing understanding of the physical properties of foods in order to optimise product quality, and research efforts should be directed towards reaction mechanisms of lipid oxidation and browning reactions in heterogeneous systems with phase separation and hindered molecular mobility (4).

Oxidation of unsaturated lipids involves initiation, propagation and termination reactions. Initiation of autoxidation depends on formation of free radicals followed by formation of hydroperoxides (5). The primary step in photooxidation is activation of oxygen to form singlet-oxygen, which (in the Type II mechanism) adds to unsaturated lipids also to form hydroperoxides (6). These mechanisms are fairly well-understood in homogeneous systems like vegetable oil and alcoholic beverages with unhidered contact between oxidation substrate and free radicals or activated oxygen (5–7). Most foods, however, have phase separation like meats, where metal catalysed formation of free radicals occurs in an aqueous phase separated from the lipids, or like frozen, dried or baked foods, where proteins or carbohydrates are able to form a glassy phase encapsulating the oxidation substrate.

Progress in design of "fresh" food with long shelf-life will depend on a successful kinetic modelling of oxidation (and browning) reactions in heterogeneous food systems. Electron Spin Resonance (ESR) spectroscopy holds the potential for further development (8, 9), as will be evident from recent investigations on reaction mechanisms in food model systems.

EXPERIMENTAL

For characterisation of early events in lipid oxidation, various methods have been adapted to food systems:

Continuous wave photolysis: Light intensities were determined by chemical actinometry for monochromatic light (wavelength typical for fluorescent light like 366 nm, 405 nm and 436 nm) selected from a high-intensity light source (18, 11). Photochemical reactions were characterised by their quantum yields (molecules reacted relative to photon absorbed).

Oximetri: Oxygen concentration in food model systems was measured non-invasively by incorporating a paramagnetic fatty acid derivative, 16-doxylstearic acid, into the samples (12). Oxygen, which itself is paramagnetic, broa-

dens the ESR signal of the probe through Heisenberg spin exchange, and the line width becomes proportional to the oxygen concentration (13).

Detection of Radicals. Three methods were used for detection of free radicals in foods by ESR: (i) direct measurement of free radicals in dried foods like milk powder (14) or freeze-dried cheese or milk (15,16); (ii) spin trapping of radicals in non-rigid systems like beer (7) and processed cheese (15); and (iii) addition of stable radicals to the food and measurement of the rate of their depletion through reaction with radicals formed in food during storage (15).

Quantification of radicals. The rate of formation of radicals (in food emulsions) was quantified by ESR using a spin trap (N-tert-butyl-∀-phenylnitrone) together with the lipid-derived spin-probe, 12-doxylstearic acid, as internal standard for "free radical concentration" (17).

RESULTS AND DISCUSSION

The primary event in lipid autoxidation is formation of the first free radicals to initiate the autocatalytic process. For photooxidation of lipids, addition of singlet oxygen yields the precursor for such radicals.

These primary radicals have been identified by ESR spin-trapping spectroscopy for certain homogeneous products; in lager beer the quantitatively most important radical during initiation of lipid oxidation was thus found to be the 1-hydroxyethyl radical (7). In homogeneous systems like beer, the rate of formation of such first radicals determines together with the presence of antioxidants the length of the lag phase and subsequently the rate of oxidation following the lag phase (18).

For dried foods like milk powder or dried chicken meat for instant soups, ESR spectroscopy was found to be non-discriminating and only to provide a broad unstructured signal (proportional to the content of free radicals), which could not be assigned to specific radicals (14, 16). Accordingly, the initiation mechanism is less certain in such products, however, progress was recently made using azo-compounds as radical initiators in a model system for dried products like egg powder, milk powder and freeze-dried starter cultures (19). Radicals were generated thermally either from a hydrophilic radical generator in the glassy carbohydrate matrix or from a lipophilic radical generator in the encapsulated lipid as confirmed from ESR-spectre of spin adducts formed by added spin traps. Formation of lipid hydroperoxides in the encapsulated lipid followed zero order kinetics and notably, the rate of oxidation was only enhanced by radicals generated in the lipid. The radicals generated in the glassy matrix was concluded to be immobilised, while oxygen was able to penetrate the glassy matrix in a rate determining step. Initiation of oxidation in such dried foods has accordingly to occur in close proximity to the lipids in the carbohydrate (or protein) matrix and oxygen permeation rate becomes critical.

In order to study the kinetics of oxygen permeation through a glassy carbohydrate matrix in more details, a non-invasive method based on ESR signal broadening in presence of oxygen (oximetry) for determination of oxygen concentration in biological systems (13), was adapted to the same carbohydrate based glassy matrix encapsulating a model lipid with the (oxygen sensitive) spin probe dissolved (12). A kinetic model for oxygen permeation from head space through the glassy matrix into the encapsulated lipid could be established based on rate data up to an equilibrium distribution of oxygen for temperatures below the glass transition temperature (65°C as determined by Differential Scanning Calorimetry). The kinetic model allows for structural heterogereity and the rather large

energy of activation of 74 kJ/mol determined (for this specific matrix) is in favour of a molecular model for oxygen diffusion (rather than the free volume model). Oxygen permeation through a protecting matrix is accordingly thermally activated and collapse of powder structure (above glass transition) is critical and depends on water activity and temperature.

The importance of the glass transition was further seen for a freeze-dried starter culture (*Streptococcus thermophilus*) for the dairy industry (21). For a matrix of ascorbic acid, casein and sucrose or mannitol, the loss of acidification activity (most probably due to oxidative processes) during storage (at constant water activity) showed Arrhenius behaviour below (but not above) the glass transition temperature (as determined by Differential Scanning Calorimetry) confirming the importance of oxygen permeation shown in the model system (12). The thermally activated loss of acidification activity had a lower energy of activation for the better radical scavenger mannitol (40 kJ/mol) compared to sucrose (57 kJ/mol).

The rate of formation of radicals in the lipid phase or at the interphase is, together with the oxygen permeation rate, critical for lipid oxidation. Attempts to quantify the rate of radical formation in term of an absolute rate has been successful so far for an oil-in-water food emulsion through a combination of the spin-trapping and the spin-probing technique (17). Radicals were trapped under pseudo-first order conditions (excess of spin-trap) ensuring linearity between ESR-signal and concentration of radicals trapped, and rate of formation of radicals during initiation of lipid oxidation could be quantified (moles/l hour) by direct comparison with a free radical standard curve based on the spin probe 12-doxylstearic acid. This method is now being further developed by the use of combinations of spin traps designed to trap radicals in different phases of complex foods with the perspective of spatial assignment of radical formation and initiation of lipid oxidation.

The combined use of spin traps and spin probes has not yet been used for real foods. For processed cheese, however, the spin trapping technique and the spin probing technique were used separately to compare (on a relative scale)

the effect of exposure to light with the effect of temperature on formation of radicals in the product during storage. For the early stages in oxidative processes, exposure to light was found to be the more important factor compared to temperature for formation of radicals in the cheese. However, storage temperature is becoming more important on longer storage, as evaluated by the level of free radicals formed. A conclusion which was confirmed by analysis of secondary lipid oxidation products (15).

The importance of exposure to light for initiation of oxidation processes in food as seen for processed cheese warrants in general quantitative investigations to replace the more qualitative approach traditionally used. In one such investigation (of a dairy spread model), the prooxidative effect of light was expressed as quantum yields for formation of hydroperoxides and for degradation of riboflavin and carotenoids (11). In order to obtain a quantitative description, a four-step strategy was recently suggested for evaluation of the sensitivity of dairy products to light: (i) identification of photosensitive compounds/photosensitizers and the resulting product defects; (ii) determination of the quantum yield for relevant photoreactions(s) at varying wavelengths; (iii) construction of an action spectrum from quantum yields and absorption spectrum to be matched by the packaging material light absorption characteristics; and finally (iv) practical storage experiments, including quality evaluation (6).

CONCLUSION

Lipid oxidation in complex foods is strongly influenced by changes in the physical structure, and deviation from Arrhenius behaviour has been found to indicate glass transition and/or collapse of structure. Oxygen permeation through glassy matrices is thermally activated and structural collapse provide better contact with radicals formed in the non-lipid phase and increases oxygen permeation. The direct quantification of the rate of formation of radicals in separate phases using the combined spin trapping and spin probing technique will further allow optimal processing conditions to be developed and aid in correct use of antioxidants (optimised hydropholbic /hydrophilic balance and partition between different phases). However, structural assignment of the quantitatively most important radicals in the different phases may depend on LC-separation of the spin adducts formed followed by MS identification.

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The Reactions Between Hydrogen Sulfide and Lipid-Derived Aldehydes in Cooked Meat

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Abstract

Hydrogen sulfide, produced from cysteine upon heating, is an important contributor to the flavour of cooked meat. During the later stages of the Maillard reaction hydrogen sulfide can also react with other volatile compounds to form additional flavour compounds. Aldehydes, formed via lipid oxidation, may react with hydrogen sulfide, to give cyclic sulfur-containing compounds, including 2-alkyl-3-thiazolines, 2-alkylthiazoles, 2-alkyl-(2*H*)-thiapyrans and 2-alkylthiophenes. All of these groups of compounds have been identified in cooked meat. 2-Alkyl-(2*H*)-thiapyrans and 2-alkylthiophenes were formed in reaction mixtures containing cysteine, ribose and methyl linoleate or methyl a-linolenate. Their formation in the reaction mixtures indicated how these compounds might be formed during cooking.

Keywords: hydrogen sulfide, meat flavour, linoleic acid, a-linolenic acid, cysteine, ribose

INTRODUCTION

Cysteine reacts with ribose during the cooking of meat to give many important aroma compounds (1). Hydrogen sulfide, released when cysteine is heated, takes part in the formation of many of these compounds. Aldehydes formed via lipid oxidation may modify the reaction between cysteine and ribose, producing further compounds.

We have identified four classes of cyclic volatile compounds in cooked beef and lamb, formed by the reaction of hydrogen sulfide with lipid-derived aldehydes (Fig. 1) (2–7). 2-Alkyl-(2*H*)-thiapyrans and 2-alkylthiophenes may be formed from the reaction of hydrogen sulfide with 2,4-alkadienals (7), while the interaction of hydrogen sulfide with alkanals, ammonia and 2,3-diones or a-hydroxyketones is a possible route to 2-alkylthiazoles and 2-alkyl-3-thiazolines (8). 2-Alkylthiophenes and 2-alkylthiazoles have been reported in a number of different foods (9). Only one 2-alkyl-3-thiazoline, 2-isobutyl-4,5-dimethyl-3-thiazoline, has been previously reported, in a yeast extract (10). No 2-alkyl-(2*H*)-thiapyrans have been previously reported as food aroma constituents.

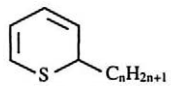
In this paper, we report the formation of 2-alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans in two model systems, both containing cysteine and ribose. One contained linoleic acid (18:2 *n*–6) methyl ester and the other contained α-linolenic acid (18:3 *n*–3) methyl ester. These systems were examined in order to explain how the aroma profiles of cooked meat from ruminants could be influenced by dietary supplements high in polyunsaturated fatty acids (PUFAs) such as 18:3 *n*–3 (3–7).

EXPERIMENTAL

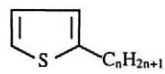
Buffered (pH 5.5) reaction mixtures, **A** and **B**, containing 0.5 mmol each of cysteine, ribose and either methyl linoleate or methyl a-linolenate respectively, were heated at 140°C for 30 min and allowed to cool. The reaction mixtures were analysed at room temperature by solid phase microextraction (SPME), using a divinylbenzene/Carboxen on polydimethylsiloxane (PDMS) fibre and a Carboxen/PDMS fibre together (Supelco, Poole, U.K.), followed by gas chromatography/mass spectrometry, using a CP-Sil 8 CB low bleed/MS fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness; Chrompack, London). This method is described elsewhere (11). Four replicates of each sample were analysed.

RESULTS

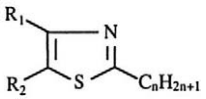
2-Alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans were identified in both reaction mixtures (Table 1). 2-Ethylthiophene, 2-methyl-(2*H*)-thiapyran, 2-ethyl-(2*H*)-thiapyran and 2-propyl-(2*H*)-thiapyran were present at much greater levels in **B** than **A**, whereas 2-butylthiophene, 2-pentylthiophene and 2-hexylthiophene were at substantially higher levels in **A** than **B**.



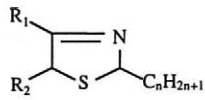
2-alkyl-(2*H*)-thiapyran
n = 1-6



2-alkylthiophene
n = 2-7



2-alkylthiazole
n = 4–10, 13–15
R_1, R_2 = Me, Et



2-alkyl-3-thiazoline
n = 4–9, 15
R_1 = H, Me, Et;
R_2 = Me, Et

Figure 1. Cyclic compounds formed from the interaction of hydrogen sulfide with lipid-derived aldehydes

Table 1 Quantities of 2-alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans (ng/100 mL sample) in the SPME extracts of reaction mixtures containing cysteine, ribose and a PUFA methyl ester

[a]Compound	LRI [b]	Quantity found 18:2 *n*–6 (A)	18:3 *n*–3 (B)
2-ethylthiophene	868	3 (1)	99 (12)
2-methyl-(2*H*)-thiapyran	912	–	19 (5)
2-propylthiophene	961	7 (12)	9 (2)
2-ethyl-(2*H*)-thiapyran	1019	–	399 (88)
2-butylthiophene	1065	8 (11)	tr
2-propyl-(2*H*)-thiapyran	1117	–	3 (0)
2-pentylthiophene	1169	21 (22)	tr
2-butyl-(2*H*)-thiapyran	1221	tr	–
2-hexylthiophene	1274	9 (11)	tr
2-pentyl-(2*H*)-thiapyran	1328	tr	–

[a]Values are the means of four replicate analyses, standard deviations are given in parentheses. [b]Linear retention index on a CP-Sil 8 CB low bleed/MS column. tr, less than 2 ng in the SPME extract from 100 mL sample; –, less than 0.5 ng in the SPME extract from 100 mL sample

Lipid oxidation on heating linoleic acid (18:2 *n*–6) leads to the formation of (*E*,*E*)-2,4-decadienal and (*E*,*E*)-2,4-nonadienal. Linolenic acid (18:3 *n*–3) is more readily oxidised, due to the extra double bond, leading to the formation of (*E*,*E*)-2,4-heptadienal and (*E*,*E*)-2,4-hexadienal (14). 2-Alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans were formed when these 2,4-dienals reacted with hydrogen sulfide. Formation of thiapyrans occurred much more readily in **B** than **A**, whereas in **A** thiophene formation was generally favoured.

The results for the reaction mixtures reflected observations of the volatiles obtained when diets high in *n*–3 PUFAs were fed to sheep and cattle. In that work increases of 2-ethylthiophene, 2-methyl-(2*H*)-thiapyran, 2-ethyl-(2*H*)-thiapyran and 2-propyl-(2*H*)-thiapyran occurred in the cooked muscle of the supplement fed animals, relative to that of the control animals (5, 6).

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Identification and Quantification of Aminophospholipid-Linked Maillard Compounds in Model Systems and Egg Yolk Products

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Abstract

Phosphatidylethanolamine (PE)-linked glucosylamines (Schiff-PE), Amadori products (Amadori-PE), 5-hydroxymethylpyrrole-2-carbaldehydes (Pyrrole-PE), and carboxymethyl (CM-PE) as well as carboxyethyl (CE-PE) derivatives were detected and quantified by liquid chromatography-electrospray mass spectrometry (LC-(ESI)MS). Model incubations of soy-PE and D-glucose were employed to firmly establish the LC-MS method. Analyses of spray dried egg yolk and lecithin products derived therefrom show one-fourth of the native D-glucose content of egg yolk to be transformed to Amadori-PE, corresponding to a PE derivatization quota of about 13 mol %. The high glycation quota of PE in egg yolk lecithin influences the emulsifying properties of the product.

Keywords: Maillard reaction; phosphatidylethanolamine; lecithin, egg yolk; Amadori compound; advanced glycation endproducts (AGEs)

INTRODUCTION

The Maillard reactión or nonenzymatic browning is initiated by an amine adding to the carbonyl function of a reducing sugar, forming glycosylamines. These compounds, as a rule, rearrange to the more stable Amadori products which undergo further reactions, producing a wide spectrum of so-called "advanced glycation endproducts" (AGEs). While the Maillard reaction of free amino acids and proteins is a well investigated process, no defined structures of glycated aminophospholipids in foodstuffs have been established so far. We have therefore independently synthesized the Maillard products **2-5** of 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine (**1**, PE (16:0-16:0), see Fig. 1) as reference material and established an LC-MS method for identification and quantification of such products in complex biological matrices [1]. We now report on the identification and quantification of PE-linked Maillard products, structural analogous to **2-5**, in model systems as well as in egg yolk products. The emulsifying properties of glycated egg yolk lecithin have also been investigated.

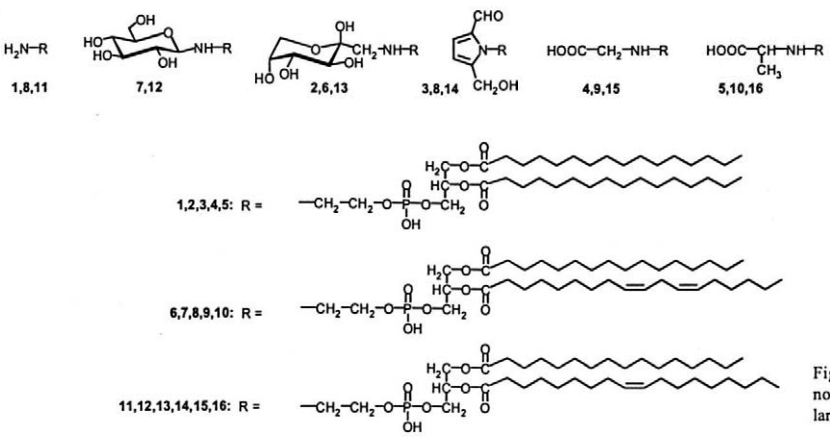

Figure 1. Phosphatidylethanolamine (PE)-linked Maillard products

EXPERIMENTAL

The Maillard compounds **2-5** were synthesized according to Utzmann and Lederer [1]. LC-(ESI)MS analyses were performed on an HP1100 HPLC system (Hewlett Packard; Waldbronn, Germany) coupled to a Micromass (Manchester, UK) VG platform II quadrupole mass spectrometer equipped with an ESI interface (conditions see ref. [1]). Model incubations of a PE isolate from soy beans (soy-PE, ~15 mM) and D-glucose (500 mM) were conducted in EtOH/phosphate buffer 0.1 M, pH 7.4 (3:2) and the reaction mixtures purified by solid phase extraction (SPE) [2]. The quantification procedures for the Maillard products and D-glucose in egg yolk and egg yolk lecithin are also given in ref. [2]. Estimation of emulsifying properties was performed according to ref. [3].

RESULTS AND DISCUSSION

For obtaining reference material to establish an LC-MS method, the following Maillard products of **1** were independently synthesized: the Amadori product (**2**, Amadori-PE [16:0–16:0]) which represents the initial stage of the Maillard reaction, the pyrrolecarbaldehyde (**3**, Pyrrole-PE (16:0–16:0)) as well as the carboxyethyl derivative (**5**, CE-PE [16:0–16:0]) being characteristic for advanced glycation reactions, and the carboxymethyl derivative (**4**, CM-PE [16:0–16:0]) which indicates oxidation processes [1].

In model reactions, soy-PE (~15 mM) was reacted with D-glucose (500 mM) for either 2 h at 65 °C or 3 h at 100 °C. The LC-MS analysis of the incubation at 65 °C showed two peaks in the ion trace for the Amadori-PE (**6**, 16:0–18:2; *m/z* 876.6). One peak could be identified as the Amadori product **6**, the other peak eluting just in front as the corresponding glucosylamine (**7**, Schiff-PE), being the precursor of the Amadori compound. Schiff-PE shows a characteristic fragmentation pattern, with loss of 120 Da from $[M-H]^-$ which can be best explained by a reverse aldol reaction (see Fig. 2). The quantitative results of both incubations are presented in Fig. 3. Whereas in the 65 °C reaction only Schiff-PE **7** and Amadori-PE **6** were formed, all investigated Maillard compounds could be identified in the 100 °C incubation.

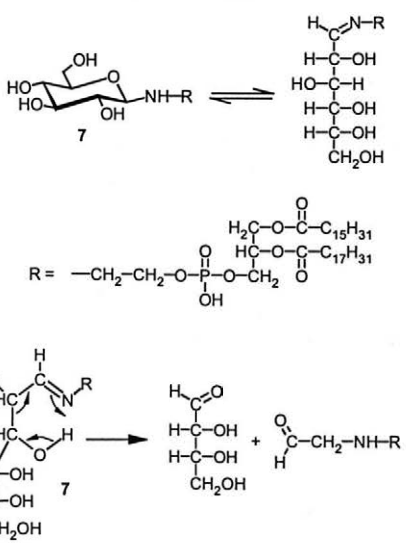

Figure 2. Fragmentation pathway of Schiff-PE 7

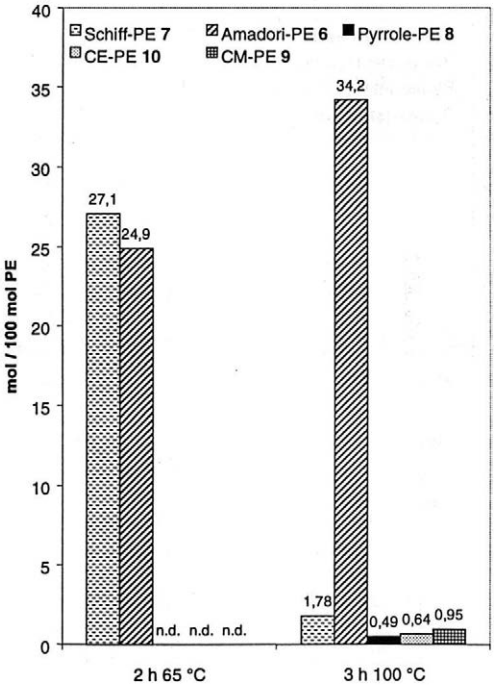

Figure 3. Quantitive results for the soy-PE/D-glucose incubations

With the method established for model incubations, we now looked for the corresponding Maillard products of PE (16:0-18:1, **11**) in egg yolk which contains about 2.8 % (w/w) PE in dry weight. From lyophilized, pasteurized, and spray dried egg yolk the PE fraction was isolated and tested for the Maillard compounds **12–16**. In freeze dried (EY1a + 1b) and pasteurized egg yolk samples (EY2a + 2b) only small amounts of Schiff-PE **12** could be detected and their content of free glucose correlated well with literature data for native egg yolk. The spray dried egg yolk (EY3a + 3b), in contrast, contained only small amounts of glucose but considerable quantities of Amadori-PE **13** (12.7 and 13.5 mol %) and traces of Pyrrole-PE **14**, as shown in Fig. 4. About one-fourth of the native glucose has thus been linked to PE. Hence, we have for the first time established the formation of glycated PE (*g* PE) in foodstuffs. Lecithin products (EYL1 + 2), obtained from spray dried egg yolk, contain similar amounts of *g* PE as the egg yolk itself. The

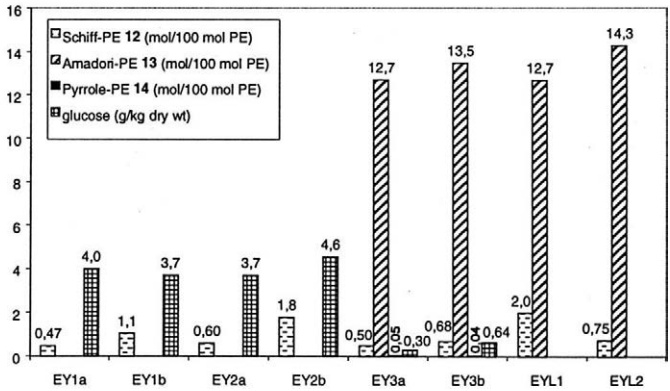

Figure 4. Glycation quota and glucose content of lyophilized, pasteurized, and spray dried egg yolk and of lecithin products derived from spray dried egg yolk

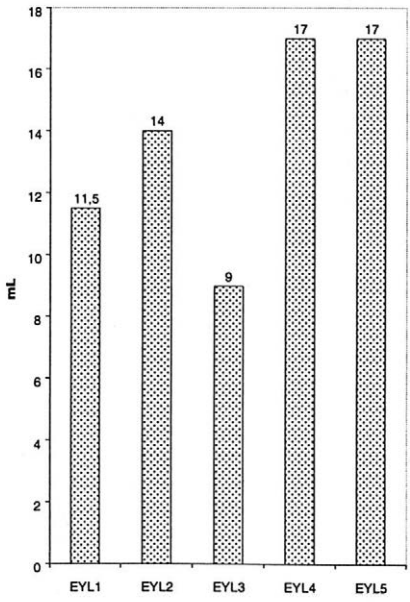

Figure 5. Emulsifying properties of different lecithin products (the given values represent mL of water that was separated from the emulsion after 24 h at 50°C)

Maillard products obviously are extracted along with the underivatized PE during industrial processing. CM- and CE-derivatives of PE could not be detected in any of the egg yolk products. The content of Amadori compounds such as **13** may serve as an indicator for heated egg yolk products. Since the polarity of the phospho head group is effectively changed by the glycation process, we have investigated the emulsifying properties of egg yolk lecithin containing *g* PE (EYL1-3) and lecithin derived from egg yolk that was desugarized before spray drying (EYL4 + 5). The EYL from the desugarized egg yolk lacking *g* PE showed poorer emulsifying properties for O/W-emulsions than the lecithin containing *g* PE (Fig. 5).

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Biomolecular Transformations of Complex Biophenols occuring at the Processing and Storage in the Table Olive and Oil Supply Chain

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Abstract

Oleuropein (**1**) undergoes numerous reactive processes during olive maturation, processing and food storage. The pathways reveal effects on olive oil and table olives, influencing the quality descriptors. The hydrolytic conversion, under abiotic conditions, generates bioactive metabolites. The secoiridoid catabolic degradation of **1** to a final lacton shows the Cannizzaro-like behaviour. The secoiridoid reactivity of **1** was shown to be dependent on the different functionalities as in the lye treatment, controlled by the experimental conditions exerted on the olive pulp and determined by the buffering capacity of the olive mesocarp and by the epicarp molecular components influencing the reactant peneration into the fruit pulp. The bioavailability, after processing and to the sensorial response into the oral cavity, was shown to be influenced by physicochemical proprieties of the BP molecules, their concentration and the supramolecular interactions with the other components comprised in the food matrix, as experimented by NMR biomimesis, with sensorial experiments, performed on single and mixed BPs, for the perception of pungent and bitter tastes.

Keywords: eleuropein; olives; olive oil; *o*-diphenols

INTRODUCTION

Nutritional and hedonic aspects of food reactive biomolecules highlight today the perceived relationship between diet and chronic diseases, such as obesity, vascular pathologies and cancer, moving beyond preventing deficiency diseases into promoting health, longevity, wellbeing, and quality of life (1).

The food market is now under a large pressure for the many changes, occurring during the *millennium* transition and calling for a repositioning of traditional production systems, e.g. the eating style, also according to the Mediterranean Aliment Culture (MAC).

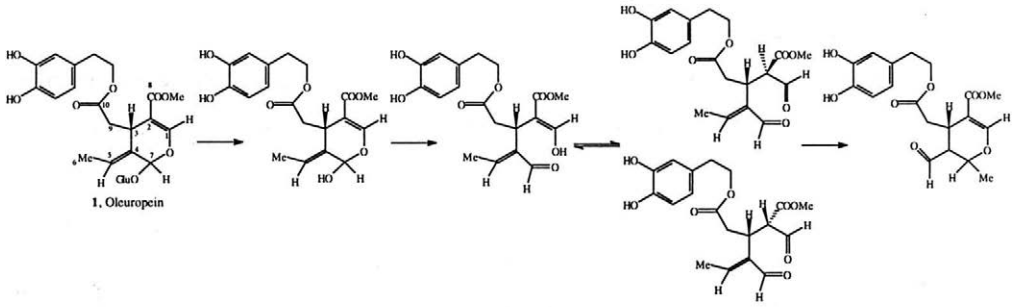
Biologically active non-nutrient minor components, having monomeric o-diphenolic mojeties, i.e. the biophenols (BPs), are widely present in all plant derived foodstuffs, as well as in EVOO-extra virgin olive oil (1a) and WOTO-wizened oinotria table olives (2), typical products of the MAC (3). The BPs of EVOO and WOTO may be of bio- or techno-origin, i.e. being naturally present in the olive drupes (4) or enzymatically or catalytically generated by the technological and storage transformation processes of the harvested fruits (5), exerting their antioxidant, free-radical antagonism and antimicrobial activity (6) for the consumer's wellbeing.

The importance of endogenous bioactive molecules, such as BPs, in the olive food supply has long been recognised (7) for their contribution to the flavour and to the functional quality (8) of the MAC products (9). The simple and complex BPs, having biomolecular structures of those contained in EVOO and WOTO, are considered to affect the hedonic-sensorial quality of the MAC foodstuffs (10), because of their pungent and bitter tastes (11).

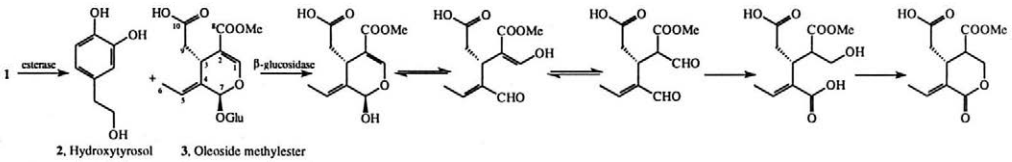
BPs are thus showing controversial effects which, until now, have not been provided an access to a single comprehensive source of critically-assessed experiments on occurrence, relation, levels and factors affecting the hedonic-sensorial and the functional qualities of MAC agrifood products, containing such biomolecules.

RESULTS AND DISCUSSION

In order to validate the BP role in the MAC foodstuffs, the biomimetic reactive system of EVOO and WOTO has been investigated towards three categories of information. The first category refers to the BP distribution in EVOO and WOTO (12) and their hydrolytic conversion under biomimetic conditions, induced by the endogenous enzymatic system of the olive fruit, assigning the molecular structures to new bioactive BP metabolites of oleuropein **1**, the hemiacetal aglycon, and the two epimeric dialdehydes, as shown in Scheme 1. They can influence the patogen antagonism of olive fruit (13) and the hedonic-sensorial characteristics (14) of the MAC agrifood products.



Scheme 1



Scheme 2

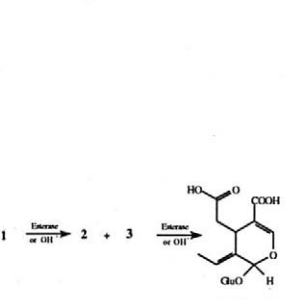
The evaluation of the metabolic process of **1** in the olive drupes, during maturation, gave the results as shown in Scheme 2, where, a part from the known esterase clavage (15), the Cannizzaro-like derivatives **4** and **5** were discovered in *Hojiblanca* cultivar from Spain.

As described from the molecular reactivity in Scheme 1–3, the major BP component of olives, a bifunctional ester at the bident carboxylic moiety of oleoside **6** (Scheme 3), results from the conjugation with MeOH and **2**. The two pending carboxylic groups of the monoterpene unit reveal different levels of reactivity toward esterases and basic attack, occurring in BP **1** (16).

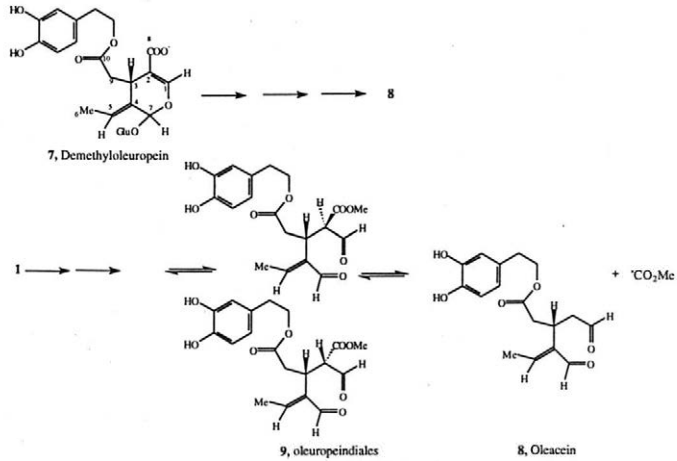
Some olive cultivars, containing a demethylated metabolite **7** of **1** (15), found in ripe fruits (17) and, available for varietal marker (18), can provide oleacein **8**, only when derived from **7**, since the demethylation process must be a prerequisite for the decarboxylation of **1**, as reported in Scheme 4.

This molecular reaction sequence was invoked from **1** after b-glucosidase attack, enol isomerisation to the oleuropeindiale **9**, and an unrealistic dynamic intermolecular equilibrium inducing $^{\bullet}CO_2Me$ expulsion (19), as a free radical leaving group. On the contrary, the decarboxylation step of the methyl ester in **1** should require hydrolysis, followed by CO_2 elimination, without any reversible reaction, because CO_2 is expelled as gas. This reaction is absent in the lipidic matrix, during the EVOOstorage (20).

The second category of information relates the bioavailability after processing and the sensorial response into the oral cavity.



Scheme 3



Scheme 4

Table 1. Thermodynamic parameters of the BP–Caf supermolecules

BPs	$K_{298\ K}$	$K_{313\ K}$	$K_{333\ K}$	$DG_{298\ K}$	$\Delta G_{313\ K}$	$\Delta G_{333\ K}$	ΔH	ΔS
Ty	4	3	2	−3.6	−2.5	−1.9	−18.9	−51.3
2	9	7	5	−5.5	−5.2	−4.7	−12.1	−22.1
HoVA	8	4	1	−5.1	−3.4	−1.0	−39.9	−117.7
3,4-DHPA[b]	16	12	5	−6.9	−6.5	−4.4	−28.4	−71.0
3,4-DHBA	57	49	19	−10.0	−9.5	−8.1	−26.3	−54.7
1	38	30	23	−9.0	−8.8	−8.7	−11.7	−9.0

[a]K = M^{-1}; DG = KJ/mol; DH = KJ/mol; DS = J/mol * deg. [b]From ref. 21

Table 2. Thermodynamic parameters of the BP–b-CD supermolecules[a]

BPs	$K_{298\ K}$	$K_{313\ K}$	$K_{333\ K}$	$DG_{298\ K}$	$\Delta G_{313\ K}$	$\Delta G_{333\ K}$	ΔH	ΔS
Ty	497	318	179	−15.3	−14.9	−14.3	−24.1	−29.5
2	355	260	116	−14.6	−14.5	−13.1	−26.7	−40.6
HoVA	137	110	97	−12.2	−12.2	−12.6	−7.9	14.4
3,4-DHPA[b]	528	358	188	−15.5	−15.3	−14.5	−24.2	−29.0
3,4-DHBA[c]	661	463	411	−16.0	−15.9	−16.6	−11.0	16.7
3,4-DHPA$^-$	191	–	–	–	–	–	–	–
3,4-DHBA$^-$	219	–	–	–	–	–	–	–

[a]K = M^{-1}; DG = KJ/mol; DH = KJ/mol; DS = J/mol * deg. [b]From ref. 21

Flavour release, during processing and eating, is influenced by physicochemical proprieties of the BP molecules, their concentration and the supramolecular interactions with the other components comprised in the food matrix, as experimented by FAB/MS and by NMR biomimesis (21).

The titration of model BPs was carried out with solution of caffeine (Caf) and β-cyclodestrine (β-CD) in D_2O; the experimental results are reported in Tables 1 and 2, where Ty = Tyrosol, HoVA=Homovanillic acid, 3,4-DHPA= 3,4-dihydroxyphenilacetic acid; 3,4-DHBA=3,4-dihydroxybenzoic acid, and 3,4-DHBA$^-$ the corresponding anion.

Sensorial biomimetic experiments, as the third category, performed on single and mixed BPs, reproducing model EVOO compositions, revealed the absence of perception mainly responsible of pungent and bitter tastes, as checked according to the panel test method and analysed by HPLC and 2D ^{1}H-NMR (22).

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Furan Fatty Acid Photooxidative Degradation Products in Dried Herbs And Vegetables

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Abstract

The influence of light exposure of dried herbs and vegetables on the formation of dimethyl furan fatty acid (DiMeFFA) photooxidative degradation products 2,3-butanedione, 2,3-octanedione, 3-methyl-2,4-nonanedione (MND), 3,4-dimethyl-5-pentyliden-2(5H)-furanone (bovolide) and 3,4-dimethyl-5-pentyl-2(5H)-furanone (dihydrobovolide) was investigated. To our knowledge, the occurrence of these compounds in tarragon, dill, basil, chervil, chive, leek, savory and onion is reported here for the first time. The flavour fraction was isolated by simultaneous distillation extraction and analysed by GC-MS. The flavour compounds were determined in relation to the exposure time as peak area ratio to an internal standard.

Keywords: furan fatty acids, photooxidation, flavour compounds, dried herbs

INTRODUCTION

The first article on furan fatty acids as precursors of flavour compounds was published by GUTH & GROSCH in 1991 (1). They reported that MND is a photooxidative degradation product of DiMeFFA and contributes significantly to the light induced off-flavour of soya-bean-oil. Shortly after, SARELSE *et al.* (2) put forth the hypothesis that bovolides are formed by photooxidation of dimethyl furan fatty acids. This was experimentally confirmed by POMPIZZI *et al.* (3). Recently, the flavour compounds 2,3-butanedione and 2,3-octanedione were identified in model experiments as additional photooxidative degradation products of DiMeFFA (4).

MND is an important flavour compound of green tea (5), dry parsley (6) and dry spinach (7) with a strawy and hay-like odour (6, 7). In these plant species, 2,3-butanedione was detected as well. Bovolide and dihydrobovolide have been identified in several foodstuffs including dried green parts of plants such as green tea (8), lamb's lettuce, garden cress and woodruff (3) and are flavour compounds with a celery-like odour (9). The sensory property of 2,3-octanedione is reported to be caramel-like and sweet (10) and sweet and fruity (11), respectively. This diketone, the origin of which puzzled several authors (see e.g. (10,11) and the literature cited therein) was identified for example in beef fat (10), sheepmeat (11) and in freshwater fish (12).

In this work, the occurrence of DiMeFFA photooxidative degradation products in different dried plant materials is reported and their relative change after light exposure is investigated.

EXPERIMENTAL

Food samples: Dried samples of tarragon (*Artemisisa dracunculus*), basil (*Ocimum basilicum*), savory (*Satureja hortensis*), chervil (*Anthriscus cerefolium*), dill (*Anethum graveolens*), chive (*Allium schoenoprasum*), onion (*Allium cepi*) and leek (*Allium porrum*) were obtained from J. Carl Fridlin Gewürze AG (Hünenberg, Switzerland) in paper bags. The samples were stored vacuum-packed in light-protected bags.

Light exposure of the food samples: The dried samples were packed in transparent PE-film bags filled with compressed air. All samples were exposed to two 36 W fluorescence lamps (BIOLUX 36[a], Osram AG, Winterthur, Switzerland) at 4500 lx at room temperature for 4 days. Samples before light exposure were taken as reference.

Extraction of the volatile fraction: Extraction of the volatiles was performed in a micro-steam distillation apparatus (Chrompack 16050, P.H. Staehelin & Cie. AG, Basel, Switzerland) for 1 hour (6 g sample, 80 ml water, 2 ml diethyl ether, 11 μg ethyl valerate and 12 μg ethyl decanoate as internal standards). The extract was dried over anhydrous magnesium sulphate and concentrated to 1 ml by means of a Kuderna-Danish concentrator fitted with a Vigreux column at 50°C.

Gas chromatography-mass spectrometry (GC-MS): GC-MS was performed with on column injection technique on a Fisons 8065 gas chromatograph directly coupled to a Finnigan MAT SSQ 710 mass spectrometer. A fused silica SW-10 column (Supelco, 60 m × 0.32 mm i.d., 0.25 mm film thickness) with a deactivated fused silica pre-column

Table 1. Change of the flavour compounds during exposure to light (peak area ratio to the internal standard ethyldecanoate)

Sample		2,3-butane-dione RI = 956	2,3-octane-dione RI = 1342	MND RI = 1728	Bovolide RI = 2164	Dihydro-bovolide RI = 2192
Tarragon	R	2	tr	nd	1	1
	L	4	1	1	7	1
Basil	R	1	tr	nd	3	4
	L	2	1	nd	4	4
Savory	R	1	tr	nd	1	nd
	L	1	tr	nd	2	nd
Chervil	R	5	1	nd	4	3
	L	6	4	4	20	5
Dill	R	1	tr	nd	1	1
	L	1	1	nd	4	1
Chive	R	4	1	tr	2	d.
	L	4	6	4	7	1
Onion	R	tr	tr	nd	tr	nd
	L	tr	tr	nd	tr	nd
Leek	R	1	tr	nd	1	1
	L	1	tr	tr	2	1

RI: retention index; R: reference; tr: traces; L: exposed to light (4 d); nd: not detected

(2.8 m × 0.53 mm i.d.) was used with helium as carrier gas (100 kPa). The following temperature programme was used: 40°C for 12 min, 40°C to 240°C at 6°C/min, 240°C for 15 min. Electron impact mass spectra were recorded with an ionisation energy of 70 eV in a mass range of 40–440 amu. The compounds were identified by comparison of mass spectra and retention indices (RI, calculated according to Van den Dool and Kratz (13)) with reference substances. For the evaluation of the ratio of the flavour compounds to the internal standard ethyldecanoate, the fragment-ions at m/e 68 (2,3-butanedione), 99 (2,3-octanedione, MND), 124 (bovolide) and 83 (dihydrobovolide) were used.

RESULTS AND DISCUSSION

The investigated herbs and vegetables have been shown to be differently susceptible to light exposure (see Table 1); onions were hardly affected at all whereas tarragon, chervil and chive exhibited the most significant changes after irradiation. Bovolide was shown to be formed in considerable amounts in almost all samples. It is interesting to note that MND, the most prominent photooxidative degradation product of DiMeFFA, could only be detected either in very small amounts or not at all in the irradiated samples, except for chervil and chive. One reason for this could be that the duration of light exposure used in the present set of experiments (4 d) was too long. MND could have been formed during the early stages of irradiation and reacted further during the latter stages. Preliminary results obtained with green tea (not shown) indicate a decrease of MND after longer irradiation times.

The present work does not give any indication about the relevance of the oxidation products to the flavour or off-flavour of the herbs and vegetables. Studies concerning this question are in progress.

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Retention of Heme Iron in Beef Cooked using Different Methods

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Abstract

Beef *M. Longissimus dorsi* was cooked using four methods: sous vide, roasting in an oven, frying in a pan in slices and mincing and frying like hamburgers. The retention of heme iron was high in all the cooking methods, although significant losses were found in all the methods, except pan frying in slices. Sous vide cooking gave the lowest retention (75%), roasting in an oven and pan frying minced meat gave higher retention (83% and 87%, respectively), while pan frying slices gave the highest retention (95%). The results indicate that the heating time-temperature course is a key factor in the loss of heme iron.

Keywords: beef; cooking method; iron; heme iron; digestion method

INTRODUCTION

Meat is an important source of iron in food, especially the highly bioavailable heme iron. Most tables of nutritional values only comprise the content of nutrients in the raw food, while it is mostly processed foods that are consumed. During processing, some loss of nutrients is to be expected, whereas the bioavailability of the nutrients may increase. Decrease of heme iron (1–3) and increase of non-heme iron (3–4) have been observed in meat during cooking, but few data on the effects of different cooking methods was found. The aim of the present study was to obtain knowledge of the effects of different cooking methods on the retention of heme iron in beef.

EXPERIMENTAL

Material and cooking methods: Beef *M. Longissimus dorsi* (LD) was divided into four parts and each part was cooked to a centre temperature of 68°C using one of four methods: sous vide (delta temperature 25°C to a centre temperature of 43°C and then 72°C constant oven temperature) for approximately 3 h; roasting in an oven at 175°C for approximately 1h 15 min; frying in a pan in 2 cm slices for approximately 6 min; mincing and frying in a pan like hamburgers for 10 min. The same LD was used in all the cooking methods (Fig. 1) and the experiment was performed in 5 replicates.

Analyses: Raw and cooked meat were analysed for heme iron according to the method of Hornsey (5) modified using a standard curve of haematin and for total iron by flame atomic absorption spectrophotometry (AAS) following dry ashing at 450°C (NMKL Method No 139, 1991). The total iron was also analysed following wet digestion with nitric acid (Chemical Analytical Method 09:Iron, Swedish Meats R&D, 1998). Bovine liver (NIST® SRM 1577b) was used to check the AAS analyses. Raw and cooked meat were also analysed for water content (NMKL Method 23, 3rd ed. 1991). The total iron and the heme iron were calculated as mg/100g of dry matter (DM) in order to overcome differences in water content before and after cooking.

Statistics: The data was evaluated using the paired comparison t-test, analysis of variance and Tukey's pairwise mean comparisons test (SYSTAT® 8.0, SPSS Inc., 1998).

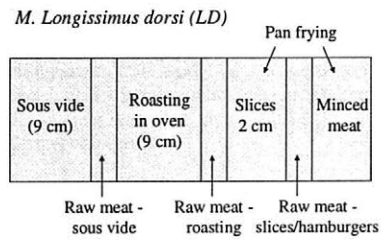

Figure 1. Parts of *M. Longissimus dorsi* assigned to the different cooking methods

RESULTS AND DISCUSSION

The retention of heme iron was high in all the cooking methods, although significant losses were found for three of the cooking methods: sous vide, roasting in an oven and pan frying minced meat (Table 1). Sous vide cooking, a long heat treatment at a low temperature, gave the lowest retention, roasting in an oven and pan frying minced meat

Table 1. Content of heme iron in raw and cooked meat, retention of heme iron after cooking and heme iron as percent of total iron

	Heme Fe (mg/100g DM)[1]		Retention of Heme Fe after cooking (%)[2]	Heme Fe (% of total Fe)	
	raw meat	cooked meat		raw meat	cooked meat
Sous vide	6.9[a]	5.2[b]	75[x]	126	110
Roasting in oven	7.0[a]	5.9[b]	83[xz]	126	109
Slices, pan fried	6.6[a]	6.2[a]	95[y]	120	123
Minced, pan fried	6.6[a]	5.7[b]	87[yz]	120	117

[1]Different letters in the same row indicate significant differences, $p \leq 0.05$ (pairwise t-test)

[2]Different letters in the same column indicate significant differences, $p \leq 0.05$ (analysis of variance)

es which are too low (8–9). Bovine liver gave correct results in the analysis of total iron by AAS and the problem was probably incomplete digestion of the meat. Dry ashing was compared to wet digestion in the analysis of total iron in one of the experiments. Wet digestion gave a significantly higher content of total iron, compared to dry ashing for the cooked meat, but not for the raw meat (Table 2). The meat was not digested completely using any of the methods, as the content of heme iron was significantly higher than the content of total iron before, as well as, after cooking (Table 2). Wet digestion using an oxidising agent has been proposed for the complete digestion of meat samples (8). Further studies of different digestion methods for the analysis of total iron in raw and cooked meat have been initiated.

Table 2. Content of heme iron and total iron after dry ashing or wet digestion

Cooking method	Raw meat			Cooked meat		
	Heme Fe (mg/100g DM)	Total Fe (mg/100g DM)		Heme Fe (mg/100g DM)	Total Fe (mg/100g DM)	
		dry ashing	wet digestion		dry ashing	wet digestion
Sous vide	8.4	6.3	5.4	5.8	5.0	5.2
Roasting in oven	7.9	6.1	6.7	7.2	5.9	6.4
Slices, pan fried	7.4	5.4	5.7	7.0	5.2	5.8
Minced, pan fried	7.4	5.4	5.7	6.0	4.5	4.9
Mean of all the methods[1]	7.8[a]	5.8[b]	5.9[b]	6.5[a]	5.2[b]	5.6[c]

[1]Different letters in the same row between means within raw and cooked meat, respectively, indicate significant differences $p \leq 0.05$ (pairwise t-test).

higher retention, while pan frying slices, a fast cooking method, gave the highest retention. The results indicate that the heating time-temperature course is a key factor for the loss of heme iron. Heme iron may be lost through the release of meat broth during cooking, or by heat destruction of the porphyrin structure leading to the formation of nonheme iron. More heme pigments leak into the broth at a low cooking temperature compared to a high cooking temperature (6) and more nonheme iron is formed during slow heating compared to fast heating (7). The differences in heme iron retention between sous vide cooking and pan frying in slices may be explained by a combination of these factors. Mincing the meat lowered the retention of heme iron, probably due to contact with the air resulting in a higher formation of nonheme iron.

The content of heme iron exceeded the content of total iron (Table 1), giving rise to questions about the analytical methods. The Hornsey method of heme iron analysis is considered accurate, repeatable and reproducible (8) and the content of heme iron agreed with earlier results on Swedish beef. Literature data indicated difficulties in the digestion of meat for analysing total iron, leading to valu-

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Influence of Technological Processes on the Chemical Structure of Cereal Dietary Fiber

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Abstract

In cereal extrusion products as well as in oat products in most cases a slight increase of soluble dietary fiber and a slight decrease of insoluble dietary fiber was observed. The kind of linkages and extent of dimerization of phenolic acids were affected by processing/extrusion in manifold ways. Effects differed not only between cereal species but also between extrusion products of one cereal. Molar ferulic acid dehydrodimers/arabinose ratios (DFA/Ara) are useful to elucidate on one hand the extent of polysaccharide cross-linking (ester-cross-links) and on the other hand cross-linking between polysaccharides and lignin (ether-cross-links). Because of decreasing molar DFA/Ara ratios in cereal extrudates an increasing importance of ester- and ether-cross-links in cereal extrusion products is supposed.

Keywords: cereals, dietary fiber, esterified and etherified phenolic acids, ferulic acid dehydrodimers, GC

INTRODUCTION

Dietary fiber has been shown to have many beneficial effects on human health. Their physiological effects such as gastrointestinal emptying (faecal bulking, intestinal transit time) and prevention of many diseases (i.e. lowering blood cholesterol, diabetes, colon cancer) are well documented (1). Dietary fiber is defined as plant nonstarch polysaccharides (cellulose, hemicelluloses, pectins, gums, mucilages) and lignin that are not digested by endogeneous enzymes in the human intestinal tract. Chemical structure, solubility and lignification are some properties of dietary fiber of great importance for their beneficial effects. Phenolic acids, especially dimeric phenolic acids like dehydrodimers of ferulic acid (Fig. 1) are important structural elements which cross-link non-starch polysaccharides or non-starch polysaccharides and lignin.

Cereals are quantitatively the most important source of dietary fiber in most diets. Because only minor amounts of cereal grains, flours and brans are consumed as raw material, analysis of processed cereal products is under nutritional aspects very important.

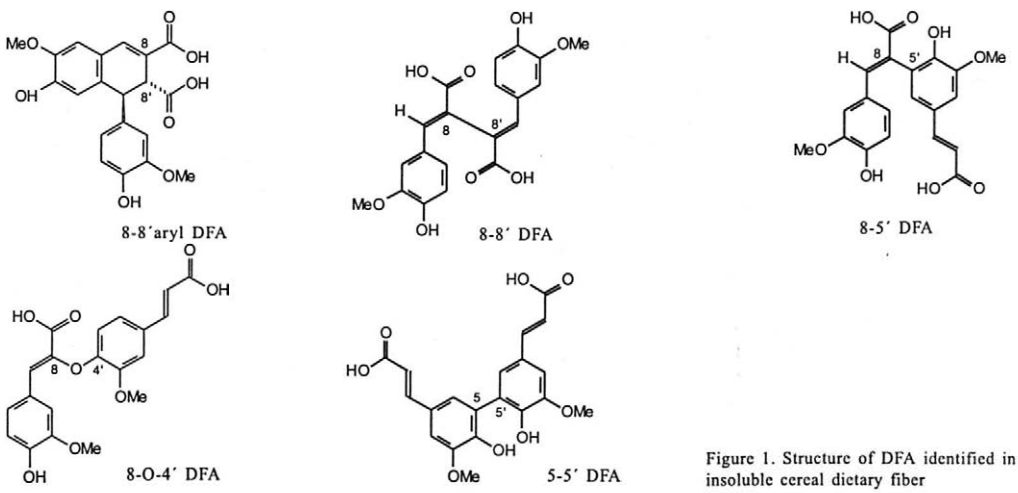

Figure 1. Structure of DFA identified in insoluble cereal dietary fiber

In modern food technology extrusion has become an important method for food processing. Most abundant product groups are breakfast cereals and snack products apart from modified starches and flours, animal feed, aromas and sweets (3). In extrusion products of wheat and maize as well as in oat products (samples of each production step and extrudates of oat flakes and oat bran) dietary fiber contents, carbohydrate composition and phenolic acid contents were determined. Esterified phenolic acids were released by mild alkaline hydrolysis while cleavage of etherified phenolic acids needed more severe conditions and high pressure alkaline hydrolysis was used.

EXPERIMENTAL

Material: Wheat bran and four extrusion products (EX 1–EX 4) were made available by the Institute of Food Technology, Technical University of Berlin (Germany), and oat products (raw kernels, kiln-dried kernels, oat flakes and its extrusion product (EXF), oat bran and its extrusion product (EXB)) were from Peter Kölln KGaA (Köllnflockenwerke, Elmshorn, Germany). Maize grits and collets were a kind gift of Bahlsen Snacks Produktions GmbH (Neu-Isenburg, Germany).

Methods: Detailed descriptions of all procedures are given in Renger & Steinhart (4).

Dietary fiber analysis: Dietary fiber was prepared by an enzymatic-gravimetric method according to a modified AOAC-method (5).

Analysis of phenolic acids: Esterified phenolic acids in insoluble dietary fiber (IDF) were released by saponification with 1M NaOH at room temperature under nitrogen and in the absence of light. Hydrolysation with 4M NaOH at 170°C in teflon pressure bombs set additionally etherified phenolic acids free. Phenolic acids were extracted by solid phase extraction (SPE) on C_{18}-cartridges and applied as TMS-derivatives to gaschromatography with flame ionisation detection (GC-FID) and gaschromatography-mass spectroscopy (GC-MS), respectively. Because no standard substances of DFA were available their content was estimated semi-quantitavely by setting the response factor to 1. Dimeric phenolic acids were additionally identified by high performance liquid chromatography – diode array detection (HPLC-DAD) (4).

Analysis of carbohydrates: Sugars were released from IDF by Saeman-hydrolysis, neutral sugars were derivatised to alditolacetates and analysed by GC-FID (4).

RESULTS AND DISCUSSION

Mainly in wheat and maize products amounts of soluble dietary fiber (SDF) increases after extrusion about 25–50% and IDF decreases about 15%. For better comparing possibilities the percentages of SDF and IDF are shown in Fig. 2. In oat extrudates a slight increase of the percentage of SDF is seen, too.

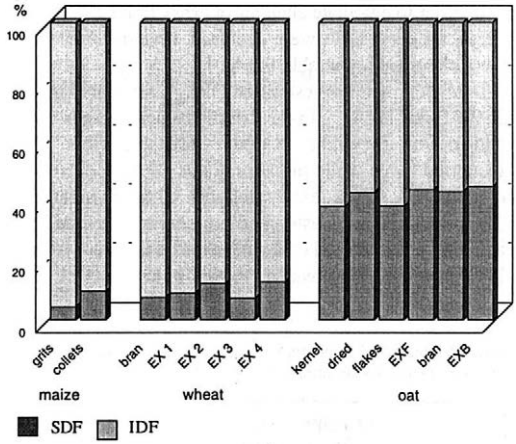

Figure 2. Percentages of IDF and SDF in cereal products

Monomeric and dimeric ferulic acid (FA) is an important structural element in cereal IDF as it is a potential cross-link between arabinoxylans on one hand, and arabinoxylans and lignin on the other hand.

In maize esterified FA amounts increase from 10.3 mg/g IDF in grits to 13.0 mg/g IDF in collets. Wheat extrudates have got smaller esterified FA amounts than wheat bran (15.5 mg/g IDF); they are $^1/_3$ to less (Table 1).

In kiln-dried oat, flakes and bran esterified FA amounts have not changed compared with that in oat kernels. In the extrudate of oat flakes (EXF) and oat bran (EXB) a higher esterified FA amount (3,5 and 3,1 mg/g IDF, respectively) than in oat flakes (2,9 mg/g IDF) or bran (2,7 mg/g IDF) is observed (Table 1). In the extrudates of maize, wheat and oat amounts of FA_{ether} are lower than FA_{ester}. Compared with the raw material there is much more etherified FA in the extrudates of maize or oat (Table 1).

Table 1. Amounts of esterified and etherified FA in IDF of cereal products

		mg/g IDF	
		FA_{ester}	FA_{ether}
Maize	grits	10.34	0.12
	collets	13.03	0.96
Wheat	bran	15.22	3.66
	EX 1	14.41	3.11
	EX 2	7.63	n.n.
	EX 3	5.57	n.n.
	EX 4	7.48	4.36
Oat	kernel	2.48	6.08
	dried	2.77	3.34
	flakes	2.89	3.85
	EXF	3.52	2.68
	bran	2.69	4.62
	EXB	3.09	2.30

In order to elucidate changes of cross-linking in IDF of cereal products DFA were analysed. Five different DFA were identified in cereal IDF (Fig. 1).

In all raw materials esterified DFA_{sum} amounted to 35–38% of total DFA_{sum}. In the extrudates percentages changed in both directions: in maize collets esterified DFA increased twice while in oat products DFA_{ester} decreased continuously. Wheat extrudates showed both tendencies. The semiquantitively estimated amounts of esterified DFA in maize, wheat and oat materials ranged from 200–800 µg/g IDF; etherified DFA was estimated in amounts of 200–3000 µg/g IDF.

Table 2. Degree of dimerization and cross-linking calculated by the appropiated molar ratios of FA, DFA and Ara

		FA/DFA (ether)	Ara/FA (ether)	Ara/DFA (ether)	Ara/DFA (ether)
Maize	grits	82	9	731	429
	collets	32	8	271	691
Wheat	bran	107	10	1078	586
	EX 1	88	12	1096	677
	EX 2	77	24	1855	1829
	EX 3	26	32	852	7515
	EX 4	62	25	1554	554
Oat	kernel	5	43	422	260
	dried	7	30	441	166
	flakes	4	32	277	71
	EXF	6	29	319	61
	bran	5	37	336	78
	EXB	8	31	471	61

Surely the degree of cross-linking depends on amounts of dimeric phenolic acids but the importance of dimers as cross-links in IDF can be better elucidated by comparing molar ratios of FA_{ester}/DFA_{ester} (degree of dimerization) (Tab-le 2). As indicator of the degree of cross-linking molar ratios between arabinose (Ara) and DFA were built (Table 2). DFA represents in its esterified form cross-links between poly-saccharide molecules (ester-cross-links) and in etherified form between polysaccharides and lignin (ether-cross-links).

In wheat extrudates the decrease of molar ratio FA_{ester}/DFA_{ester} went along with an increase of Ara/FA_{ester} (Table 2) which indicates an increasing importance of DFA as cross-links. Therefore in oat extrudates effects were vice versa: a slight increase of FA_{ester}/DFA_{ester} and a decrease of Ara/FA_{ester} occurred. Besides molar ratios of Ara/DFA_{ester} decreases so that a smaller importance of ester-cross-links in oat extrudate is supposed. In maize collets Ara/DFA_{ester} decreases as well while in wheat extrudates a decrease in EX 3 and an increase in EX 2 and 4 is seen.

DFA are more important as cross-links between polysaccharides and lignin than as cross-links between arabinoxylans which can be seen by comparing the mentioned molar ratios for esterlinked and etherlinked DFA. Especially in oat products Ara/DFA_{ether} are much smaller than Ara/DFA_{ester}. In oat flakes, oat bran and their extrudates a continuous decrease of molar ratios Ara/DFA_{ether} is observed which means an increasing importance of DFA as „ether-cross-links". The same tendency can be seen in maize collets. In most wheat extrudates the effect is vice versa: Ara/DFA_{ether} increases which means an decreasing importance of DFA as „ether-cross-links".

Changes in molar ratios can only be explained by the following phenomenons:

- hydrolysation of ester- and ether-linkages (consider: hydrolysation of an etherlinked DFA can lead to an increase of the amount of esterlinked DFA)
- structural changing of arabinoxylans by partly solubilisation of xylooligosaccharides (enrichement of arabinose in arabinoxylans)

In view of processing effects on dietary fiber the following conclusions can be drawn:

- in most extrudates of maize, wheat and oat a slight increase of SDF and a decrease of IDF is observed;
- in all raw materials and some extrudates etherified DFA predominates esterified DFA ? cross-linking between polysaccharides and lignin is an important aspect in IDF;
- increase of the degree of dimerization and change of the degree of cross-linking in most extrudates ? tendency that in extrudates importance of DFA as cross-links increases.

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Degradation of Steroid Glycoalkaloids by Lactic Fermentation

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Abstract

The influence of tomatine on the *Lactobacillus* sp. growth was evaluated. The growth curves of pure strain in the special ATP medium and strain with a small addition of tomatine were compared. It was found that the growth rate of the strain with addition of tomatine was changed in the second stage of growth curve. It indicates the inhibitic influence of tomatine on the *Lactobacillus* sp. strain. The changes in steroid glycoalkaloid content were observed by HPLC on the Supelcosil LC-NH_2 column. Also the amount of organic acids were analysed by isotachophoresis and calculated from the experimental data.

Keywords: glycoalkaloids; tomatine; solanine; *Lactobacillus*

INTRODUCTION

A group of nitrogenous compounds of the plant origin, marked out by their basic character, ranks among the alkaloids. The chemical structure of many alkaloids is rather complicated, since alkaloids often contain more heterocyclic, perhaps carbocyclic nuclei in their molecule and this nuclei bond to one another by different ways. Alkaloids exert a broad physiologic effect on animal organism, mainly on its nerve system. This is the distinctive alkaloid feature. Alkaloids found in plants are the free ones, but more often they occur in the forms of salts. They bond to various plant acids, e.g. malic, tartaric, citric, oxalic etc.

The basic skeleton of the nitrogenous glycoalkaloids (also called steroid alkamines) of bi-uterine plants from the family of solanaceous (*Solanaceae*), lilies (*Liliaceae*) and silkweeds (*Asclepiadaceae*) is derived from cholestan, which consists of there condensed sextuple and one pentamerous circles. Steroid glycoalkaloids consist of alkaloid and saccharide components, which are bond to each other by a glycosidic bond. They are decomposed by hydrolysis into aglycon with the steroid core and a mixture of monosaccharides, which come into the existence by the hydrolysis of the original oligosaccharide.

Only the plants belonging to the solanaceous family, namely the genus of nightshade (*Solanum*) and tomato (*Lycopersicon*) comprising potatoes (*S. tuberosum*), eggplants or aubergines (*S. melogena*) and potatoes (*L. esculentum*) are of the food processing importance (1).

Concentrations of steroid glycoalkaloids differ in various parts of the plant. They are created as the secondary metabolism products. The greatest ability to synthesise alkaloids is observed in young plants, but this decreases with the maturation of fruits. Alkaloids are most widely distributed in vegetative plant organs, least widely in maturing fruits. Steroid glycoalkaloids were supposed to be transported from the vegetative organs to the fruits. The analysis of the juice obtained from the plant stems proved that alkaloids are not transported from the vegetative organs to the fruit. The accumulation of alkaloids in the fruits means that they probably synthesise here (2).

Solanine streroid glycoalkaloids were identified in tomatoes likewise in other plants of the *Solanaceas* family. Tomatine is considered the most important glycoalkaloid of tomatoes, but there are also solanine and demissine.

One-shot mechanized tomato harvest, which represents one of the conditions of their rational mass production, causes the processing of a certain share of unripe fruits, which have not often acquired either their final colour or size. The share of green and orange coloured tomatoes has increased mainly in the recent years owing to the deterioration of the climate.

That is why the technologies must find the ways to utilise these unripe fruits and consider not only their less favourable superficial qualities, but also their compositions, which differ from the ripe ones. From this point of view, tomatine, whose distinct embryotoxical impact has been proved, is particularly important. Topical concentration of this substance can differ significantly in the products as it depends not only on the rate of the fruit maturity, but also on the time and way of their storage.

The given amount of tomatine was not proved in the ripe red and partially ripe orange fruits. But tomatine was always detected in unripe fruits. The amount of tomatine was studied on after four conservation treatments, namely freezing, sublimation drying, heat sterilisation and conservation applying benzoic acid.

The problem of the unripe tomatoes and their products has not been definitely solved yet and that is why it remains still topical. But the results suggest that immature to-

matoes can be used in the canning industry since on the appropriate conditions the tomatine amount of products can be low enough not to threaten the consumer's health. Since the tomatine amount, which is relatively high in freshly harvested tomatoes falls down significantly during the storage, it is recommend not to consume the unripe tomato products sooner than 60 days after their harvest (3).

Majority of the works on biodegradation which have been published so far deal with the biodegradation of the organic pollutants, e.g. polychlorinated biphenyls. The study on biodegradation of steroid glycoalkaloids has not been paid to the sufficient attention so far. Lactic fermentation is one of the possible techniques of SGA biodegradation. The studies to find out the influence of lactic fermentation on the solanine amount in unripe tomatoes were conducted. The amount of solanine was determined in unfermented green tomatoes, fermented tomatoes and pickle. The highest solanine amount was detected in unfermented green tomatoes (11.1/100 g), the lower amount was detected in the fermented green tomatoes (7.2mg/100g) and 0.9 mg/100 was detected in pickle. Only 65% of the original solanine concentration was detected in the tomatoes and 5% in the pickle. The remaining 30% was not determined at all. These results suggest that the contribution of lactic fermentation lies in the reduction of SGA amount in green tomatoes (4).

MATERIAL AND METHODS

Green tomatoes, mechanically intact, without the signs of diseases and untouched by pests were subjected to the analysis. 50 g of homogenised tomato sample were extracted in 100 ml of the methanol:chloroform mixture (2:1) for 30 minutes. Then the filtration using the Büchner funnel followed and the solid share was repeatedly extracted in 100 ml of the methanol : chloroform mixture for other 30 minutes. The filter cake was rinsed with 25 ml of extraction mixture and united filtrates were vacuum thickened to the volume approximately of 8 ml. The thickened sample was centrifuged and then acidified with 15 ml of 0.2 M HCl. After the addition of 12.5 ml of NH_4OH the sample was heated for 30 minutes at 70°C at the water bath and then centrifuged again. The centrifuged sample was vacuum evaporated to a dry place, dissolved in 5 ml of methanol and analysed employing the HPLC method (Shimadzu LC-10 AD) on the column of Supelcosil LC-NH_2, 5 µm, mobile phase of methanol:acetonitrile:0.005 M-KH_2PO_4 at the wave length of 208 nm.

Fermentation: The fermentations of *Lactobacillus plantarum* at varied temperature in ATP medium were performed. The growth curves show the characteristic shape corresponded to the individual stages of strain growth (Fig. 1). Also the influence of tomatine addition to the fermented media in amount of 0,15 g/l and 0,25 g/l was studied (Fig. 2).

RESULTS AND DISCUSSION

The *Lactobacillus sp.* strains ferment the nutriment to lactic acid, which evokes the pH value decreasing. The hydrolysis of tomatine in the acid environment can occur. This phenomenon should be used by lactic fermentation of green tomato and significantly decrease the content of steroid glycoalkaloids.

The influence of tomatine on the *Lactobacillus* sp. growth was evaluated. The growth curves of pure strain in the special ATP medium and strain with a small addition of tomatine were compared. It was found that the growth rate of the strain with addition of tomatine was changed in the second stage of growth curve. It indicates the inhibitic influence of tomatine on the *Lactobacillus* sp. strain.

The changes in tomatine concentration in model solutions were studied. Based on the mathematical model of lactic acid production its hydrolytic effect on steroid glycoalkaloid degradation was considered.

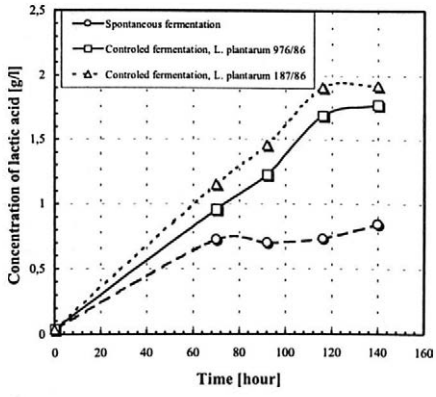

Figure 1. The changes of lactic acid concentration during fermentation

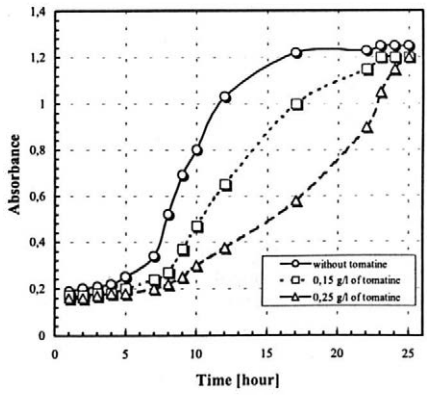

Figure 2. The influence of tomatine on growth rate of *Lactobacillus plantarum*

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Ascorbigens in *Brassica* and *Sinapis* Based Food

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Abstract

Ascorbigens may be formed during degradation of arylmethylglucosinolates in food and plant material containing ascorbic acid (vitamin C). Recently, methods based on normal phase supercritical fluid chromatography (SFC) have been developed both for analytical determinations and preparative purifications of the ascorbigens formed in cabbage and cruciferous related plant material. Up to now, four different ascorbigens, namely ascorbigen, neoascorbigen, 4-methoxyascorbigen and 4-hydroxybenzylascorbigen have been structurally identified from MS, 1D and 2D NMR.

Keywords: NMR; ascorbigens; glucosinolates; supercritical fluid chromatography; sinalbin; glucobrassicin.

INTRODUCTION

Ascorbigens [2-C-(arylmethyl)-∃-L-xylo-3-hexulofurasonic acid (-lactone derivatives] are a group of compounds formed as a result of reactions between ascorbic acid (vitamin C) and degradation products of some arylmethylglucosinolates (Fig. 1). The arylmethylglucosinolates acting as efficient precursors of ascorbigen formations are those which give unstable isothiocyanates that immediately releases the thiocyanate ion and a potential free carbonium ion. Glucosinolates of this type comprise hydroxy-, methoxy and/or glycopyranosyloxybenzylglucosinolates and indol-3-ylmethylglucosino-lates. These glucosinolates occur widely in crucifers (*Brassicaceae*) used as food. As myrosinase (thioglucoside glucohydrolase; E.C. 3.2.3.1) and vitamin C co-occur with the glucosinolates, several ascorbigens can be formed during processing and storage of food containing cruciferous plant materials. Ascorbigens formed from substituted benzylglucosinolates as 4-hydroxybenzylglucosinolate (sinalbin) seems to be structurally simple (1) whereas various oligomeric indol-3-ylmethylascorbigen derivatives (2, 3) might also be formed during autolysis processes in matrix systems containing cruciferous vegetables or seed materials. Limited informations on the various types of ascorbigens in food or cruciferous plant material are available, owing to lack of previously used methods of analyses, but these problems can now be overcome by use of supercritical fluid chromatography (SFC) (4, 5).

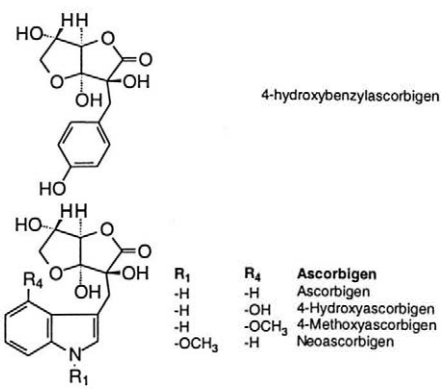

Figure 1. Structure of ascorbigens

EXPERIMENTAL

Material: Various types of cabbages (Broccoli, white cabbage ect.) were obtained from local markets. 3-hydroxybenzyl alcohol, 4-hydroxybenzyl alcohol, indol-3-ylmethanol, methane sulfonic acid (MSA) and ascorbic acid were from Sigma-Aldrich (Steinheim, Germany). Other reagents were of analysis grade.

Procedures: Various ascorbigens were produced either by condensation between indol-3-ylmethanol/4-hydroxybenzyl alcohol in aqueous buffers containing ascorbic acid or by enzymatic degradation of the respective arylmethylglucosinolates in presence of ascorbic acid. The glucosinolates were either purified or present in intact plant material (no ascorbic acid added).

SFC method: Ascorbigens were analysed on a Gilson SF3 Supercritical fluid chromatograph equipped with a 200 × 4.6 mm (I.D.) HP Hypersil Si at 2 mL/min (CO_2). The column back-pressure was held isobaric at 17 MPa and the column temperature at 35°C. Initially, the mobile phase was 4 vol% methanol:MSA (99:1) in CO_2 for 3 min, followed by

a linear gradient to 12 vol% modifier in 4 min. This was held for 6 min run and then returned to initial conditions.

RESULTS AND DISCUSSION

Myrosinase catalysed degradation of sinalbin initially lead to formation of 4-hydroxybenzylisothiocyanate, which easily react with nucleophiles such as ascorbic acid or water. 4-Hydroxybenzylascorbigen was preferentially formed if ascorbic acid was present in the buffer, whereas absence lead to formation of 4-hydroxybenzyl alcohol, if no stronger nucloephiles were present. The same is true for the heteroaromatic glucosinolates, where the isothiocyanates are highly unstable and spontaneously reacts with water or other nucleophiles. The compounds formed during reaction with water (indol-3-ylmethanols) are also unstable due to resonance stabilization of an intermediate carbonium ion, and therefore may either selfcondence with formation of oligomerics, or act as a good electrophile, e.g. react with ascorbic acid. It may therefore be discussed whether the same occurs for 4-hydroxybenzylisothiocyanate: does it initially react with water, form a stabilized carbonium ion and then react with ascorbic acid ?, or does it directly react with ascorbic acid ? As shown in Fig. 2, the reaction between 4-hydroxybenzyl alcohol and ascorbic acid proceeds very slowly at pH 6.0, whereas the reaction between 4-hydroxybenzylisothiocyanate (formed during degradation of sinalbin) and ascorbic acid proceeds spontaneously and nearly quantitative (only little formation of 4-hydroxybenzyl alcohol) (Fig. 2).

The structure of 4-hydroxybenzylascorbigen, ascorbigen, neoascorbigen and 4-methoxyascorbigen were confirmed from various 1D and 2D NMR techniques (^1H, ^{13}C, COSY, HMQC and HMBC) after purification by preparative SFC (Fig. 3). The developed normal phase SFC method were applied to determination of ascorbigens formed in autolysates of various cabbages, e.g. broccoli and savoy cabbage using 3-hydroxybenzyl alcohol as internal standard. Generally ascorbigen was the quantitatively dominating ascorbigen followed by neoascorbigen and 4-methoxyascorbigen (Fig. 4). In broccoli, the amount of ascorbigen, neoascorbigen and 4-methoxyascorbigen were, respectively, 420 mg/kg dry weight (DW), 300 mg/kg DW and 27 mg/kg DW, and in savoy cabbage 660 mg/kg DW, 54 mg/kg DW and 44 mg/kg DW. In galega, a Portuguese cabbage, the amount of ascorbigen, neoascorbigen and 4-methoxyascorbigen were as high as 1750 mg/kg DW, 460 mg/kg DW and 47 mg/kg DW, respectively, corresponding to approximately 260 mg/kg fresh weight (FW), 69 mg/kg FW and 7 mg/kg FW (5).

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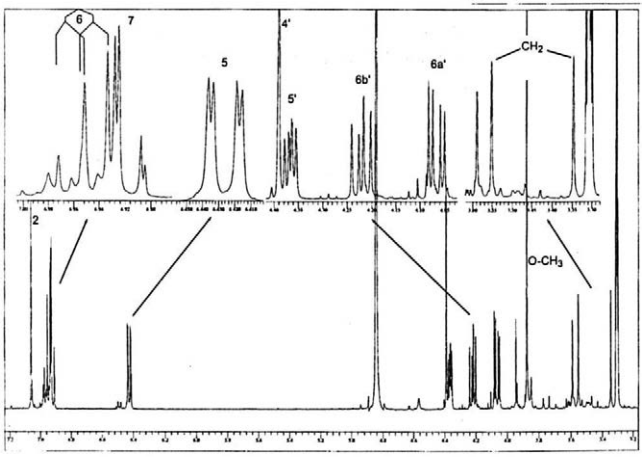

Figure 2. Formation of ascorbigen from either 4-hydroxybenzylisothiocyanate or 4-hydroxybenzyl alcohol and ascorbic acid, at pH 6.0. Labels: 1:4-hydroxybenzylisothiocyanate, 1a: 4-hydroxybenzyl cyanide, 1b: 4-hydro-xybenzyl alcohol, 1c: 4-hydroxybenzylascorbigen

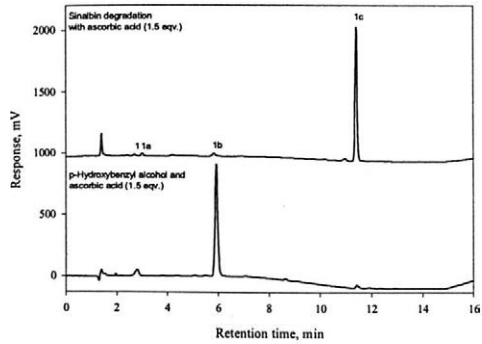

Figure 3. ^1H NMR (left) and COSY of 4-methoxyascorbigen (spectra were recorded in MEOH-d_4)

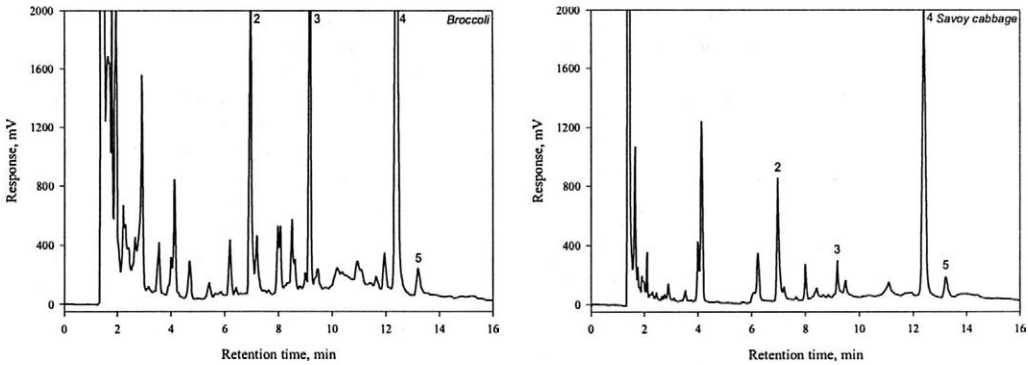

Figure 4. SFC-Chromatograms of broccoli (left) and savoy cabbage (right) autolysates. Labels: 2: 3-Hydroxybenzylalcohol (i.std.); 3: neoascorbigen; 4: ascorbigen; 5: 4-methoxyascorbigen

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Oligomeric Compounds Formed under Acidic Processing of Food Containing Hydroxybenzyl and Indol-3-Ylmethylglucosinolates

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Abstract

Supercritical fluid chromatography (SFC) using packed normal phase columns has been used for analytical determinations and preparative purifications of various products formed during acidic condensation of indol-3-ylmethanols. Preparative purification of the individual indol-3-ylmethyl oligomer-derivatives by SFC has resulted in identification of a number of oligomeric compounds with up to five heteroaromatic rings. The structures have been confirmed by 1D and 2D NMR-spectroscopy.

Keywords: NMR; supercritical fluid chromatography; glucosinolates, indol-3-ylmethanols; ascorbigens

INTRODUCTION

Hydroxybenzylglucosinolates, such as sinalbin (4-hydroxybenzylglucosinolate), and indol-3-ylmethylglucosinolates i.e. indol-3-ylmethyl-, N-methoxyindol-3-ylmethyl-, 4-hydroxyindol-3-ylmethyl- and 4-methoxyindol-3-ylmethylglucosinolate are well-known precursors of unstable and reactive isothiocyanates. These glucosinolates occur in relatively high concentrations in mustard (sinalbin), and the indol-3-ylmethylglucosinolates occur in nearly all types of cabbages belonging to the plant family *Brassicaceae*. They co-occur in the plant with the enzyme myrosinase (E.C. 3.2.3.1) which after damage of the plant cells catalyse the hydrolysis of glucosinolates to their respective isothiocyanates (autolysis). In the case of sinalbin, the autolysis initially produces 4-hydroxybenzylisothiocyanate (1), which in relatively fast reactions react with a number of nucleophiles, either by reactions directly with the isothiocyanate group, or with release of the thiocyanate ion and simultaneously formation of 4-hydroxybenzyl alcohol, 4-hydroxybenzylascorbigen, various thioethers or dithiocarbamates (1). The indol-3-ylmethylglucosinolates also releases isothiocyanates during the myrosinase catalysed hydrolysis, but these compounds are so unstable that they are difficult to detect. However, the indol-3-ylmethylisothiocyanate-derivatives will thus spontaneously releases

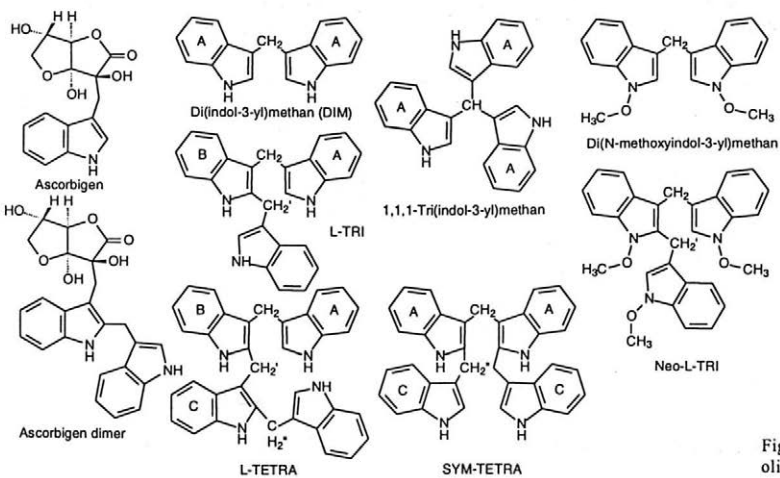

Figure 1. Structure of some of the oligomeric compounds identified

the thiocyanate ion and intermediate indol-3-ylmethylcarbonium ions, which, depending on pH of the reaction medium, react with a wide range of electrophiles or with another indol-3-ylmethyl derivative at C-2 or C-3 to produce various oligomeric compounds (2–4) (Fig. 1). The present paper presents some of the newest results obtained through our research in arylmethylglucosinolate chemistry.

EXPERIMENTAL

Material: All reagents were of analysis grade. Indol-3-ylmethanol (I3C) and ascorbic acid were obtained from Sigma-Aldrich (Steinheim, Germany). N-methoxyindol-3-ylmethanol (NI3C) was synthesised from 2-nitrotoluene.

SFC method: Oligomeric compounds and oligomeric ascorbigen compounds were analysed on a Gilson SF3 Supercritical fluid chromatograph equipped with a 200 × 4.6 mm (I.D.) HP Hypersil Si using a flow rate of 2 mL/min (CO_2). Column temperature was set at 50°C and the column back-pressure was 20 MPa. The initial mobile phase composition was carbon dioxide with 7 vol % methanol, after 13 min run at isocratic conditions, the modifier concentration was increased linearly to 15 vol in 7 min followed by 6 min isocratic run at 15 vol %. The modifier was then increased to 25% in 3 min and returned to initial conditions after 3 min hold at 25%.

RESULTS AND DISCUSSION

When the indol-3-ylmethylglucosinolates are enzymatically degraded in aqueous solutions, the main initial products are indol-3-ylmethanols (indol-3-ylmethanol, N-methoxyindol-3-ylmethanol, 4-hydroxyindol-3-ylmethanol and 4-methoxyindol-3-ylmethanol). These compounds are, however, more or less stable in acidic buffer systems, where they exist in equilibrium with the corresponding indol-3-ylmethylcarbonium ions, which acts as electrophiles. The uncharged indol-3-ylmethanols, ascorbic acid and several other compounds may act as nucleophiles, and therefore a complex mixture of oligomeric compounds may be produced, of which a few is shown in Fig. 1. I3C and NI3C have been used as model compounds to investigate some of these oligomerisation processes. Generally, NI3C is more stable in acidic media than indol-3-ylmethanol probably because the -O-CH_3 group is more electronegative than -H. The –O-CH_3 group attached on the heteroaromatic N decreases the ability of NI3C to form a stabilised carbonium ion which are required to start the oligomerization process. NI3C, however, still form oligomeric compounds, such as dimeric and trimeric, in acidic media, as has been confirmed from NMR (Fig. 2).

I3C readily forms oligomeric compounds, even in slightly acidic solutions (Fig. 3 and 4). If ascorbic acid is present in the buffer, also similar homologues of ascorbigen are formed.

When no ascorbic acid is present in the buffer, the main compounds formed are DIM and L-TRI, besides small amounts of higher oligomeric compounds as tetrameric and pentameric (Fig. 4, left). When ascorbic acid is present in the solution, the dominating compounds are ascorbigen and its oligomeric homologues (Fig. 4, right). The amount of higher oligomeric compounds formed is lower than ascorbigen oligomers, likely because of the low solubility of these compounds in aqueous solutions compared to the solubility of ascorbigen oligomerics.

Normal phase SFC is preferable to RP-HPLC as method of analysis due to the lipophilic nature of these compounds. No problems with slow post-equilibration of the Si-column, as may be a problem in NP-HPLC, has been observed, probably due to the high diffusivity of carbon dioxide, and the better solubility of residual water and alcohols in supercritical carbon dioxide than in a typical NP solvent as hexane.

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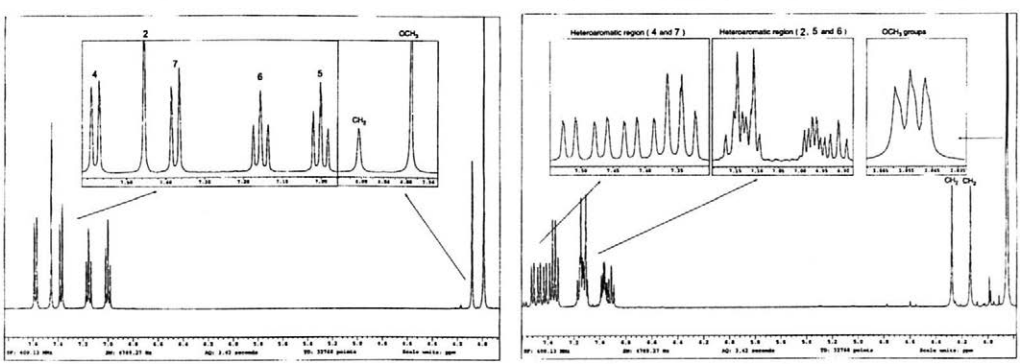

Figure 2. ^{1}H NMR spectra of di(N-methoxyindol-3-yl)methan (left) and the similar linear trimer (Neo-L-TRI) (right). Spectra recorded in DMSO-d_6

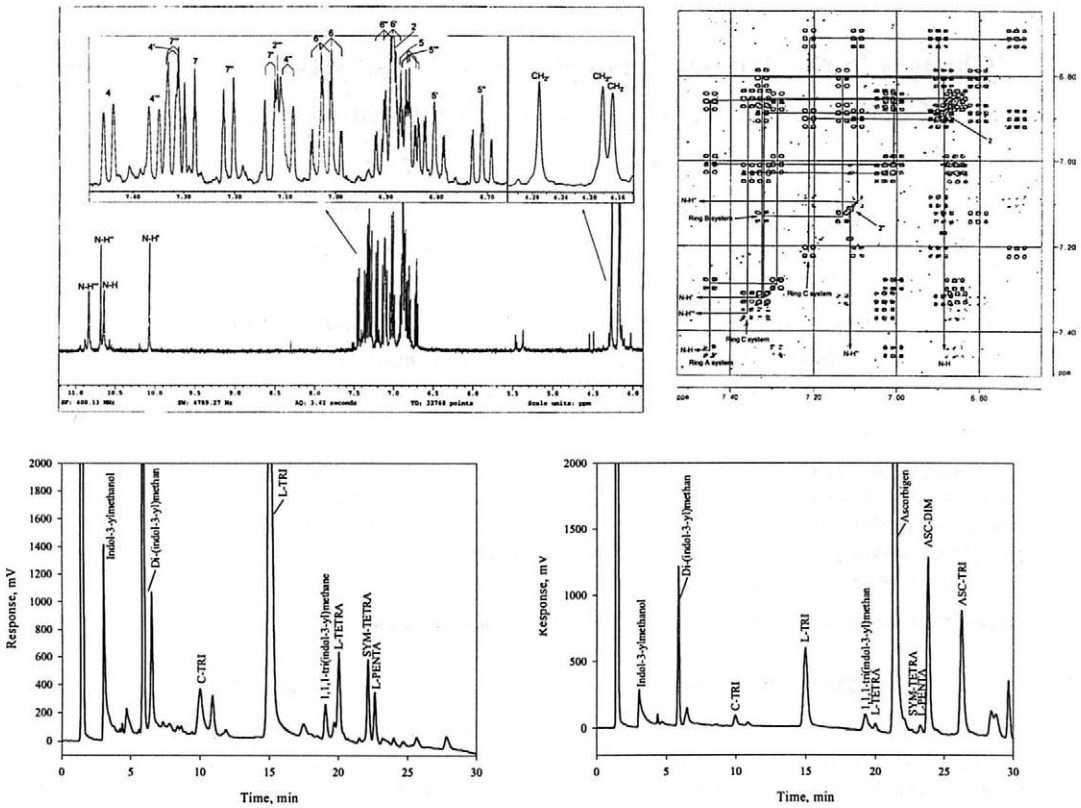

Figure 4. SFC chromatograms of I3C dissolved in buffers (pH 3) either without ascorbic acid (left) or with ascorbic acid (right)

cation of Plant Protein Binders and Co-Binders in Paper and Paints, and these financial supports are gratefully acknowledged by the authors.

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Changes in the Sensory Quality of a Strawberry Drink during Storage – Correlation of Results Obtained from Instrumental Techniques and Sensory Analysis

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Abstract

In this study we investigated the flavour of a commercially available strawberry drink with special regard to the changes in the sensory properties during storage of the juice, which has not been described in literature before. The experiments were performed using instrumental analytical techniques (GC-MS with different sample preparation techniques) as well as a trained sensory test panel. Significant changes of certain flavour compounds could be determined after a rather short storage period which were highly correlated to changes of the aroma observed by the sensory test panel.

Keywords: strawberry juice, aroma, storage, GC-MS, SPME, sensory evaluation

INTRODUCTION

Recently, strawberry fruit juices and nectars have become very popular. There are several companies offering such drinks. It is generally known, that the flavour of fruit juices changes drastically during storage. Various compounds have been reported to degrade over time, whereas other compounds are formed. In most cases these degradation and formation reactions lead to a deterioration of the original aroma of the product. Consequently, the shelf-life time of fruit juices is not limited by any microbial reactions but by a drastic decrease of the sensory attributes of the products (1, 2).

In literature there are several citations dealing with the aroma of fresh strawberries (e.g. [3–5]), but the aroma of strawberry juice has not been studied intensively (6). In the presented study a commercially available and very popular strawberry drink was investigated with special regard to changes in the sensory attributes in course of storage under different conditions (i.e. 4°C for the reference samples and 37°C to achieve acceleration of the ageing process). Investigations were performed regarding the changes in concentration of flavour compounds by the use of gas chromatography-mass spectrometry (GC-MS) of an organic extract. Solid phase micro-extraction (SPME) (7, 8) was used as an alternative sample preparation technique. The sensory relevance of the extracted flavour compounds was evaluated by means of aroma extract dilution analysis (AEDA) [9]. Furthermore, the juice samples were evaluated by a trained sensory test panel throughout the whole storage period.

EXPERIMENTAL

Preparation of the aroma extracts: The aroma extracts were prepared by liquid-liquid extraction using fluorotrichloromethane ("Kaltron" [10]) as organic solvent. 200 mL of juice were extracted twice with 20 mL Kaltron; the extracts were combined and reduced to a final volume of 0.5 mL. Camphor was used as internal standard.

Gas chromatography – mass spectrometry (GC-MS): The GC-MS analyses were performed on a system from Hewlett Packard (gas chromatograph HP 5892 II plus) with a mass selective detector (HP MSD 5972). The used capillary column was a HP 5 MS (30 m, 0.25 mm, 0.25 μm). Helium was used as carrier gas. The following conditions were used: column head pressure: 0.81 bar; temperature programme: 35–5.3°C/min – 280°C (2 min); injection volume: 2 μL (splitless), injector temperature: 250°C, detector temperature: 300°C, ionisation mode: EI (70 eV), mass range: < 20 min (35–200 amu), > 20 min (35–300 amu).

GC-Olfactometry (GCO, GC-sniffing): The sniffing experiments were carried out using a system from Hewlett Packard (gas chromatograph HP 5890 II). The analytical column was a HP Ultra 2 (30 m, 0.32 mm, 0.25 μm). At the end of the analytical column the effluent was split into two parts (used ratio 1:1); one part was connected to the FID, the second part into a sniffing port (SGE). The experimental conditions were as follows: injector temperature: 250°C; column head pressure: 1.0 bar, injection volume: 2 μL (splitless injection); injector temperature: 220°C; temperature program: 35–6.6°C/min – 280°C (2 min); detector temperature (FID): 310°C; sniffing-port: addition of humidified air (200 mL/min).

SPME coupled with GC-MS: A Carboxen/PDMS fiber (75 µm film thickness; Supelco) was used. 10 mL of samples were equilibrated at 25°C for 10 min; the SPME fiber was exposed into the headspace for 20 min. 1,2,3-Trichloropropane was used as internal standard. The compounds were thermodesorbed from the fiber directly into the injection system of the GC-MS. The used capillary column was a HP 5 MS (30 m, 0.25 mm, 0.25 µm). Helium was used as carrier gas. The following conditions were used: column head pressure: 0.54 bar; temperature program: –30°C (2min)–10°C/min – 250°C; injector temperature: 270°C, detector temperature: 280°C, ionisation mode: EI (70 eV), mass range: < 20 min (20–200 amu), > 20 min (35–300 amu).

Sensory analysis: Sensory tests were performed weekly by a trained panel of 12–14 subjects. The samples were presented as a duo-trio test. Samples stored at 37°C were tasted in comparison to reference samples that were stored at 4°C.

RESULTS AND DISCUSSION

The results show that the aroma properties of the investigated strawberry drink change very quickly. In summary, a drastic loss of the fresh and fruity flavour properties that are responsible for the typical aroma of the product was observed.

In analytical terms, these changes are expressed in decreasing amounts of various esters and linalool, as well as increasing amounts of certain acids (e.g. hexanoic acid or 2-ethylhexanoic acid), various degradation products of linalool (α-terpineol and linalool oxide) and diverse furan derivatives. Figure 1 and figure 2 show the concentration curves observed during storage at 37°C for a few aroma active compounds. Results obtained by GC-MS measurements of the organic aroma extracts and the results of SPME complement each other well. Compounds with very high volatility can be investigated easily by SPME, whereas polar compounds like acids can be monitored better in the organic extract.

In most cases, the aroma properties of the compounds that show decreasing concentrations are correlated with pleasant, fresh and fruity attributes, whereas most of the compounds with increasing concentrations show more or less unpleasant, fatty or terpentine-like aroma notes. These results were also confirmed by the results from comparative AEDA comparing the flavour-dilution-factors of several compounds of the fresh sample and the sample at the end of the storage experiment. Again a drastic decrease of the pleasant, fruity notes and an increase of some unpleasant aroma attributes could be observed.

The results obtained from GC-MS measurements and GC-olfactometry correlate well with the data obtained from sensory evaluation of the juice. The test panel indicates a significant difference between the sample stored at 37°C and the reference sample after a storage period of 3 weeks (simulating a storage time of about 6 months at room temperature). After this time significant changes in concentration could be observed from most compounds.

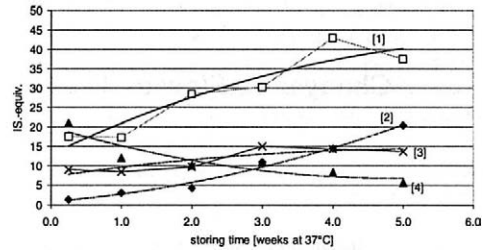

Fig. 1. Concentration curves of several compounds during storage at 37°C, [1] DMS, [2] furan carboxaldehyde, [3] linalool, [4] α-terpineol

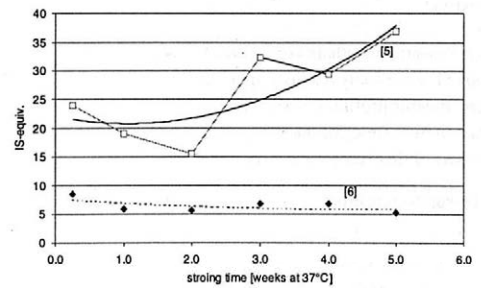

Fig. 2. Concentration curves of several compounds during storage at 37°C, [5] hexanoic acid, [6] ethyl hexanoate

As a summary it can be stated that the aroma of strawberry juice is very sensitive to the external environment which results in a short shelf life of such products. It must strongly be recommended to store strawberry juices at low temperatures to maintain the quality and to shorten the storage time as much as possible.

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Changes in Flavour Profiles of Mushrooms during Cooking

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Abstract

The flavour constituents of 23 edible strains of *Agaricus bisporus* (commercially available and wild varieties), fresh and oven-cooked, were analysed instrumentally (HPLC, dynamic headspace GC-MS), in order to evaluate the effect of heat treatment on their flavour profile. For sensory analysis, the cooked mushrooms were presented to a trained sensory analytical panel. Using Quantitative Description Analysis (QDA^{O}) the mushrooms were scored for 15 taste/aroma and 6 mouthfeel/texture attributes. As a result of this comparative study, new mushroom varieties with potentially interesting flavour profiles were identified.

Keywords: mushrooms; *A. bisporus*; flavour profile; sensory analysis; heat treatment

INTRODUCTION

The cultivation, trade and processing of common mushrooms (*Agaricus bisporus*) are economically important horticultural activities in the European Union and United States. The consumption of fresh mushrooms has increased in recent years along with an increased emphasis on improving the quality and availability of fresh products. However, the genetic basis of the currently used commercial strains in The Netherlands is relatively small, leading to little variation. New varieties with improved sensory characteristics are requested by the market.

A key factor in increasing the sale of fresh mushrooms is the improvement of the quality available to the consumer. Flavour (including mouthfeel) represent an important quality attribute contributing to the widespread consumption of edible mushrooms (1).

This research describes a combined sensory and instrumental approach to characterise several strains of *A. Bisporus* and to assess the influence of cooking on their flavour profiles.

EXPERIMENTAL

Material – Sample preparation: 23 edible strains of *A.Bisporus* (4 commercially available and 19 wild varieties) were provided by local growers. The stems of the mushrooms were removed. The oven cooking was done by heating the fresh mushrooms in a closed "baking-bag" for 8 minutes at 230°C. The fresh and cooked mushrooms were cut into pieces of approximately 1.5 cm, dropped immediately into liquid nitrogen and stored at –80°C to prevent possible degradation.

High pressure liquid chromatography (HPLC): Determination of dry matter content and extraction and subsequent HPLC analysis of sugars and organic acids was performed according to LUNING *et al.* (2). HPLC analysis of amino acids was done by means of the Waters accq-taq method.

Gas chromatography – Mass spectrometry: Dynamic headspace isolation of volatile compounds was performed using Tenax TA as adsorbent. The GC and GC-MS analysis was performed as described in (3).

Sensory analysis: A sensory analytical panel of 18 trained panellists was used to assess the taste, aroma and texture of the selected cooked mushrooms. The Quantitative Descriptive Analysis (QDA^{O}, STONE & SIDEL 1993) was used to score the mushrooms for 15 taste/aroma attributes (sweet, salt, bitter, mushroom, fungi, metallic, earthy/musty, meat-stock, chicken-stock, sharp, anise, nutty, boiled egg, spicy, total flavour) and 6 texture/mouthfeel attributes (solid, cleaveable, tough, elastic, chewable, fibrous), on a 5–95 linear scale.

RESULTS AND DISCUSSION

Mannitol and trehalose were detected to be the most important sugars in all samples analysed. Mannitol, a C6 polyol, is the main storage carbohydrate to be found in fresh mushrooms and amounts between 18–36% dry matter. The amount of trehalose was much lower between 0.5–3.4% dry matter. During cooking the level of both sugars is reduced.

Malic acid and fumaric acid were the main organic acids identified, ranging between 1.5–3% dry matter and 0.2–0.5% dry matter, respectively. Citric acid (0.07–0.2%),

pyroglutamic acid (0.04–0.3%) and α-ketoglutaric acid (0–0.03%) were also identified in all samples. During cooking the amounts of organic acids are not significantly changed.

Larger amounts of sugars and organic acids were found for the wild varieties as compared to the commercial varieties for both fresh and cooked mushrooms.

There is a significant difference in the amino acids profile between the different mushrooms. The total amount ranges from 4% to 11% dry matter with higher value for commercial strains as compared to the wild ones. In all samples Glu, Gln, Asp and Orn were the most abundant amino acids representing around 60% of the total amino acids content. All the samples analysed proved to be deficient in the sulphur-containing amino acids. No Cys was identified and the level of Met was between 0.3% and 1.16% of the total amino acids content. During cooking the amount of amino acids is decreased especially for Asp, Gln, Orn.

Following the GC-MS analysis 66 components were separated and quantified and 57 were identified. Among them 2-methyl-butanal, 3-methyl-butanal, pentanal/2-3-pentandione, 2-methyl-3-buten-2-ol, hexanal, 3-octanone, pentanol, 1-octen-3-ol, and benzaldehyde account for 60-90% of the total FID signal of fresh mushrooms. 1-octen-3-ol, a key aroma component of mushrooms, was the main volatile constituent identified in the fresh samples (4). There are significant differences in the aroma profiles between the analysed samples.

In general, higher levels of 1-octen-3-ol were found for the wild strains as compared to the commercial ones. During cooking the aroma profiles are changed. The amount of 1-octen-3-ol is drastically reduced while the amount of benzaldehyde is much higher in the cooked mushrooms.

A total of 15 taste/aroma attributes and 6 texture/mouthfeel attributes were generated for the sensory evaluation of the selected mushrooms. Multivariate statistics were used to classify the samples according to their perceived flavour. The PCA analysis presented in Fig. 1 gives an overview of the sensory analysis and the relationships between the sensory and the instrumental data obtained for the cooked mushrooms.

The 2nd PC separates the samples mainly according to their texture. Based on texture two main groups can be identified (the wild varieties are marked with a star):

Group 1: CU1, CS600, CB63*, CB81*, CB117*, CB209*, CB217*, having a more *solid* texture and a higher dry matter content;

Group 2: CA2400, CS856, CX16/1*, CB65*, CB214*, CB215*, CB222*, CB223*, CB225*, B226*, B227* having a more *elastic, chewable, fibrous* texture and a lower dry matter content;

These 2 groups can be further separated according to the 1st PC on basis of their taste and aroma:

Group 1A: CU1, CS600, CB117* more aromatic (higher scores for *mushroom, boiled egg*) and with a higher content of amino acids;

Group 1B: CB63*, B81*, B209*, B217* more *sweet* and having a higher amount of sugars and organic acids;

Group 2A: CA2400, CS856, CX16/1*, CB65*, CB215*, CB227* more aromatic, with higher scores for *mushroom, meat-stock, chicken-stock, spicy, total taste* and having a higher content of amino acids and volatile constituents;

Group 2B: CB214*, CB222*, B223*, CB225*, B226*, more *sweet* with a higher content of sugars and organic acids;

The result of this research reveals the potential of the wild varieties either to replace the commercial ones or to provide new mushroom varieties with different and potentially interesting flavour profiles.

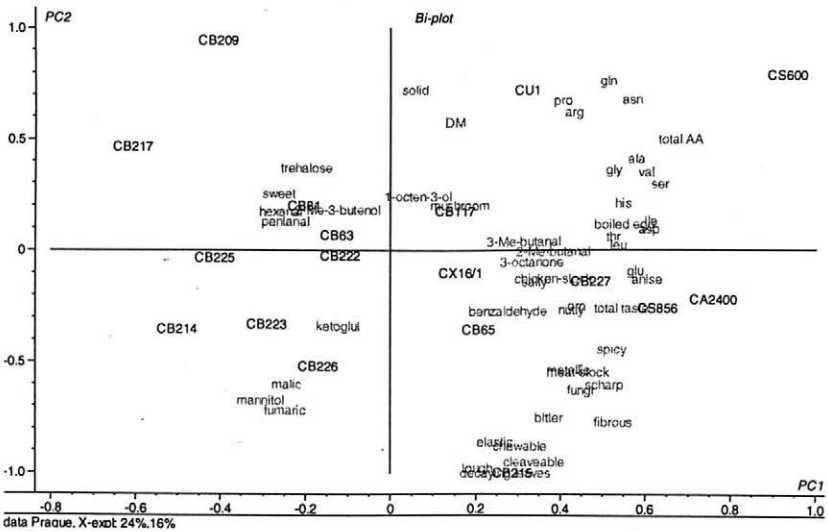

Figure 1. PCA bi-plot (scores and loading) of the combined instrumental and sensory data for cooked (C) mushrooms

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Reactions of Amino Acids in Foods: Formation of Flavour Compounds

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Abstract

S-alk(en)yl-L-cysteine sulfoxides, i.e. *S*-methyl-L-cysteine sulfoxide (methiin), *S*-propyl-L-cysteine sulfoxide (propiin), *S*-allyl-L-cysteine sulfoxide (alliin) and (*E*)-*S*-(1-propenyl)-L-cysteine sulfoxide (isoalliin), occurring naturally in alliaceous and brassica vegetables were heated under various conditions and the generated volatile products isolated, analyzed and their sensory properties described. The major reaction pathways leading to these volatiles are presented and discussed.

Keywords: *S*-alk(en)yl-L-cysteine sulfoxides; *S*-methyl-L-cysteine sulfoxide (methiin); *S*-propyl-L-cysteine sulfoxide (propiin); *S*-allyl-L-cysteine sulfoxide (alliin); (*E*)-*S*-(1-propenyl)-L-cysteine sulfoxide (isoalliin); Strecker aldehydes; garlic; onion

INTRODUCTION

There are more than 200 amino acids found in nature of which 20 occur in proteins as their building units and, to a minor extent, as free amino acids. Amino acids are very reactive compounds which readily decompose or react with some other food constituents, especially with reducing sugars, oxidized lipids and oxidized polyphenols under the formation of numerous flavour-active and coloured compounds.

The most significant reactions of amino acids include oxidation, isomerization, elimination and other reactions that may take place either enzymatically or nonenzymatically. These reactions can generate many volatile flavour-active products depending on the reaction conditions (Strecker aldehydes, ammonia, hydrogen sulfide, amines and other products). Another very important group of compounds derived from amino acids are thermally generated volatiles arising mainly as the products of the Maillard reaction. Sulfur amino acids (i.e. cysteine, methionine) and other sulfur compounds yield numerous *S*-containing flavour-active volatiles. In addition to cysteine and methionine, foods of plant origin contain several other sulfur amino acids, in particular, *S*-alkyl(en)yl-L-cysteines and their sulfoxides. Free amino acid *S*-methyl-L-cysteine sulfoxide, sometimes trivially called methiin, appears to have very wide distribution in plants as it occurs in many genera of the *Brassicaceae* and *Liliaceae* family to which many important vegetables (such as cabbage, broccoli, garlic, onion) belong. *S*-allyl-L-cysteine sulfoxide (alliin), and (*E*)-*S*-(1-propenyl)-L-cysteine sulfoxide (isoalliin) are well known free amino acids of the genus *Allium* (*Liliaceae*), mainly associated with garlic and onion, respectively. *S*-propyl-L-cysteine sulfoxide (propiin) is a major constituent of onion while *S*-ethyl-L-cysteine sulfoxide (ethiin) was found as a minor constituent of some garlic varieties (1, 2). Several other *S*-alk(en)yl-L-cysteine sulfoxides have been identified in higher plant (1). *S*-alk(en)yl-L-cysteines and their sulfoxides are precursors of powerful and unusual flavours of many alliaceous and brassica vegetables and precursors of various biologically active substances. This is the reason why they attract the attention of food chemists, technologists, physiologists and pharmacologists for many decades.

The aim of this work was to identify and quantify S-containing volatiles arising from oxidized and thermally degraded methiin, propiin, alliin and isoalliin.

EXPERIMENTAL

Material. *S*-alk(en)yl-L-cysteines and their sulfoxides have been synthesized by alkylation of L-cysteine with alk(en)yl halides (3, 4), (+)-*S*-allyl-L-cysteine sulfoxide was a gift from TNO, Zeist, NL. All the other chemicals used were commercial products of highest available quality.

Thermal decomposition. Amino acids were heated at temperatures ranging from 80°C to 200°C in the presence of variable water amount. The resulting volatiles extracted to diethyl ether were analyzed by GC/FID and GC/MS (5–7).

RESULTS AND DISCUSSION

Decomposition of *S*-alk(en)yl-L-cysteines and their sulfoxides at high temperatures is strongly influenced by time

of heating, temperature and water content. Deoxyamino acids seem to be essentially more stable in comparison with the corresponding sulfoxides.

A total of 37 compounds generated from methiin were identified (5). Among the compounds identified 24 sulfur-containing volatiles were present but neither the Strecker aldehyde nor its transformation products were detected. Dimethyl disulfide was the predominant compound representing more than 60% of the total volatiles. Dimethyl trisulfide, the second most abundant volatile, was formed in considerably smaller scale. Significant amounts of various pyridines (both alkyl- and alkylthiosubstituted) were also found, in particular at higher temperatures. The resulting odour of the isolated volatiles could b generally described as unpleasant, spoiled, cabbage-like with strong ammonia and pyridine-like notes.

The first step involved in the volatiles formation is a cleavage of methiin into methanesulfenic acid and 2-aminoacrylic acid (Fig. 1).

The latter compound can spontaneously hydrolyze giving ammonia and pyruvic acid which further eliminates carbon dioxide and forms acetaldehyde. The self-condensation of methanesulfenic acid leads to the formation of dimethyl thiosulfinate, the key breakdown product. This can easily decompose into dimethyl disulfide and dimethyl thiosulfonate or alternatively might form 2,3,5-trithiahexane oxides. reduction of 2,3,5-trithiahexane oxides could lead to the formation of methyl (methylthio)methyl disulfide. The pathway leading to dimethyl trisulfide seems to be more complex and a few mechanisms have been proposed (e.g. reaction of 2 molecules methanesulfenic acid with hydrogen sulfide, reaction of dimethyl disulfide with elemental sulfur, decomposition of dimethyl disulfide, reaction of dimethyl thiosulinate, dimethyl thiosulfonate and hydrogen sulfide) (11, 13). The most important reaction seems to be the self-degradation of dimethyl thiosulfinate (8).

The stability of propiin is comparable with that of methiin. Generally, propiin appears to decompose in the analogous manner proposed for methiin. *S*-Propylcysteine (deoxypropiin), α-alanine and pyruvic acid were identified among the nonvolatile products but neither cysteine or cystine arised (9, 10).

A total of 53 volatile compounds generated from propiin were identified (12). Among them, 23 sulfur-containing volatiles were present, with dipropyl disulfide, dipropyl trisulfide, and at higher temperatures also propanethiol being generated as the wholly predominant volatiles (more than 79% of the total volatiles isolated). A complex mixture of various pyridines (alkyl- and alkylthio-substituted) is also formed and a total of 32 different derivatives were identified.

In general, propiin decomposes in a similar way to methiin. It breaks down to propanesulfenic acid and 2-aminoacrylic acid that is sponaneously hydrolyzed to ammonia and pyruvic acid. The self-condensation of propanesulfenic acid leads to the formation of dipropyl thiosulfinate. Thiosulfinate can readily decompose to dipropyl disulfide and dipropylthiosulfonate. Its further transformations lead

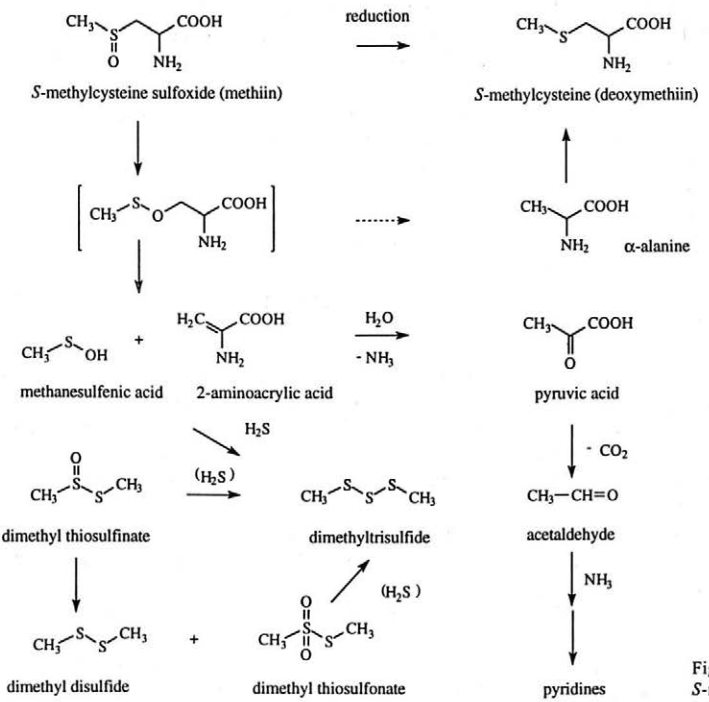

Figure 1. Proposed thermal degradation of *S*-methyl-L-cysteine sulfoxide

to dipropyl trisulfide. Two unsaturated aldehydes (2-methyl-2-butenal and 2-methyl-2-pentenal) arised by either aldolization and subsequent dehydration of acetaldehyde (formed by the breakdown of pyruvic acid) or acetaldehyde and propanal (formed by elimination of hydrogen sulfide from propanesulfenic acid), respectively (14). The carbonyls identified are proposed to participate significantly in the formation of the pyridines found in the model systems (15).

The overall odour of degraded propiin samples could be generally described as quite pleasant, resembling boiled onion or leek. Anyway, at higher temperatures burned and pyridine-like notes prevailed.

Also alliin is a very unstable amino acid and, in comparison with its methyl and propyl analogue possessing the saturated side chain, it seems to be even more susceptible to heating as it quickly decomposes almost completely at moderate temperatures (6). Similarly to methiin and propiin, it appears to be more stable when heated dry or in diluted samples. A total of 57 volatile compounds, of which 50 were sulfur-containing ones, were identified in models with alliin (Fig. 2).

The predominant sulfur compounds arising at temperatures lower than 140°C were diallyl mono-, di-, tri- and tetrasulfides. At higher temperatures, these very labile sulfides decomposed to form rather unusual cyclic compounds, especially 2,5-dimethyl-1,4-dithianes, 2-methyl-1,4-dithiepane and dimethyl-1,2,5-trithiepanes etc. Considerable amounts of allyl alcohol were also generated at all the temperatures studied.

S-allylcysteine (deoxyalliin) and cysteine were identified in the nonvolatile fraction. Deoxyalliin was probably generated through the reduction of alliin by allyl mercaptan, while cysteine was formed *via* a [2,3]-sigmatropic rearrangement (4). This rearrangement of alliin might lead to the intermediate sulfenate. The reduction of sulfenate would consequently yield allyl alcohol and cysteine. Cysteine can further decompose giving hydrogen sulfide, ammonia, acetaldehyde, various thiazoles etc.

The main degradation pathways of alliin markedly differ from those of the analogues having the saturated side chain, namely methiin and propiin. Alliin does not decompose to give allylsulfenic acid, pyruvate and ammonia. This mechanism results in the absence of alanine and especial-

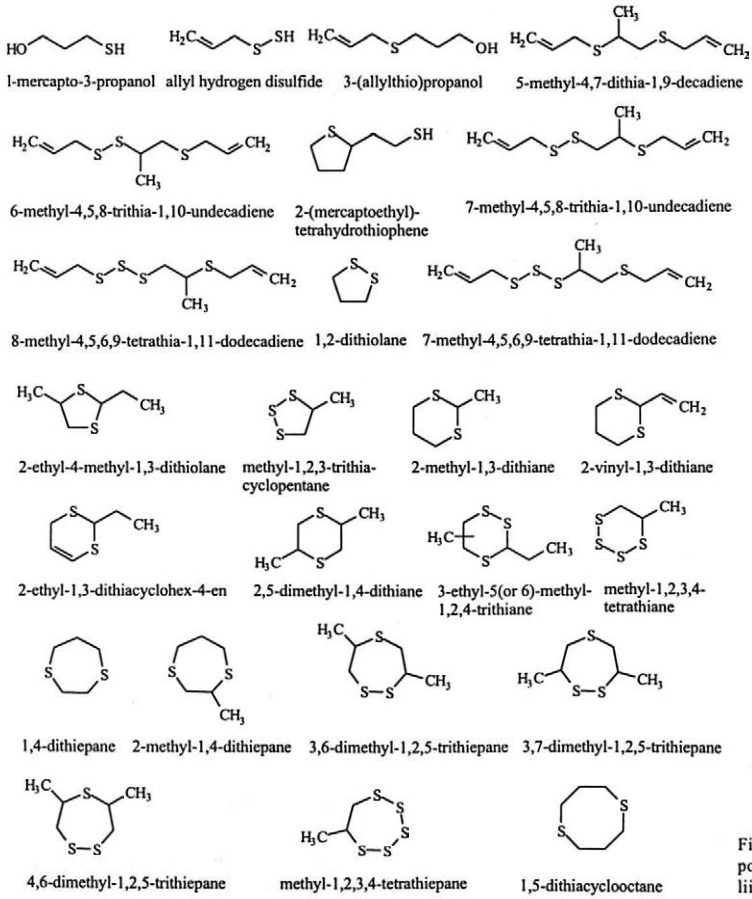

Figure 2. Some of the volatile compounds thermally generated from alliin

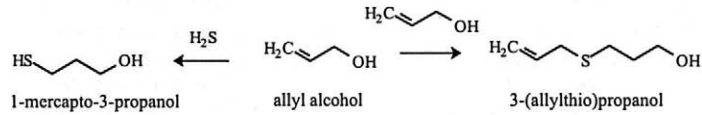

Figure 3. Formation of 3-(allylthio)propanol and 1-mercapto-3-propanol

ly of 2-vinyl-4*H*-1,3-vinyldithiin and 3-vinyl-4*H*-1,2-dithiin, the typical products of allicin (diallyl thiosulfinate) transformation. Allyl alcohol is formed from alliin through a [2,3]-sigmatropic rearrangement and partly it may also arise by hydrolysis of diallylsulfides. Allyl alcohol seems to be a key compound (16). It can react with the allylthio radical, allyl mercaptan or alliin/deoxyalliin to give diallyl sulfide and also with allyl mercaptan or hydrogen sulfide to form 3-(allylthio)propanol and 1-mercapto-3-propanol, respectively (Fig. 3).

Another important reaction mechanism is probably the reduction of alliin to deoxyalliin (e.g. by allyl mercaptan) and a consequent homolytic cleavage of the labile C-S bond, resulting in the formation of allylthio and propenyl radicals (17). The allylthio radicals can combine giving diallyl disulfide which easily undergoes disproportionation to diallyl sulfide and diallyl trisulfide. Allyl mercaptan plays a pivotal role in the formation of many of the volatiles. By self-reaction of 2 molecules, rather unusual cyclic compounds are formed, such as 2-(mercaptoethyl)tetrahydro-thiophene, 2,5-dimethyl-1,4-dithiane, 2-methyl-1,4-dithiepane and 1,5-dithiacyclooctane. Many others, e.g. dimethyl-1,2,5-trithiepanes, are presumably generated by the reaction of allyl mercaptan with hydrogen sulfide and some low-molecular weight carbonyls (formaldehyde and acetaldehyde) (4). The formation of unsaturated acyclic sulfur compounds (such as 5-methyl-4,7-dithia-1,9-decadiene and other dithiaalkadienes) can be best explained by addition of the

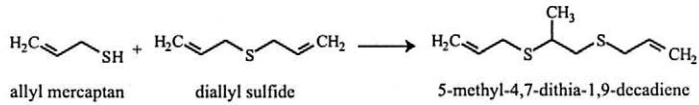

Figure 4. Proposed formation of acyclic dithiaalkadienes from allyl mercaptan

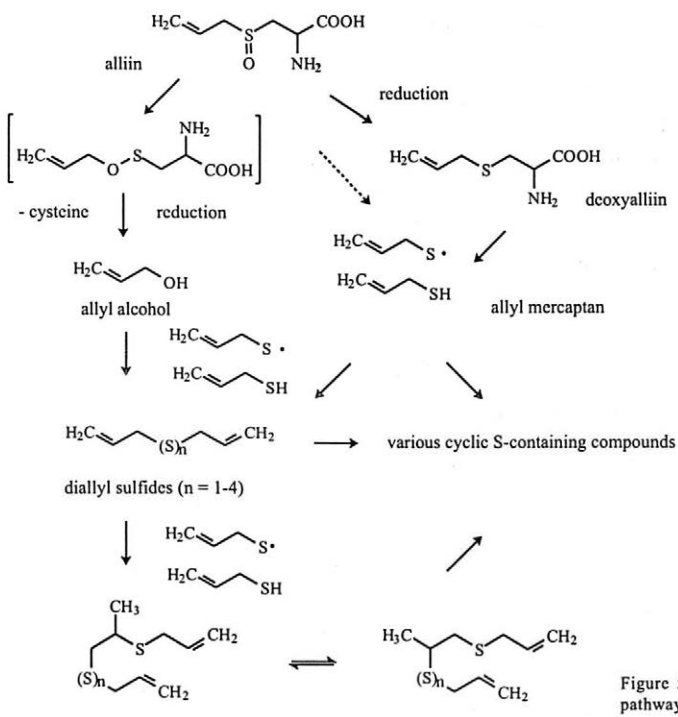

Figure 5. Proposed major nonenzymatic degradation pathways of alliin

allylthio radical or allyl mercaptan (Fig. 4) in the Markovnik fashion to the double bond of diallyl sulfides (17). Simplified reaction pathways are presented in Fig. 5.

The overall aroma of the degraded alliin samples is strongly dependent on the temperature of heating. At lower temperatures, it can be described as a typical garlic-like, slightly biting with ammonia and pyridine-like notes. At higher temperatures it becomes less acceptable, with sulfury, burned, gummy, mushroom-like and biting odours prevailing.

Thermal degradation of isoalliin leads to the formation of a very limited number of volatile products as only 4 volatiles were generated at significant levels, namely 2,4-dimethylthiophene and 3,4-dimethylthiophene (both considered to be the typical constituents of fried onion aroma) and 2 unsaturated aldehydes, 2-methyl-2-butenal and 2-methyl-2-pentenal (1). Nevertheless at higher temperatures, trace amounts of various alkylthiazoles (e.g. propyl ethyl methyl, ethyl dimethyl, diethyl methyl, dimethyl propyl and ethyl methyl propylthiazoles) were detected.

The most important degradation pathway of isoalliin seems to be internal Michael condensation to thermally much stable cycloalliin. The reduction to deoxyisoalliin takes place in a considerably smaller extent. The latter compound gives rise to the thiophenes *via* a homolytic cleavage of the C-S bond, followed by the condensation of the propenylthio radicals and elimination of hydrogen sulfide (Fig. 6) (18). The proposed simplified mechanism of isoalliin thermal degradation is given in Fig. 7. It can be assumed that isoalliin undergoes a rearrangement to the intermediate sulfenate (similarly to alliin). The reduction of this compound would yield 1-propenol and cysteine. These compounds can subsequently provide propanal and

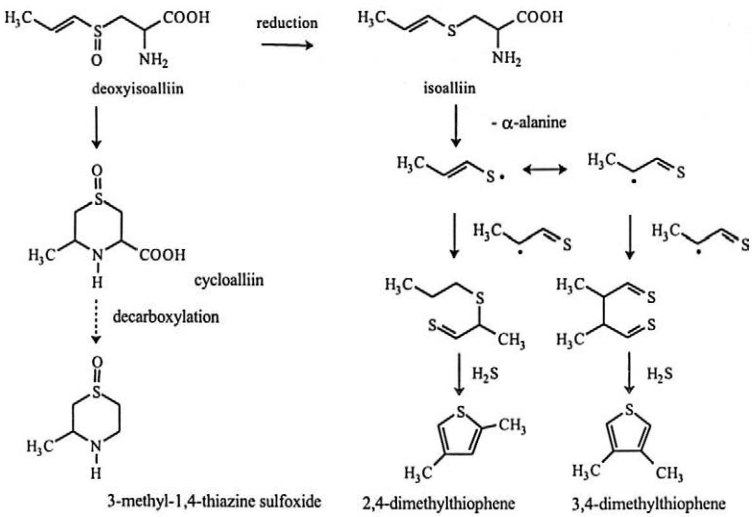

Figure 6. Transformation of isoalliin to cycloalliin and dimethylthiophenes

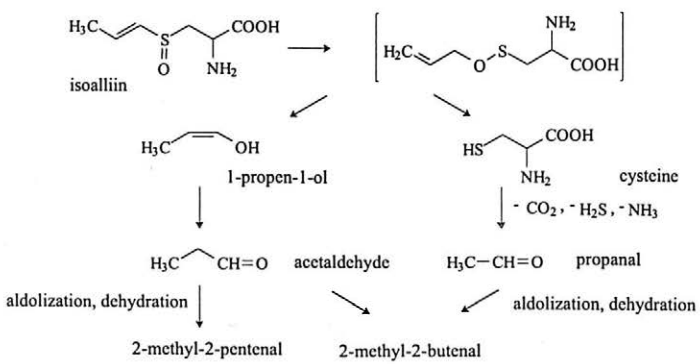

Figure 7. Proposed major nonenzymatic degradation pathways of isoalliin

acetaldehyde, whose aldolization leads to the above mentioned unsaturated aldehydes.

The overall aroma of the thermally degraded isoalliin samples did not much resemble the typical aroma of cooked or fried onion. At lower temperatures it could be described as a potato-like or vegetable-like. At higher temperatures, it became less acceptable, with sulfury, burned and biting odours prevailing.

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Studies on the Key Odorants Generated by Maillard-Type Reactions from Sulfur-Containing Precursors

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Abstract

The sulfur containing amino acid cysteine is known as an important precursor of very potent food aroma compounds, such a 2-methyl-3-furanthiol, 2-furfuylthiol, 2-acetyl-2-thiazoline or the recently identified 5-acetyl-3,4-dihydro-2H-1,4-thiazine. Although cysteamine is a well-known degradation product of cysteine, when reacted in the presence of sugar degradation products, only a few data are available on its role as precursor of sulfur-containing aroma. Compounds, in particular, in comparison with cysteine. In the lecture, results of the application of an Aroma Extract Dilution Analysis on fructose/cysteamine models thermally treated under different conditions will be presented. Furthermore, results of experiments aimed at clarifying reaction pathways leading to key aroma compounds from cysteamine/cysteine by means of ^{13}C-labeled intermediates will be discussed. Finally, data obtained in Maillard systems with rare sulphur containing amino acids will be presented.

Keywords: Maillard reaction; cysteine; S-containing compounds; thiols

Isolation and Characterization of Glyoxal-Arginine Modifications

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Abstract

Three structures were identified as the major modifications in incubation mixtures of arginine with glyoxal. Initially, 1-(4-amino-4-carboxybutyl)-2-imino-4,5-dihydroxy-4,5-dihydro-imidazolidine is formed very rapid as the single most important product, which is slowly degraded to N^7-Carboxymethylarginine. This novel molecule represents the final stable end product under regular Maillard conditions. Both structures can be converted to 1-(4-amino-4-carboxybutyl)-2-imino-5-oxo-imidazolidine only at strong acid conditions. This study also provides insights to kinetics and reaction mechanisms.

Keywords: Maillard reaktion; arginine; glyoxal; carboxymethylarginine

INTRODUCTION

Glyoxal is generated in foods and in vivo by autoxidation of unsaturated fatty acids and by the Maillard reaction of reducing sugars and amines. Due to its high reactivity the α-dicarbonyl easily reacts with proteinbound lysine and arginine residues. The quantitatively most important modification of the ε-aminogroup of lysine is N^{ε}-carboxymethyllysine (1). Reaction with lysine also leads to crosslinking of proteins by formation of amide, imidazolium and imidazolinone type structures (glyoxal-amide-crosslink, GOLD, GODIC) (2, 3, 4). Concerning the reaction of glyoxal with the guanidino group of arginine, inconsistent results have been published in the literature. Recently, an imidazolinone was identified as the only reaction product in heated reaction mixtures of N^{α}-acetylarginine with glyoxal after an acidic work-up procedure (5). This stands in contrast to older publications, where a single product was isolated, which showed strong affinity towards borate and released arginine upon treatment with *o*-phenylenediamine (6). Such properties prompted the authors to suggest a dihydroxyimidazolidine structure. This controversy led us to reinvestigate the products of the reaction of arginine with glyoxal and the related mechanisms (7).

EXPERIMENTAL

Authentic reference materials were obtained from reaction mixtures of glyoxal with arginine after ion exchange chromatography and preparative HPLC. Structures were assigned based on ^{1}H-, ^{13}C-, DEPT-, HMBC-, HMQC-NMR experiments and high resolution mass spectrometry. Quantification and identification in incubation mixtures was performed by HPLC with fluorescence detection after *o*-phthaldialdehyde postcolumn derivatization. In selected cases the identity of the material detected was verified by LC/MS. For BOC-deprotection samples were acidified with 3N HCl at room temperature prior to analysis.

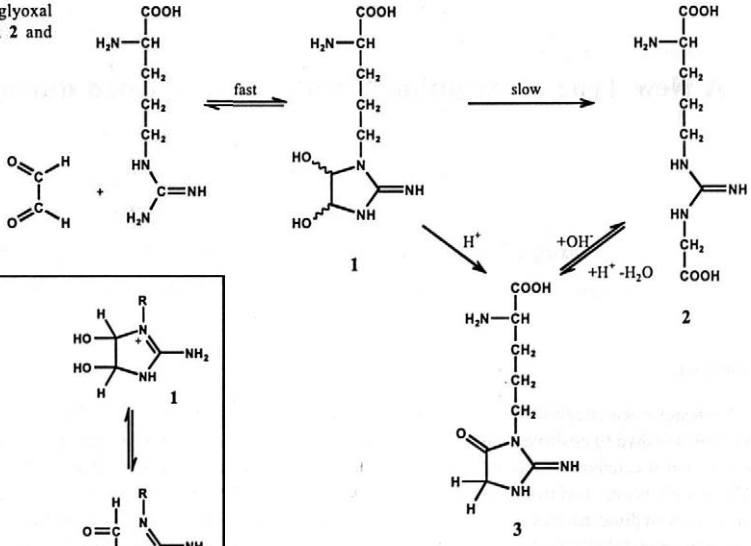
RESULTS AND DISCUSSION

Investigations of reaction mixtures of N^{α}-t-BOC-arginine with glyoxal in 0.1 M phosphate buffer with pH 6–9 and temperatures of 28–50°C unequivocally identified dihydroxyimidazolidine **1** as the primary single structure (figure 1). This early product is converted to carboxymethylarginine **2** (CMA) in a much slower reaction.

In contrast, other authors have verified imidazolinone **3** as the only direct reaction product (5). However, they used strong acid conditions (2M HCl, 110°C, 100 min) to deacetylate incubations of N^{α}-acetylarginine and glyoxal. We found this conditions to almost completely convert dihydroxyimidazolidine **1** and CMA **2** to imidazolinone **3**. **3** was also described in glyoxal modified casein after enzymatic hydrolysis. The first step of the procedure includes treatment of the protein with pepsine in 0.02 N HCl for 24h at elevated temperature. In our experiments **1** was stable under such conditions, but CMA partially converted to **3**, especially during the acidic incubation step. Therefore, the detection of imidazolinone **3** must be evaluated as an artefact of the acidic sample work-up procedures applied.

Dihydroxyimidazolidine **1** was identified as a cis/trans-isomere mixture of ca. 1:4, each consisting of two diastereomeres. Cis- and trans-isomere pairs could be separated chromatographically using mild acid eluents, but quickly found the equilibrium again once the pH is raised. This means, that cis- and trans-isomeres can convert to each other via the open chained form and, ultimatively, that gly-

Figure 1. Reaction of arginine and glyoxal to dihydroxyimidazolidine **1**, CMA **2** and imidazolinone **3**



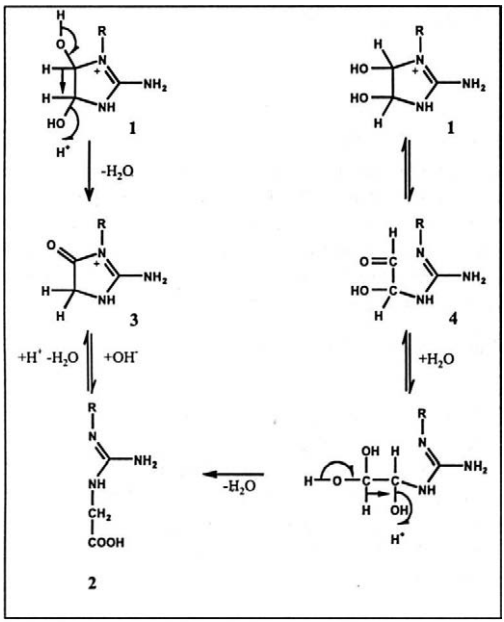


Figure 2. Reaction pathways explaining the formation of CMA **3** from dihydroxyimidazolidine **1**

oxal might be liberated again. When **1** was incubated in the presence of *o*-phenylendiamine or aminoguanidine, formation of arginine was observed indeed. The action of the α-dicarbonyl trapping reagents was confirmed by GC/MS-quantification of the corresponding glyoxal quinoxaline and triazine derivatives, respectively. In presence of borate the cis-isomeres form stable adducts, thus altering their chromatographic properties. This is in line with publications describing only one structure from reaction mixtures of arginine with glyoxal in the presence of borate, while absence generated a mixture of products (6). Obviously borate forms a complex only with the cis-isomeres and thereby shifts the product generation totally towards the otherwise sterically unfavored structure.

Two reaction pathways are possible for the synthesis of CMA **2** from dihydroxyimidazolidine **1**. CMA could be generated by ring opening and intramolecular Cannizzaro reaction (Fig. 2, right side), similar to the generation of N^{ε}-carboxymethyllysine from glyoxal (1). This hypothesis is supported by accelerated CMA synthesis under alcaline conditions, which favors the ring opening. In addition, CMA formation is suppressed in the presence of *o*-phenylendiamine. This reagent should react with the free carbonylfunction of **4** and, thus, omit CMA formation. Theoretically, disproportionation could also occur in the cyclic form of **1**. The resulting imidazolinone **3** would then hydrolyse to give CMA **2** (Fig. 2, left side). As the hydrolysis must be assumed to be much faster than the rearrangement, absolute concentrations of **3** in reaction mixtures would be negligible. This would explain why we were unable to detect **3** in reaction mixtures. However, this would also mean that presence of *o*-phenylenediamine would not influence CMA formation. As the opposite is the case, the latter pathway is of no relevance. In further support, we could verify that CMA formation directly from **3** is not influenced by o-phenylenediamine.

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A New Type of Arginine Modification Formed during Food Processing

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Abstract

After heating acetylarginine with varying amounts of lactose for 1 to 4 hours at 100°C and subsequent hydrolysis, a previously unknown ninhydrin positive compound could be detected in the amino acid chromatograms. Following preparative isolation, the compound was unequivocally identified as N^{δ}-[5-(3-hydroxypropyl)-4-oxo-imidazolon-2-yl]-L-ornithine (PIO) by ESI-TOF-MS as well as one- and two-dimensional ^{1}H- and ^{13}C-NMR. PIO was exclusively formed during incubation of peptide-bound arginine with disaccharides containing a 1–4-glycosidic linkage. 3-deoxypentulose proved to be the direct precursor of PIO. The ornithinoimidazole PIO represents a new type of post-translational protein modification during food processing, which might be responsible for the major part of arginine derivatisation in disaccharide-containing foods like milk.

Keywords: Maillard reaction; arginine; N^{δ}-[5-(3-hydroxypropyl)-4-oxo-imidazolon-2-yl]-L-ornithine; imidazolinone

INTRODUCTION

During heating and storage of foods, Maillard reactions between proteins and carbohydrates can have a significant influence on the nutritional and functional quality of final product. Several compounds resulting from the modification of lysine residues (e.g. Amadori products, carboxymethyllysine, pyrraline) have already been identified and quantified in several foods (1, 2). Despite the fact that also arginine can become extensively modified during several heating processes, only one arginine derivative of the Maillard reaction, an ornithinoimidazolinone resulting from the reaction of the guanidino side chain of arginine with methylglyoxal, has been identified and quantified in severely heated foods like coffee or certain bakery products up to now (3). Here, we describe isolation and identification of a previously unknown disaccharide-specific arginine modification.

EXPERIMENTAL

Materials: All Chemicals were of highest purity available.

Isolation of PIO and HipPIO: The free amino acid PIO 1a was isolated as hydrochloride after incubating a solution containing lactose and acetylarginine (molar ratio 5:1, pH 7, 100°C, 4 h) followed by acid deacetylation (2M HCl, 120 min at 110°C) via preparative ion-exchange chromatography on DOWEX 50W-X8. Peptide bound HipPIO 1b was isolated after heating hippurylarginine and lactose with semi-preparative RP-HPLC, using isocratic elution (0,1% TFA-methanol, 84:16) (5).

Amino-acid analysis: Ion-exchange chromatography (IEC) was performed according to (7).

NMR-Spectroscopy: ^{1}H and ^{13}C-NMR one and two dimensional spectroscopy was performed in D_2O on a Bruker DRX 500 spectrometer according to (5).

ESI-TOF-MS: MS studies were performed using a Micromass LCT according to (5).

RESULTS AND DISCUSSION

After heating acetylarginine with lactose for 4 h at 100°C followed by acid deacylation, a previously unknown ninhydrin positive compound could be detected in the amino-acid chromatogram, eluting between phenylalanine and histidine. The formation of this unknown peak correlated with the observable arginine modification and increased with increasing amounts of lactose (Fig. 1).

Using preparative cation-exchange chromatography, sufficient amounts of the putative arginine derivate were isolated. ESI-TOF-MS analysis showed one molecular mass of 272.30. Based on one- and two-dimensional ^{1}H- and ^{13}C-NMR analysis the structure could unambiguously identified as N^{δ}-[5-(3-hydroxypropyl)-4-oxo-imidazolon-2-yl]-L-ornithine (PIO, 1a in Fig. 1). To prove that PIO was not formed during acid hydrolysis, hippurylarginine was heated with lactose and the reaction mixture was directly analysed with RP-HPLC and detection at 220 nm (Fig 2). Using semi-preparative RP-HPLC, the hippurylderivative of PIO was isolated. Peptide bound HipPIO (1b in Fig 2) was identified using ESI-TOF-MS and NMR analysis.

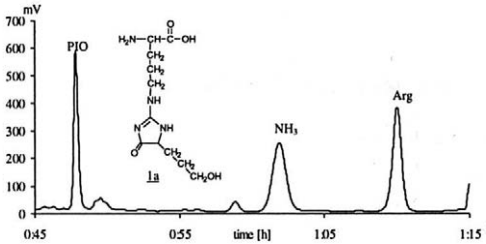

Figure 1. Ion exchange chromatogram (detail): Acid hydrolysis of Incubation mixture consisting acetylarginine and lactose (4 h; 100°C)

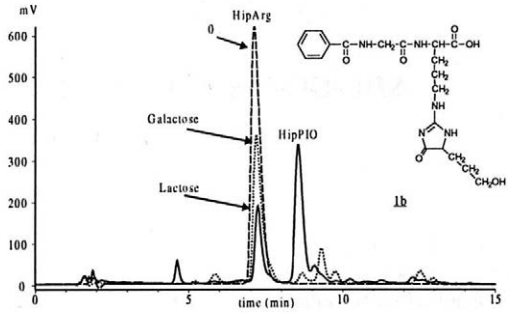

Figure2. RP-HPLC-chromatogram: Heating of hippurylarginine (HipArg) without (0) and with galactose or lactose (110°C, 3 h)

As can be seen from Fig. 2 HipPIO is exclusively formed during incubation of hippurylarginine with lactose or other disaccharides heaving a 1,4-glycosidic link, were it represents the dominant reaction product. No HipPIO or only minor amounts are formed from monosaccharides.

The surprising fact that with PIO an ornithinoimidazolinone resulting from a reaction between guanidine group of arginine and a C5-compound was identified, might be explained by results published by TROYANO *et al.* (6) and BERG and VAN BOEKEL (4); who reported on the formation of 3-desoxypentulose in heated milk. We speculate that 3,4-didesoxypentosulose might be the direct precursor for 1a in heated milk. Studies elucidating this mechanism are recently in progress.

PIO might be responsible for the major part of arginine derivation in disaccharide containing foods like milk. As PIO is nearly completely destroyed during conventional acid hydrolysis, forming arginine as main hydrolysis product, the extent of arginine derivatisation in heated or stored milk products might be significantly underestimated when determinded by routine amino acid analysis.

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SDE-GC-MS Profiling of Green and Roasted Coffee Beans from Different Origins

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Abstract

The volatile compositions of 3 Arabica and 3 Robusta coffees were studied using a combination of simultaneous steam distillation-extraction (SDE, Likens-Nickerson) and gas chromatography-mass spectrometry (GC-MS). Principal component analysis (PCA) was used to visualise the relationships between the volatile patterns of the different green and roasted coffee beans. Some meaningful clusters were obtained, allowing interesting conclusions concerning the influence of origin on the aroma pattern of coffee.

Keywords: coffee; aroma; simultaneous steam distillation-extraction (SDE); gas chromatography-mass spectrometry (GC-MS); principal component analysis (PCA)

INTRODUCTION

As aroma is one of the most appreciated attributes of coffee, the composition of the volatile fraction of roasted coffee has been intensively studied. Several hundreds of compounds have been reported as constituents of coffee aroma (1, 2). At the same time the composition of green coffee beans and the changes occurring during roasting have been subjects of many investigations (3). Several reactions play an important role in the aroma formation during roasting of coffee: such as, Maillard reaction, Strecker degradation, degradation of trigonelline and of phenolic compounds, auto-oxidation of lipids, caramelisation of sugars, etc. (4). However, coffee processors are aware of the fact that, besides the roasting conditions, also the origins of the green coffee beans have a major impact on the final aroma. For instance, it is well known that significant aroma differences exist between the 2 commercially important botanical species, *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta). Additionally, processing methods of the green coffee beans, such as dry and wet processing, result in different aroma patterns.

The aim of this study was to evaluate whether analytical methods could be used to measure variability and interchangeability of green and roasted coffee beans from different origins and to measure the effect of the roasting process on their aroma composition. Therefore, the volatile composition of 3 Arabica and 3 Robusta coffees were studied using SDE-GC-MS.

EXPERIMENTAL

Materials: Green and medium-roasted coffee beans from 3 Arabica coffees (Brazil, Java and Kenya) and 3 Robusta coffees (Ivory Coast (Grain Noir), Vietnam and Tanzania (Soft African)).

Isolation of volatiles by simultaneous steam-distillation-extraction (SDE, Likens-Nickerson): A sample of 25 g ground green or roasted coffee suspended in 600 mL water was extracted for 2 hours in a modified Likens-Nickerson apparatus using 60 mL dichloromethane as extraction solvent and 154.9 µg of nonane as internal standard (IS). After cooling to ambient temperature the combined dichloromethane fractions were concentrated to a final volume of 0.5 mL (green) or 5.0 mL (roasted) in a Kuderna Danish concentrator.

Gas chromatography-mass spectrometry (GC-MS): The SDE-extracts (1 µL) were analysed by injection on a HP 5890 gas chromatograph coupled to a HP 5971A MSD mass spectrometer (Hewlett-Packard, USA): fused silica column (HP PONA cross-linked methyl silicone, 50 m × 0.21 mm × 0.5 µm); carrier gas He (1 mL/min); column temperature: 40°C iso during 5 min, 40 to 160°C at 3°C/min, 160 to 220°C at 5°C/min; split injection (1:5 split ratio); injector lines (250°C), transfer lines (280°C); electron impact (70 eV); mass range 40–260 amu; solvent delay 6.8 min. Identification of the volatiles was based on comparison of the spectra with the spectra of the NBS49K library and of a self-made library. Semi-quantitative determinations of the volatile constituents were calculated by relating the peak intensities to the intensity of nonane as IS and were expressed as ng/g of green coffee bean or as µg/g of roasted coffee bean.

Statistical analysis: To visualise the complex data matrix and the relationships between the coffee beans of different origins and their volatile compounds, a principal component analysis (PCA) was performed using The Unscrambler® 6.1 (Camo, Norway) statistical software.

RESULTS AND DISCUSSION

In comparison with the roasted coffee beans much less volatiles were found in the green beans. Besides an important amount of aliphatic aldehydes (pentanal, hexanal, nonanal, 2-octenal, 2-decenal and 2,4-decadienal), derived from the degradation of aliphatic fatty acids, some typical fermentation products (2,3-butanedione, 2,3-pentanedione, 3- and 2-methylbutanal, 3- and 2-methylbutanol and 3- and 2-methylbutanoic acid) were found. Only a few typical roasted coffee compounds, such as furans and pyrazines (2-furancarboxaldehyde, 2-furanmethanol, methylpyrazine, 2,6-dimethylpyrazine), were identified. The level of phenolic compounds in green beans (phenol, 2-methoxyphenol, 4-ethenyl-2-methoxyphenol) already explained the aroma difference between roasted Arabica and Robusta coffees.

The different roasted coffee samples had a rather similar qualitative but a different quantitative composition. The 58 identified volatile compounds were classified according to their chemical structure (pyrazines, furans, pyrroles, phenolics, esters, ketones, acids, aldehydes, S-compounds, ...) and their presence could often be related to chemical reactions during roasting. Comparison of the GC-MS profiles revealed a significant difference between the roasted Arabica and Robusta coffee beans at the level of the phenolic compounds (phenol, 2-methoxyphenol, 4-ethyl-2-methoxyphenol and 4-ethenyl-2-methoxyphenol). Robusta coffee showed 4 a 5-fold higher concentrations of phenolics compared to Arabica coffee.

In order to visualise the volatile composition matrix, PCA was performed on the semi-quantitative data, with the duplicate analyses of the 6 coffees as objects and the 58

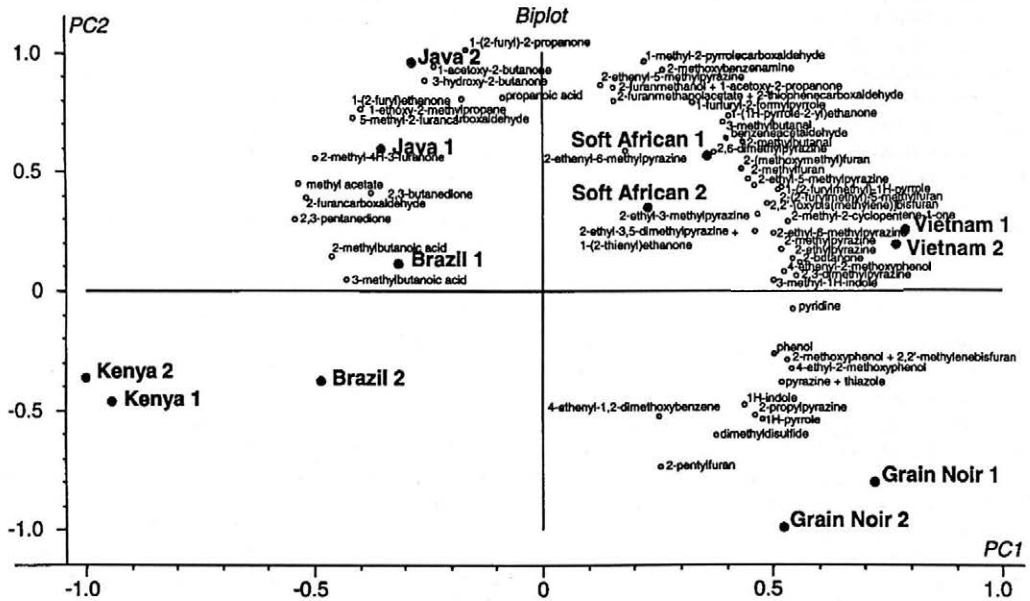

Figure 1. PCA-biplot of the volatile composition of 3 roasted Arabica (Brazil, Java, Kenya) and 3 roasted Robusta (Grain Noir, Vietnam, Soft African) coffees

volatile compounds as variables (Fig. 1). The score plot showed a good classification between the roasted Arabica (negative PC1) and the roasted Robusta (positive PC1) coffees, explaining 78% of the total variance (PC1 50%, PC2 28%). Furthermore PC2 clearly differentiated the Arabica and Robusta coffees mutually. This was in good accordance with sensory analysis, from which it was clear that the 6 coffee origins had a quite different sensory pattern. Robusta coffees, especially Grain Noir, were dominated by phenolic compounds, which corresponded with their higher 'phenolic' note. Soft African and Vietnam were mainly characterised by pyrazines. Furans were found throughout the loading plot. Due to the wet processing (except for Brazil) Arabica coffees were characterised by some fermentation products. In accordance with the results from sensory testing and titratable acidity, Kenya showed a higher degree of volatile acids (2- and 3-methylbutanoic acid).

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"The Untypical aging Off-Flavor" (UTA) in Wine – Formation and Possible Preventions

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Abstract

2-Aminoacetophenone (AAP) is known as the character impact compound being responsible for the so called "untypical aging off-flavor" (UTA) in white wines. AAP can be formed by radical oxidation of indole-3-acetic acid (IAA) triggered by sulfuration. This report presents investigations on the mechanism of AAP-formation. After synthesis of possible intermediates it could be shown that AAP is formed by indole ring cleavage via 3-(2-formylamino)-phenyl-3-oxo-propionic acid (FAPOP) and N-formylaminoacetophenone (FAP). The degradation of IAA to AAP was suppressed in the presence of radical scavengers. However, analysis of 48 grape musts and their corresponding wines revealed that the amount of IAA or the antioxidative capacity did not correlate with the UTA-intensity of the wine, whereas the nitrogen content did. Differences in the release of IAA during fermentation of musts with different nitrogen contents indicate a correlation between the nitrogen supply of the vines and the UTA-formation.

Keywords: untypical aging off-flavor (UTA); wine; 2-aminoacetophenone; indole-3-acetic acid; sulfite; radical scavanger

INTRODUCTION

The tryptophan metabolite indole-3-acetic acid (IAA) is considered as a potential precursor of 2-aminoacetophenone (AAP), an aroma compound which causes in concentrations of more than 1 µg/L the "untypical aging off-flavor" (UTA) in white wines (1–4). The UTA develops within a few months of wine-storage in contrast to the typical aging which is a slow oxidation and change of specific aroma compounds during several years. Wines with UTA show odor taints like "floor polish, old wood cabinet, naphthalin or acacia flowers". For some years, about 20% of the rejected white wines in the German vintage wine certification exhibited the UTA which consequently causes losses of high economic values. Studying the UTA-formation in different grape varieties, degree of ripening, and climatic conditions revealed significant correlations between the appearance of high AAP-concentrations and wines from grapes which were put under stress by insufficient water or nitrogen supply as well as wines produced from high yield or early grape vintages (5, 6). Studies on the mechanism of AAP-formation in wine have shown that oxidative degradation of IAA by cooxidation with sulfite leads to the formation of AAP which is inhibited by a high total antioxidative status (TAS) of the

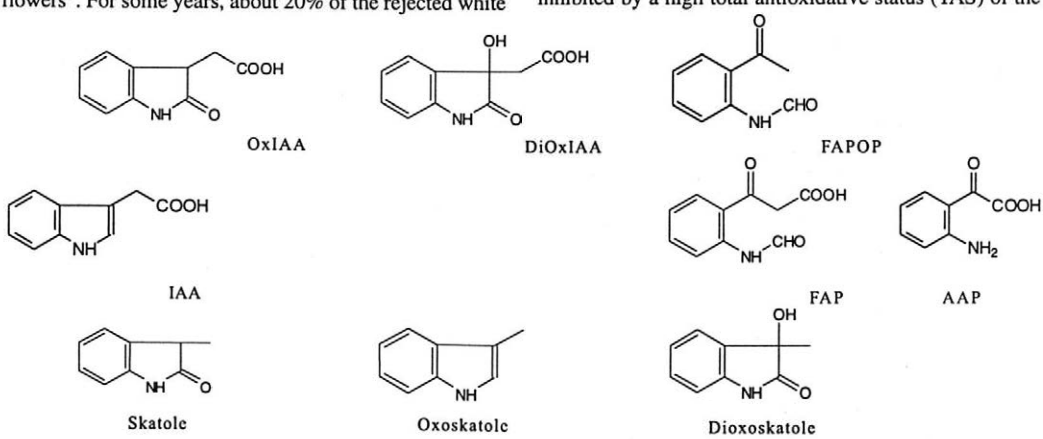


Figure 1. Possible degradation pathways and inter-mediate products by the cooxidation of IAA with sulfite to form AAP

wine (7). Fig. 1 shows the possible pathways and intermediate products by the oxidation of IAA to form AAP.

The purpose of this study was to elucidate the mechanism of AAP-formation in order to find oenological or technological possibilities for a prevention or at least a reduction of the AAP-formation during or after winemaking.

EXPERIMENTAL

Materials: 48 grape musts and their corresponding wines (vintage 96-98) were provided by the Bayerische Landesanstalt für Weinbau und Gartenbau (Veitshöchheim, Germany). Vines were cultivated under oenological parameters simulating different stress-situations. Fermentations of two grape musts with different content of total nitogen (N) were conduced in 1L flasks, incubated on a shaker (100 U/min) at 20°C after inoculation with 15 g/hL *S. cerevisiae*. Fermentations were monitored by refractive index, samples were taken daily.

AAP, ascorbic acid, 2,2´-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), caffeic acid, catechin, IAA, and quercetin were obtained from Sigma (Deisenhofen, Germany), Folin-Ciocalteau-reagent, formol and gallic acid from Merck (Darmstadt, Germany), 1,6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and myoglobin were purchased from Fluka (Neu-Ulm, Germany) and tryptophan was donated by Degussa (Hanau, Germany).

Synthesis of reference substances: Oxoskatole was prepared by treating skatole with dimethylsulfoxide and hydrochloric acid (8), 2-oxindole acetic acid (OxIAA) by treatment of IAA with N-bromsuccinimide, followed by a hydrogenation (9). 3-(2-Formylamino)-phenyl-3-oxo-propionic acid (FAPOP) was prepared by treating IAA with methanolic tetrahydrofurane and ozone and hydrogenation afterwards (10). N-Formylaminoacetophenone (FAP) was synthesized by formylation of AAP with formic acid and acetic acid anhydride (11). (2-Sulfoindole)-3-acetic acid (IAA-HSO_3) and dioxindole-3-acetic acid (DiOxIAA) were prepared by oxidation of IAA with bisulfite (12) and isolation by preparative HPLC (Merck, NovaPrep 200).

Modell system for elucidation the mechanism of AAP-formation: A wine-like model solution (10 vol% ethanol, 5 g/L tartaric and malic acid; pH 3.3) was spiked with 200 mg/L IAA and sulfurized with 100 mg/L potassium bisulfite. Samples were stored at 45 °C for 2 h and analyzed by HPLC-UV/ESI-MS (Hewlett-Packard Series 1100).

UTA-Intensity Analysis: The UTA-intensity of the wines were evaluated by the "UTAFIX-Test-Kit" (Schliessmann, Schwäbisch Hall, Germany).

TAS and Nitrogen Status (NS) Measurement: TAS of the wines were evaluated by determination of the relative ability to scavenge the radical cation $ABTS^{\bullet+}$ in comparison with Trolox (13). Total phenolics were estimated by the method of Folin-Ciocalteau (14) and expressed as gallic acid equivalent (GAE). NS of the grape musts and their corresponding wines were evaluated by the formol value (15).

Determination of free and conjugated IAA: IAA was determined by RP-HPLC-Fl (Nucleosil 120-3 C_{18}, 250×4 mm; Merck F-1080 fluorescence detector). Free plus ester- and amide-linked IAA was isolated after alkaline hydrolysis (5 M NaOH, vacuum vessel, 5 h at 120 °C) by a clean-up procedure using a C_{18}-SPE (Varian, Bond Elut LRC C-18/OH) followed by a SAX-SPE (Merck, LiChrolutO SAX). Free IAA was determined after isolation by a SAX-SPE.

RESULTS AND DISCUSSION

HPLC-MS analysis of the model wine solution spiked with IAA revealed that AAP is formed by radical oxidation of IAA, triggered by sulfuration, where indole ring cleavage of IAA leads to FAPOP, which is decarboxylated to FAP. FAP finally is hydrolyzed to AAP. Furthermore IAA was degraded into indolecarbaldehyd, OxIAA, DiOxIAA and IAA-HSO_3 as well, but it was turned out that these compounds were no precursors of AAP. However, decarboxylation of IAA via skatole, oxoskatole, and dioxoskatole was not involved in this sulphite-mediated destruction (see Fig. 1). In the presence of ascorbic acid or other polyphenolic compounds like gallic acid, caffeic acid, quercetin, catechin or tannins, the degradation of IAA following the AAP-pathway was suppressed.

The content of total phenolics as determined by the Folin-Ciocalteau method varied from 300 to 600 µg GAE/mL in the grape musts and 190 to 340 µg GAE/mL in the wines. TAS of the wines ranged between 0.1 and 0.7 mM Trolox equivalents which was mainly due to the total phenolics. The formol value varied from 4 to 17 in the grape musts and 0.7 to 15 in the wines. However, the content of total

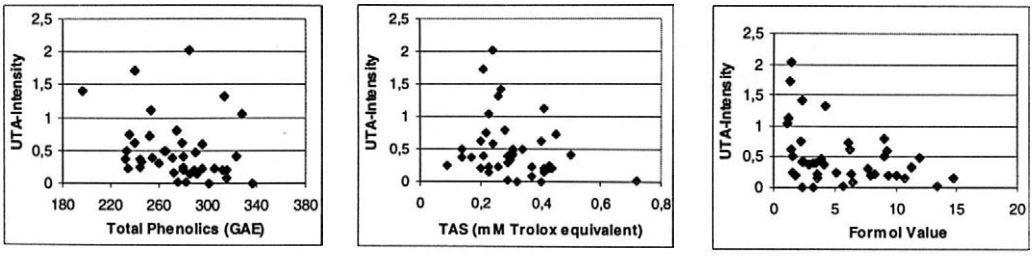
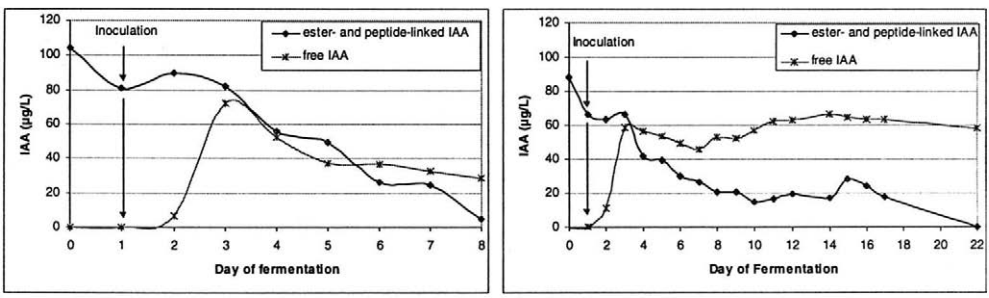


Figure 2. Correlation between total phenolics, TAS or formal value and the UTA-intensity of the investigated wines (n = 48)

phenolics and TAS of investigated wines did not correlate with UTA-intensity but the formol value did: Wines with a high UTA-intensity were characterized by al low NS of the grape must as well as of the wine (Fig. 2).

Determination of free and conjugated IAA in 48 grape musts and wines indicated that in grape must there were only traces of free IAA (< 10 µg/L) and higher amounts of conjugated IAA (20–150 µg/L). During fermentation the concentration of free IAA increased to about 20–50 µg/L in the wine while the concentration of ester- or amide-linked IAA decreased to about less than 30 µg/L. Investigations on the changes of free and conjugated IAA in different grape musts during fermentation revealed that there were significant differences in the release of free IAA (Fig. 3).

While the fermentation of the must with a total N of 462 mg/L was finished after 9 days with an amount of free IAA of 29 µg/L, the fermentation of the must with a total N of 200 mg/L was finished only after 22 days with a yielding of 58 µg/L free IAA. At the end of fermentation just traces of conjugated IAA could be detected in the source material with a high nitrogen content and no in the source material with a low nitrogen content. A formation of IAA could be detected at the day of turbulent fermentation (second day after inoculation) by the yeast and not by a release from conjugated IAA. These different reactions leads to the presumption that the nitrogen supply of the must and the



must A must B

Figure 3. Determination of free and conjugated IAA during fermentation (must A: total N = 462 mg/L, 8 days fermentation; must B: total N = 200 mg/L, 22 days fermentation)

resulting yeast metabolism play a part in the formation of the UTA in wine.

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Migration of Epoxy Monomers in Canned Foods and their Reaction with Food Components

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Abstract

Epoxy compounds like bisphenol A-diglycidylether (BADGE) have been reported to migrate from the internal coating of cans and lids into the packed foods. BADGE and its hydrolysis and hydrochlorination derivatives were determined in different foods by RP-HPLC-Fl using bisphenol A-di-3-hydroxypropylether (BADHPE) as internal standard. The reaction of BADGE with different food components was studied in sunflower oil, tuna and peaches. Analyses were done subsequently after homogenisation (control), after 1 day storage at room temperature or after heat treatment simulating sterilisation. While the spiked BADGE could be completely recovered in sunflower oil even after heat treatment, more than 95% disappeared in tuna without detectable formation of known derivatives. In peaches more than 60% of the added amount of BADGE was hydrolysed after heat treatment yielding BADGE·2H_2O. These results indicate reactions of BADGE besides hydrolysis mainly with N-containing food components.

Keywords: canned foods, migration, epoxides, hydrolysis, bisphenol A-diglycidylether (BADGE)

INTRODUCTION

Most food and beverage cans and lids are coated by epoxy resins, combinations of epoxies with phenolic resins or by organosols (PVC) stabilised with epoxy compounds (scavenger for hydrochloric acid). These coatings may release epoxy compounds like bisphenol A-diglycidylether (BADGE), phenols like bisphenol A (BPA) as well as BADGE oligomers and several other derivatives into the packed food (1–3). Additionally migrating epoxy compounds are likely to react with food components during heat treatment (sterilisation) or storage. Besides hydrolysis the reaction with chloride in foods and gastric simulants was shown (4). In the final opinion of the Scientific Committee on Food (SCF) the migration of BADGE and its hydrolysis and hydrochlorination derivatives is limited to 1 mg/kg food (5). In our workgroup an RP-HPLC-Fl method for the determination of all SCF-relevant BADGE derivatives and BPA in foods using bisphenol A-di-3-hydroxypropylether (BADHPE) as internal standard (IS) was developed (6).

In order to enlighten the reaction of BADGE with food components besides hydrolysis and hydrochlorination RICHARD *et al.* (4) added BADGE to different foods and determined BADGE and its derivatives after extraction with methyl-tert.-butyl ether, acylation and NPLC-Fl on a cyano phase. They found that at least 97% of the BADGE added to tuna in water had disappeared and was not detectable as hydrolysis or HCl-derivative. The aim of our present investigation was to confirm the conversion of BADGE to unknown compounds in foods mainly consisting either of triacylglycerines , or carbohydrates, or proteins and N-containing substances in order to find out the main reaction partners of BADGE.

EXPERIMENTAL

MATERIALS

Apparatus. HPLC-FLD: TSP AS 100 autosampler (Thermoquest, Germany), L-6200 pump, F-1080 fluorescence detector (Merck, Germany), Kroma System 2000 for data acquisition (Bio-Tek Instruments, Germany); HPLC-ESI-MS: HP 1100 system consisting of an autosampler, a binary pump, a column oven, UV/VIS detector and an AP-ESI-MSD (Agilent Technologies, Germany).

Chemicals. BADGE, BADGE·2H_2O and BADGE·2HCl were purchased from Fluka (Buchs, Switzerland). BADGE·H_2O, BADGE·HCl, BADGE·H_2O·HCl and BADHPE (IS) were synthesised as has been published previously (Note: Fluka made the first 3 substances available in Feb. 2000) (6).

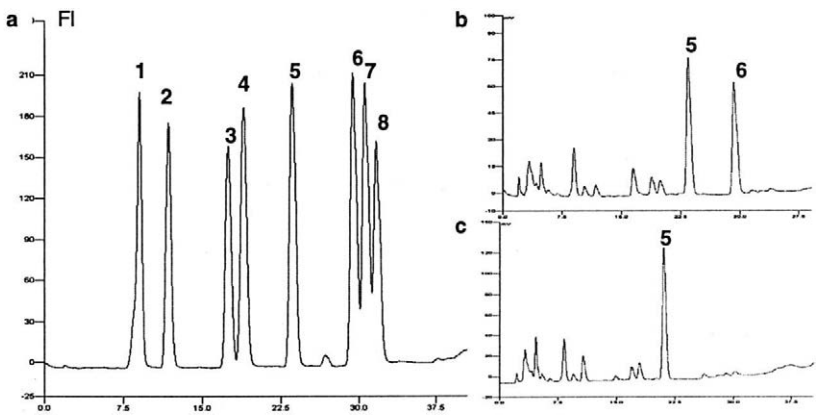
Samples. Sunflower oil, tuna – canned in water (74.5% water, 25.5% N-substances, 0.8% lipids, 1.5% ash), tuna – canned in oil (59.8% water, 29.1% N-substances, 8.2% lipids, 2.8 % ash) and peaches - canned in light syrup (84.7% water, 0.45% N-substances, 0.03% lipids, 14.6% carbohyd-

rates (glucose, fructose, sucrose), 1.4% total dietary fibre, 0.25% ash, pH about 3.0) (7) were analysed to contain less than 40 µg/kg BADGE and its derivatives. The samples were homogenised and divided in 10 g portions. BADGE and BADHPE were dissolved in acetonitrile (MeCN) (20 g per L), 250 µL were added to the food samples and homogenised subsequently resulting in BADGE concentrations of 1, 10, 100 and 500 mg/kg homogenate.

Treatment before Analysis: As a control the samples were analysed subsequently after homogenisation. In order to observe the reaction of BADGE with food components the samples were either stored at room temperature for 24 h or heated (10 g in a 50 mL DURAN bottle with thread) at 121 °C for 30 min in an autoclave (Wolf TKL-MCA 123, Germany) to simulate sterilisation (Fig. 1b, c).

Analytical procedure

The samples (except for sunflower oil) are dried by grinding with the same amount of Kieselguhr. The powder is transferred into a chromatographic column (300 × 25mm) and extracted with 150 mL diethylether. After evaporation of the extract the remaining lipid (or the sunflower oil) is transferred into a Pyrex tube and extracted with 5 mL MeCN under vigorous shaking. A filtration through a C18-SPE column is used to remove residual lipids from the MeCN extract. The SPE-column is finally rinsed with 2 mL MeCN/water 90:10 (v:v). Separation is performed by RP-HPLC (Multospher 250 × 4 mm, 5–120 RP18; gradient: water/MeOH/MeCN, 0 min 40:40:20 → 30 min 25:50:25) and detection by fluorescence at Ex/Em 275/305nm (Fig. 1a). BADGE, BADGE·H_2O, BADGE·$2H_2O$, BADGE·HCl, BADGE·2HCl



Figgure 1. RP-HPLC separation of BADGE derivatives: 1 BADGE·$2H_2O$, 2 BPA, 3 BADGE·H_2O, 4 BADGE·H_2O·HCl, 5 BADHPE (IS), 6 BADGE, 7 BADGE·HCl, 8 BADGE·2HCl. a) standards, 1 mg/L, b) tuna in water spiked with BADGE and BADHPE (1 mg/kg) and subsequent analysis, c) tuna in water spiked with BADGE and BADHPE (1 mg/kg) after 30 min at 120 °C

and BADGE·H_2O·HCl are calculated using the internal standard BADHPE. The IS has been shown to exhibit a similar recovery rate to the analytes (6) and is not supposed to react with the food matrices. Confirmation of identity of BADGE and its derivatives was done by ESI-MS (SIM).

RESULTS AND DISCUSSION

The internal standard BADHPE could be proved not to react with the foods chosen: Irrespective of the matrix its recovery rates after sterilisation ranged from 70–104%, resulting from losses that typically occur during the extraction and clean-up steps. This demonstrates the suitability of BADHPE as IS for determinations nearly independent of matrix and treatment influences.

The conversion rates of BADGE to its hydrochlorination derivatives BADGE·HCl, BADGE·2HCl and BADGE·H_2O·HCl was < 1% under the conditions chosen and in all matrices. RICHARD *et al.* (4) reported a formation of HCl-adducts after addition of 1–4% of NaCl to the respective food.

As expected nearly no loss of BADGE was observed for sunflower oil, a matrix mainly consisting of triacylglycerines (Fig. 2). By contrast more than 80% of the BADGE spiked to tuna in water disappeared during one-day storage and about 30% of the BADGE spiked disappeared from tuna in oil. After sterilisation the loss of BADGE amounted to more than 95% and 80%, respectively (Figs. 1b, c and 2). These losses occurred irrespective of the amount of BADGE added (1–500 mg/kg) indicating an excess of reactive matrix components. The oil in the latter tuna sample (8.2% vs. 0.03% in tuna in water) seems to protect BADGE from reaction with other food components. Since the matrix nearly exclusively consists of N-containing substances (see *samples*) these are very likely to be the major reaction partners of BADGE. This is supported of by the fact that BADGE is reported to be a contact allergen, which implies a reaction between BADGE and skin proteins (8).

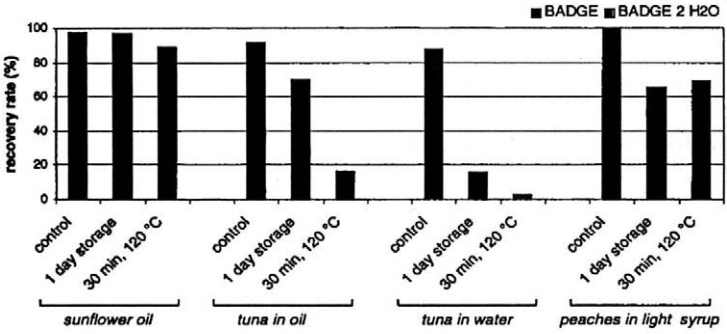


Fig. 2. Conversion of spiked BADGE (1 mg/kg) in 4 food samples under 3 different treatments

For analyses of epoxies in protein rich foods it may be concluded that the samples have to be extracted immediately after homogenisation. In accordance with the results of Richard et al. (4) no new peaks were detectable in the chromatograms of the tuna in water extracts although more than 95% of the initial amount of BADGE had disappeared (Fig. 1b, c). Identification of adducts of BADGE with N-containing substances are currently in progress.

A significant formation of BADGE·$2H_2O$ was observed in spiked peaches after sterilisation (Fig. 2). This is due to the water content and the acidic pH of this matrix. However, after one-day storage or sterilisation about 30% of the spiked BADGE disappeared without formation of other known derivatives.

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Structure and Function of Melanoidins in Foods and Food-Related Systems

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Abstract

The structures of melanoidins are poorly understood. They may contain a repeating unit to which low molecular weight (LMW) chromogenic structures are attached and, in proteins, these chromogenic structures can cross-link peptide chains. Melanoidins may have several functions in foods, the better known ones being their contribution to colour and antioxidative effects. In addition, they may bind metals, affect the growth of bacteria and exhibit desmutagenic activity.

Keywords: melanoidins, Maillard reaction, colour, antioxidants, binding properties, colonic bacteria, antibacterial effects, desmutagenic effects

INTRODUCTION

The Maillard reaction occurs in foods when carbohydrates, which possess a free carbonyl group, interact with compounds possessing a free amino group, such as amino acids, in either the free or protein-bound form. It occurs when most foods are heated, including malt, coffee, bread and other bakery items. The colour that develops is due to melanoidins and their coloured precursors. Melanoidins are the final, high molecular weight (HMW) products of the Maillard reaction and they have been defined as brown nitrogen-containing polymers (1). In some foods, e.g., coffee, phenolic precursors are also involved in reactions and melanoidins are formed containing phenolic sub-units.

Melanoidins are widespread dietary components in a typical western diet. However, their structures and functions, the latter being very diverse, are still largely unknown (2).

Structures of melanoidins

The structures of melanoidins have eluded scientists for many years. They remain poorly defined but important progress has been made recently. In contrast, progress has been rapid concerning the structures and formation of low molecular weight coloured Maillard products, a selection of which is given in Fig. 1.

There are various hypotheses regarding melanoidin structures. One possibility is that melanoidins are formed by more or less random reactions between LMW coloured reaction products, such as those in Fig. 1, and other LMW reaction products. Alternatively, a repeating unit (which may be colourless or contribute little to colour) may form the backbone of melanoidins, with chromogenic LMW structures attaching themselves to this backbone, resulting in HMW coloured structures. Either of these alternatives would be possible in model systems. While there is no experimental evidence for the random association of smaller coloured compounds into melanoidins, the case for some type of repeating unit is easier to make. A repeating unit, **V**, formed by the aldol condensation of the Schiff base of 3-deoxyosone, is one possibility and was proposed in 1981, based on experimental evidence obtained by studying the pyrolysis and oxidation of melanoidins prepared from aldoses and butylamine (7).

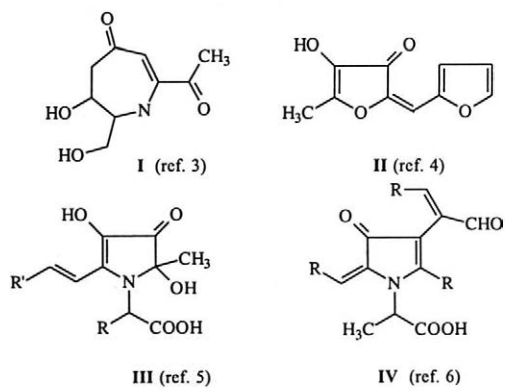
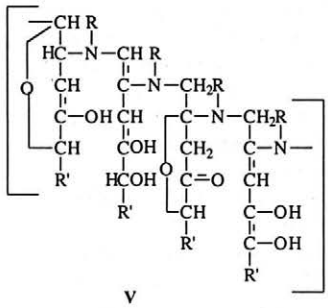


Figure 1. A selection of low molecular weight coloured Maillard reaction products



V

A stoichiometric approach to probing melanoidin structure showed that melanoidins (> 12 000 daltons) prepared from glucose and glycine possessed very similar extinction coefficients at 450 nm that were independent of the sugar to amino acid ratio. The starting materials became incorporated into melanoidins in similar amounts, regardless of their ratio, suggesting that melanoidin formation followed a specific pathway (8).

Tressl and co-workers have made important progress concerning the structures of polymeric materials prepared from (a) 2-hydroxymethyl-*N*-methylpyrrole in trichloromethane and (b) *N*-methylpyrrole with 2-formyl-*N*-methylpyrrole (or furfural) in methanol (9). Examples of the structures are given in Fig. 2. Since these structures were synthesised from one or two selected precursors, they are not likely to represent the structures of melanoidins in foods. Nevertheless, this type of repeating unit could exist as domains in some food melanoidins.

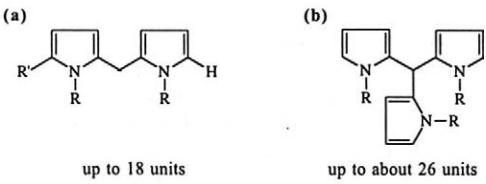


Figure 2. Examples of structures formed from (a) 2-hydroxymethyl-*N*-methylpyrrole and (b) *N*-methylpyrrole and 2-formyl-*N*-methylpyrrole (9)

Most of the work on melanoidin structure has been concerned with materials isolated from model systems based on single sugars and single amino acids. In many foods, proteins are the greatest source of free amino groups. A recent investigation of the structure of casein after heating with glucose has shown that the protein becomes modified and cross-linked by low molecular weight coloured Maillard products such as **III** and **IV** (10). The identification of structures of this type supports the idea that melanoidins may form by small coloured Maillard products becoming attached to a colourless skeleton. In addition, it seems that, in foods, these reactions may result in cross-linking and browning of proteins, and the term 'melanoproteins' has been coined (11).

Function of melanoidins

Melanoidins have many functions and the main ones are discussed here.

Colour: The most obvious feature of melanoidins is their brown colour. The simplest way of monitoring colour development is to measure absorbance at a single wavelength in the visible region or to obtain a visible absorption spectrum. However, the ultraviolet-visible spectrum obtained for melanoidins is fairly featureless, especially in the visible region, where extensive tailing is observed. More information is obtained by measuring colour in, e.g., CIELAB space (12). While no studies have been published specifically on melanoidins, it is useful to describe an investigation of total MRPs (12). Aqueous solutions of glucose or xylose (1, 3 and 5%) and lysine or glycine (1%) were refluxed at pH 8 for up to 6 h and their L*a*b* values obtained. L* values decreased with heating time and the rate of decrease was greater with increasing sugar concentration. Chroma (a*,b*) plots are shown in Fig. 3. As heating progressed, coloured compounds formed and the locus moves along the b*+ axis (apart from xylose/glycine), indicating the development of a yellow colour. Further heating caused the locus to form a loop as the hue changed to orange at the point of greatest brilliance (highest chroma) at a hue angle of h* = 67°. After this point, the a*+ value continued to increase (towards reddish brown) whi-

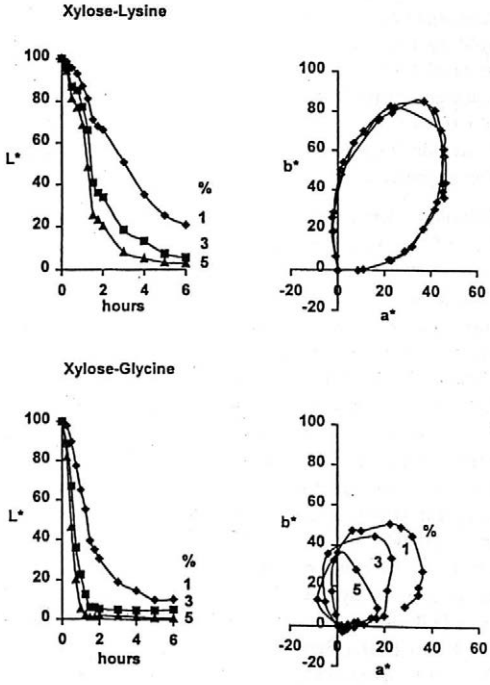


Figure 3. CIELAB chroma plots of colour development of glucose or xylose - lysine or glycine mixtures (u: 1% sugar + 1% amino acid; n: 3% sugar + 1% amino acid; s 5% sugar + 1% amino acid.) (With permission from ref. 12)

le the b*+ value decreased. Finally, with the development of very dark colours of low chroma, the locus turned towards the origin. The near superimposition of the loci for the three sugar concentrations of each sugar-amino acid combination (except xylose/glycine) indicates that the quality of the colour is not altered by sugar concentration. The loci for the two lysine systems are also very similar while those for glucose/glycine and glucose/lysine are slightly different, indicating differences in the pigments and the possibility that the amino acid has a greater effect than the sugar on the pigments produced. On heating, xylose/glycine initially gave a green colour, which then changed to brown and, finally, to blood red. It appears that a green coloured compound is produced in the xylose/glycine system. The data illustrate that the nature of the colour changes with heating time, and this correlates with the progress of the reaction, including the formation of melanoidins.

Antioxidant properties

There are several reports in the literature of the antioxidant activities of melanoidins (13, 14). Antioxidants are able to delay or prevent oxidation processes, typically involving lipids, the oxidation of which frequently limits food shelf-life. Melanoidins have been reported to act as antioxidants by all the following mechanisms (14):

- scavenge free oxygen,
- scavenge active oxygen, i.e.,
- hydroxyl radicals ($HO^{\bullet}$),
- superoxides ($O_2^{\bullet-}$),
- scavenge peroxyl radicals ($ROO^{\bullet}$),
- peroxides,
- act as reducing agents (i.e., donate hydrogen or electrons),
- chelate metals.

Although there are several reports of the antioxidative effects of MRPs in heated foods (13), few have focused on melanoidins. An investigation of the degree of roasting on the antioxidative activity (assessed by oxygen consumption and peroxyl radical scavenging activities) of coffee brews showed that antioxidative activity increased with roasting (attributed to the development of MRPs) up to the medium-dark roasted stage and then decreased with further roasting (15). All the roasted coffees possessed higher antioxidative activity than the green beans but at the longer roasting times, some degradation of MRPs possessing antioxidative activity probably occurred.

In model systems, melanoidins prepared from glucose (or fructose) and lysine inhibit the oxidation of linoleic acid, as assessed by the thiobarbituric acid reactive substances (TBARS) measurement (16).

The antioxidant activity of melanoidins varies according to the profile of reaction precursors as well as the reaction conditions, although insufficient data are available to draw conclusions regarding the effects of these variables on antioxidant properties.

The effect of dietary components on the antioxidant status of the body is of increasing interest. One study, using the retentate obtained on dialysis of heated glucose-protein (casein or soy protein) showed that the livers of rats fed the browned material had lower TBARS values, suggesting that these melanoproteins may exhibit antioxidant effects *in vivo* (17).

Binding properties: Of the possible groups of ligands, most attention has been paid to the binding properties of melanoidins by metals. A range of melanoidins was prepared by heating aqueous solutions of glucose or fructose with lysine over a range of conditions, i.e., 80–159°C, 30–119 min, pH 6.14–8.51, a_w 0.57–0.95, and separating the melanoidins by dialysis (16). Almost all could bind copper, with 0.016-2.267 μmol copper per mg melanoidin being bound, according to the sugar and the processing conditions. Coffee pigments are reported to have a greater affinity for copper with 84 mmol copper being bound per gram of freeze dried coffee (18).

There appear to be no reports in the literature of other compounds, such as flavour components or IQ compounds, binding to melanoidins.

Effect on colonic bacteria: Feeding studies with rats have shown that only small proportions of melanoidins prepared from glucose and glycine (or lysine) are absorbed through the gut, the majority being excreted in the faeces (19). Another study on rats was concerned with the effect of total Maillard reaction products (HMW and LMW) on the growth of gut bacteria (20). Increases in the growth of lactobacilli and no effects on numbers of enterococci, coliforms and clostridia were observed.

Recently, melanoidins were prepared from glucose and lysine heated in aqueous solution at pH 5 and their effects on human colonic bacteria were determined (19). Anaerobic batch culture fermenters were inoculated with faeces as the source of colonic bacteria. Melanoidins were the only fermentable carbon source in the growth medium and resulted in significant increases in some groups of gut bacteria. Using a phenotypic approach (assessing numbers of viable bacteria), significant increases ($p < 0.05$) in total anaerobes, bacteroides and clostridia after 6 h incubation (compared to time zero) were observed, while bifidobacteria increased significantly ($p < 0.05$) after 24 h. A genotypic method (enumerating both viable and non-viable cells) gave no significant differences after 6 h incubation but, after 24 h, bacteroides, clostridia and lactobacilli all increased significantly ($p < 0.05$).

If bacteria are able to degrade melanoidins in the gut, this implies that other dietary components may be released and made available for absorption through the gut wall. This may be desirable in the case of nutritionally limiting dietary components, such as certain vitamins and trace metals, but if melanoidins are able to render undesirable components unavailable for absorption, obviously their release in the gut would be undesirable.

Antibacterial activity: The effects of MRPs, both with and without dialysis (MWt cut-off 1000 Da), from arginine and xylose or from histidine and glucose on some pathogenic and food spoilage-causing bacteria have been investigated (20). Total MRPs inhibited growth by prolonging the lag phase, in many cases. The precise effect varied with the type of bacteria and the MRPs. The HMW fractions of MRPs from both sugar-amino acid samples, heated for 5 h, were more inhibitory towards *Bacillus subtilis* than either the total or LMW material. When arginine and xylose were heated for 20 h, the HMW fraction was most inhibitory towards *B. subtilis, Escherichia coli* and *Staphylococcus aureus*.

Desmutagenic activity: The Maillard reaction results in the formation of mutagens, notably certain heterocyclic amines (21). Desmutagenic compounds result in a loss of mutagenicity in a system and nondialysable melanoidins prepared from glucose and glycine have desmutagenic effects against certain heterocyclic amines (22). Desmutagenic activity was also apparent for molecular weight fractions of 1000–5000 Da and above 5000 Da, but not for below 1000 Da. It was suggested that reductone structures and antioxidative properties of melanoidins may be linked to the observed desmutagenicity. Further work using a wider range of mutagens, including aflatoxin B_1, 4-aminobiphenyl and 2-nitrofluorene, showed both nondialysable melanoidins and the 500–1000 Da fraction prepared from glucose and glycine to possess desmutagenic effects (23).

CONCLUSION

Progress is now being made concerning the structures of melanoidins as well as understanding their diverse functions. Much more work remains to be done before findings can be applied to the improvement of food quality.

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Elucidation of Chromogenic Pathways in Non-Enzymatic Browning of Pentoses and Hexoses

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Abstract

Besides the unique aroma, the typical brown colour of thermally processed foods mainly originating from the Maillard reaction between reducing carbohydrates and amino acids or proteins, is highly desirable and is intimately associated in consumers minds with a delicious, high-grade product. To further improve the quality of processed foods, e.g., by controlling the non enzymatic browning reactions more efficiently, a better understanding of the structures and the formation of chromophores from carbohydrates is required. To gain insights into these browning reactions, amino acids or proteins and carbohydrates as well as carbohydrate degradation products have been thermally under conditions used in food processing, the key chromophores have been identified by application of the recently developed colour dilution analysis. In addition, quantitative precursor studies and [^{13}C] labelling experiments have been performed to clarify to formation routes of these chromophores helping to unravel the puzzling network of browning reactions.

Keywords: pentoses; hexoses; Maillard reaction; aroma

Colour Development in Glyoxal – Valine Model System

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Abstract

Fractionation of Maillard reaction products and identification of some low molecular weight (<1000 Da) coloured compounds arising in model aqueous solution of glyoxal and valine (95°C, 40 hours) is reported in this study. Fractionation revealed that the low molecular weight compounds are responsible for about 32% of the absorbance at 420 nm related to the water soluble colorants, while nearly 56% of the colour come from polymeric chromogens (> 3000 Da). Prevailing part of the low molecular weight coloured compounds extractable with organic solvents is ionizable possessing acidic character. Several coloured together with non-coloured products were identified or tentatively identified as compounds with molecular weights ranging from 165 to 389 Da, being comprised mostly of pyrazine skeleton, e.g., 2-(5-hydroxy-5*H*-pyrazin-2-ylideneamino)-3-methyl-but-2-enoic acid (I). The investigated fractions contained limited amounts of imidazoles; only colourless 2-[3-(1-carboxy-2-methylpropyl)-imidazolium-1-yl]-2-(2-methylethyl)-ethanoate (II) was found.

Keywords: Maillard reaction; coloured compounds; pyrazines; imidazoles

INTRODUCTION

The Maillard reaction is considered as the chief source of colour in processed food as well as of discolorations associated with food deterioration. Despite of extensive investigations, the actual chemical species responsible for colour changes remain still largely undefined.

Several simultaneously active mechanisms leading to colorants formation seem to work even in binary model mixtures (usually reducing sugar – amino acid). A few advanced Maillard reaction intermediates were found to be penultimate precursors of numerous low-molecular-weight coloured compounds (1). Alternative reaction scheme leading to very early colour formation, probably by radical-assisted mechanisms, was also proposed (2, 3). It implies formation of glyoxal directly from hexoses due to oxidative and/or dicarbonyl cleavages of the monosaccharides (4), and its reduction to glycoladehyde. Besides, glyoxal, similarly to many other short chain α-hydroxycarbonyl and dicarbonyl compounds, belongs to the precursors with great efficiency in colorants formation also in non-reducting media (5, 6). It is also well known, that reactions of this highly reactive degradation product of saccharides and lipids with amino compounds lead to the formation of a series of *N*-containing heterocyclic compounds such as pyrroles, imidazoles, pyrazin(on)es and other flavour-active compounds (7).

The use of sugar fragment seems advantageous in connection with supposed reduction of pathway multiplicity and of (coloured) products heterogeneity. In this paper, a heated glyoxal – valine aqueous reaction mixture was investigated. Valine was chosen as a simple aliphatic amino acid with moderate polarity and rather low browning activity (5). Study on colour distribution into defined fractions of the reaction mixture, separation and identification of premelanoidins and mainly low molecular coloured products from the model system were performed in this work.

EXPERIMENTAL

Model experiments. Non-buffered aqueous solution with equimolar 0.5M concentrations of glyoxal (as trimeric dihydrate, Sigma) and valine (Fluka) was air-heated at 95°C in closed bottles for 40 hours. These experimental conditions were chosen due to relatively high levels of the target compounds, fair stability during sample treatment and sufficient repeatability of the heated reaction mixture composition (8).

Fractionation. The prepared reaction mixture (250 ml) was filtered (0.45 μm) and the filtrate was fractionated with ultrafiltration (cellulose membranes, 3 kDa or 1 kDa). The latter ultrafiltrate (100 ml) was extracted with diethyl ether (3 × 100 ml) and the aqueous layer once again with ethyl acetate (3× 100 ml). The both collected and concentrated extracts were then applied on the silica gel 60 (0.063–0.200 mm, Merck) column (2 × 60 cm, in toluene) and eluted with a series of 12 solvent mixtures (100 ml) with increasing polarity (adapted from [9]) to give 12 fractions being transferred into methanol.

HPLC analyses. Nova-Pak® C18, 250 × 4.6 mm, 4 μm, and Guard-Pak™ Nova-Pak® C18 (Waters) with gradient elution (water/acetonitrile), f = 0.6 ml/min, 20 μl loop, and HPLC system consisting of Waters 2 × 515 HPLC pump and 996 PDA detector system were used. The MS detector was Perkin-Elmer API 3000 electrospray with triple quadrupole. The pure target products were collected after separation of the fractions by means of semipreparative chromatography (Lichrospher 100 C_{18} in LichroCart 250-10, Merck).

Structure confirmation. Structure and purity of the isolated compounds were confirmed by ^{1}H- and ^{13}C- NMR spectrometry (Bruker Avance DRX 500).

RESULTS AND DISCUSSION

Several separation steps based on differences in molecular weight, solubility, polarity and/or volatility of the components were applied to simplify sufficiently the reaction mixture (Table 1). Ultrafiltration (with membrane of 1 kDa limit) revealed about one-third colour (as A_{420}) belonging to the low molecular compounds soluble in water, while the oligomeric fraction (1-3 kDa) amounted to about 12% of the colour. About one-third of the ultrafiltrate (1 kDa) colour exhibited extractability to diethyl ether. The aqueous layer was further extracted with ethyl acetate to give slightly lower yield of the extractable colorants. When pH adjustment of the ultrafiltrate from the primary (~ 2.5) towards higher values before extraction was done, the extractability was dramatically lowered (Table 1). This finding implies acidic character most of the coloured low molecular weight compounds being included in the organic fractions.

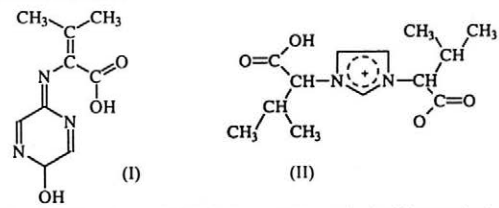
The organic extracts were subjected to additional fractionation on silica gel column in order to isolate some of the significant low molecular weight colorants. The fractions were analysed on HPLC-diode array system to search out the target compounds. LC-MS analyses were carried out with the frac-tions containing sufficiently resolved significant peaks, that absorb in visible region with well structured (not melanoidin-like) spectra. Only five fractions obtained from the both extracts and separated under chromatographic conditions used have met the above requirements. The target compounds were further isolated from a semipreparative HPLC system and their structures were proposed after NMR spectroscopic measurements.

Table 1. Colour distribution into differently defined fractions of the heated glyoxal – valine model mixture (averaged, n = 3)

Fraction	A_{420}/A_{420}(total)*(in %)
Insoluble in water (I)	23 (in DMSO); 17 (m/m) yield
Soluble in water (S)	77
Soluble in water < 1 kDa (1 kDa UF)	24
Soluble in water < 3 kDa	33
Soluble in water > 3 kDa	44
Diethyl ether (DEE) extract from 1kDa UF	8.7
DEE extract from 1kDa UF with pH 6.5	3.6
DEE extract from 1kDa UF with pH 10	1.5
Ethyl acetate extract from 1 kDa UF	5.7
Heated glyoxal only solution	9.0
1 kDa UF of heated glyoxal only solution	4.0

*A_{420}(total) = absorbance at 420 nm of the whole glyoxal – valine reaction mixture

One of the low molecular coloured compounds dominant mainly in toluene:ethyl acetate (7:3, v/v) fraction from the diethyl ether extract with molecular weight 209 Da and pyrazine-like UV/VIS spectrum (l_{max3} = 351 nm) was tentatively identified as 2-(5-hydroxy-5*H*-pyrazin-2-ylideneamino)-3-methyl-but-2-enoic acid (I) (Fig. 1). The compound, similarly to most of the other colorants identified, is a poor chromogen due to low extinction at wavelengths > 385 nm. The compound represents not more than 2% of the diethyl ether extract colour potential, which demonstrates ongoing complexity of the final fractions analysed. The solid pure compound has shown pale yellow hue and marked roasty and malty odour. The substance was present in two chromatographically resolved diastereomers. Several close derivatives of (I), such products of decarboxylation (M.w. 165), oxidation (dehydrogenation, M.w. 207), dehydration (M.w. 193 and 191, resp.), with another isoprop(en)yl moiety bound (M.w. 249–255, resp.), and other were also found. A slightly UV/VIS absorbed imidazole derivative, viz. 2-[3-(1-carboxy-2-methylpropyl)-imidazolium-1-yl]-2-(2-methylethyl)-ethanoate (II) (Fig. 1), was one of the dominant components of more polar fractions of the ethyl acetate extract.



(I) (II)

Figure 1. Structures of 2-(5-hydroxy-5*H*-pyrazin-2-ylideneamino)-3-methyl-but-2-enoic acid (I) and 2-[3-(1-carboxy-2-methylpropyl)-imidazolium-1-yl]-2-(2-methylethyl)-ethanoate (II)

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Studies on the Formation of Protein-Bound Amino Acid Derivatives as Markers of the Maillard Reaction under High Hydrostatic Pressure

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Abstract

The influence of high hydrostatic pressure up to 600 MPa on the Maillard reaction was studied in model systems consisting of amino acids or β-casein and sugars. Pentosidine was found to correlate positively with the increase of pressure in both systems, while the formation of pyrraline is reduced. Results suggest, that under high pressure the Maillard reaction has to be regarded from a different point of view than in heat treated systems.

Keywords: Maillard reaction; high pressure; pentosidine; pyrraline

INTRODUCTION

The application of high hydrostatic pressure is an interessting alternative for food preservation (1). Furthermore, the use of this new technology is promising new protein properties and therefore may lead to new products. Beside the well known inactivation of microorganisms (2–4), high hydrostatic pressure influences chemical reactions (5). Although it seemed likely, that the Maillard reaction, as one of the major reaction schemes in food chemistry, is also affected, very few studies on that subject have been published up to now, focusing mainly on the measurement of browning reactions (6, 7). Model studies with a system consisting of tryptophan and glucose or xylose revealed that the formation of Amadori products is favoured under high pressure, while the rate of their degradation is reduced (8). Very recently, it could be shown that the formation of volatiles in the course of the Maillard is reduced by the application of pressure (9, 10). Currently, no information is available about the protein-bound formation of amino acid derivatives in the reaction between proteins and carbohydrates under high hydrostatic pressure. In this study the influence of high hydrostatic pressure on the formation of pentosidine and pyrraline of reaction mixtures consisting of amino acids with sugars or b-casein with sugars, respectively, was investigated.

EXPERIMENTAL

Chemicals: All chemicals used were of highest purity available. 3-deoxyglucosulose (3-DG) and N^{α}-acetylpyrraline were synthetised according to (11).

High pressure plant: Pressure application was performed in stoppered polystyrene test-tubes (5 ml), used on a high pressure pilot plant (Dunze and add-Hochdrucktechnik, Bad Homburg, Germany).

Analysis of pentosidine: Pentosidine was analysed according to (12).

Analysis of pyrraline: A RP-HPLC system with a Eurospher 100 C18 column (Knauer, Berlin, Germany) and UV-detection at 295 nm was used for analysing N^{α}-acetylpyrraline in amino acid mixtures. The eluent consisted of 7.5 mM sodium pentanesulfonate in 10% ethanol. The pH was adjusted to 3.0 using propionic acid.

Amino acid analysis: This was performed as described in (13).

Preparation of model systems: Solutions of N^{α}-acetylarginine (acarg), N^{α}-acetyllysine (aclys), glucose (glc), ribose (rib) and 3-DG (0.1 mmol/ml each) were prepared in 0.5M imidazole buffer (pH 6.8). Mixtures (acarg/aclys/rib; acarg/aclys/glc; aclys/glc; aclys/3-DG) were made by mixing equal volumes of the solutions. Solutions of β-casein with ribose or glucose in imidazole buffer were prepared, adjusting a molar ratio between lysine residues and sugar of 1:10. On the resulting mixtures, high hydrostatic pressure up to 600 MPa was applied for 2 h at 60°C. Where necessary analysis was performed after enzymatic or acid hydrolysis of the samples.

RESULTS AND DISCUSSION

Fluorescence emission spectra (excitation: 350 nm) were recorded on solutions of β-casein incubated with glucose or ribose, respectively. Although differences in spectra

could not be regarded as significant, glucose seemed to hinder the formation of fluorophores while ribose showed a tendency to promote it. This increase in measurable fluorescence led us to verify the formation of pentosidine, a known fluorescent crosslink amino acid of the advanced Maillard reaction. For that purpose solutions containing equal molar amounts of each N^{α}-acetylarginine, N^{α}-acetyllysine and ribose were subjected to high pressure and then analysed on the amino acid analyser with fluorescence detection ($\lambda_{ex}/\lambda_{em}$ = 335/385 nm) after acid hydrolysis. As shown in Fig. 1 the pentosidine content in the samples markedly increased with pressure (14).

The enhanced formation of pentosidine under elevated pressure could be reproduced on a system consisting of b-casein and ribose. Levels of protein-bound pentosidine rose from not detectable (< 30 µg/100 g protein) at air pressure up to 4.8 mg/100 g at 600 MPa.

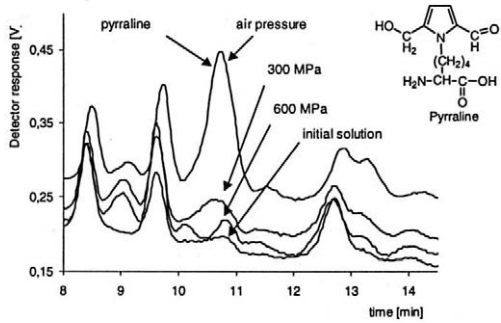

Figure 1. Overlay chromatograms (amino acid analysis with fluorescence detection; detail)

As a further important derivative of protein glycation, the formation of pyrraline from 3-DG and N^{α}-acetyllysine was studied. In this samples we could notice a continuous decrease in the pyrraline content with rise in pressure (Fig .2).

Under the choosen conditions at air pressure, a noticeable amount of pyrraline is formed from N^{α}-acetyllysine and 3-DG, while under high pressure this reaction is suppressed down to the detection limit (300 µg/100 g protein).

In this work for the first time the influence of high hydrostatic pressure on post-translational modification of proteins could be demonstrated via the quantification of individual amino acid derivatives of the advanced Maillard reaction. Our results point out that various individual reaction steps in the course of protein-carbohydrate reactions significantly differ in their pressure dependence. Corresponding studies are currently in progress.

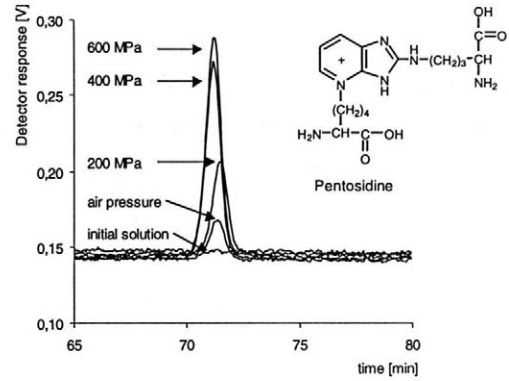

Figure 2. Overlay chromatograms (RP-HPLC with UV-detection; detail)

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Reinvestigation of the Reaction Between 2-Furancarboxal-Dehyde and 4-Hydroxy-5-Methyl-3(2H)-Furanone

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Abstract

Besides 2-(2-furanylmethylene)-4-hydroxy-5-methyl-3(2H)-furanone and 5-[2-(2-furanyl)-ethenyl]-2-(2-furanylmethylene)-4-hydroxy-5-methyl-3(2H)-furanone, the reaction between 2-furancarboxaldehyde and 4-hydroxy-5-methyl-3(2H)-furanone produced four novel compounds containing two residues derived from the furanone structure.

Keywords: 2-furancarboxaldehyde; Maillard reaction; coloured compounds

INTRODUCTION

Although the development of colour is one of the two most important sensory consequences of the Maillard reaction in foods, the knowledge about the structures of the coloured compounds is still rather scarce, mostly because the major coloured compounds are melanoidins, polymers which appear to be particularly difficult to separate and purify. Only low molecular weight coloured compounds have been fully characterised (1–5).

The strategies to study this problems may involve the isolation of coloured compounds from foodstuffs or from mixtures obtained reacting amino acids (or proteins) and sugars or Maillard intermediates. Following the last approach, we decided to reinvestigate the reaction between 2-furancarboxaldehyde **1** and 4-hydroxy-5-methyl-3(2H)-furanone **2**, that had been studied for the first time by Ledl and Severin (6). They isolated and characterised 2-(2-furanylmethylene)-4-hydroxy-5-methyl-3(2H)-furanone **3**, one of the first coloured compound ever isolated, and 5-[2-(2-furanyl)ethenyl]-2-(2-furanylmethylene)-4-hydroxy-5-methyl-3(2H)-furanone **4**, deriving from a double condensation of 2-furancarboxaldehyde on 4-hydroxy-5-methyl-3(2H)-furanone **2.** Our interest was aroused by the fact that, when this work was started, none had been able to detect **4** in sugars/amino acids model systems.

EXPERIMENTAL

Reaction of furanone 2 and 2-furancarboxaldehyde 1 in anhydrous conditions. Compound **2** (0.88 mmol) was dissolved in ethanol (1.0 mL) and warmed at 50°C. In sequence 2-furancarboxaldehyde (0.70 mmol), piperidine (18 mL), and acetic acid (18 mL) were added and the mixture was reacted at 50°C for 2 h. After evaporation of the solvents, the residue was purified by flash chromatography using hexane/ethyl acetate from 6:4 to 2:8. In these conditions the following compounds were isolated: **3** (42.5 mg, 31.6 % yield), **4** (only trace), and, besides these expected products, four novel ones, **5a,b** (8 mg, 4 % yield), that appeared as a double peak with t_R 15.21, 15.40 min, and **6a,b** (10 mg, 5 % yield), a double peak with t_R 16.71, 16.90 min.

Reaction of furanone 2 and 2-furancarboxaldehyde 1 in water. Compound **2** (50 mg, 0.438 mmol) was dissolved in distilled water (22 mL) at pH 2 or 3, 2-furancarboxaldehyde (36.5 µL, 0.44 mmol, 1:1 ratio, or 146 µL, 1.75 mmol, 4:1 ratio) was added and the mixture was heated at 100°C under reflux for 2 or 4 h. The reaction mixtures were cooled and analysed in HPLC, without any treatment.

RESULTS AND DISCUSSION

Reaction of furanone 2 with 2-furancarboxaldehyde 1 in anhydrous conditions. In order to obtain samples of the coloured compounds **3** and **4** to be used as standards for quantification in model systems, the condensation of furanone **2** with 2-furancarboxaldehyde was firstly conducted in the presence of piperidine and acetic acid in ethanol. With the aim to minimise the formation of **4,** it was decided to use an excess of furanone **2**: after 1 h only a very small amount of **4** was formed and some novel compounds were detected. The first two **5a,b** appeared as a double peak with t_R 15.21 and 15.40 min, the other two **6a,b** as a double peak with t_R 16.71 and 16.90 min. Compound **5a,b**, and **6a,b** were separated by flash chroma-

tography on silica gel and their structures were assigned by NMR and MS experiments. As in HPLC, the ^{1}H-NMR and ^{13}C-NMR in $CDCl_3$ data indicated in each case the presence of two products in ratio 1:1, probably two isomers. Chemical shifts, coupling constants, one-bond and multiple bond correlations permitted to assign to compounds **6a** and **6b** and **5a** and **5b** the structure shown in the Figure. In both cases it was not possible to discriminate between compound **a** and **b**.

Reaction of 2-furancarboxaldehyde and 4-hydroxy-5-methyl-3(2H)-furanone 2 in water. In order to find the best conditions for the formation of compound **3** and **4** in water, solutions containing different molar ratios (1:1 e 4:1) of 2-furancarboxaldehyde and 4-hydroxy-5-methyl-3(2H)-furanone **2** were heated at 100°C. The experiments were performed at pH 2 and 3, where the formation of **4** should be favoured, because 2-furancarboxaldehyde is the major Maillard reaction product at low pH. HPLC analysis of the reaction mixtures without any purification or concentration, permitted to quantify **3, 4**, and the novel compounds **5a, 5b, 6a,** and **6b**. The conversion of the reagents in these conditions is not very high and traces of compound **4** were observed only after 4 h heating. The consumption of the starting substrates is faster at pH 2 than 3. At pH 2, compound **3** is formed more with a 4:1 than with a 1:1 reagent ratio, and continues to accumulate during further heating; at pH 3, on the contrary, compound **3** is formed more when the reagent ratio is 1:1, and tends to decrease at longer times. Either **5a,b** or **6a,b** are favoured by a lower pH. In respect to **5a** and **5b**, compounds **6a** and **6b** are increased by prolonging the heating time: this suggests the fact that **5a** and **5b** are spontaneously converted into **6a** and **6b** by air oxidation, which was confirmed on a pure sample of **5a,b** exposed to air in ethanol/water under stirring.

It seems unlikely that **5a, 5b**, and **3** derive from a common intermediate, because the double bond of **3** indicates that the elimination of water from the intermediate alcohol deriving from the condensation of furanone **2** and 2-furancarboxaldehyde is very easy. Therefore, a mechanism involving the condensation of two furanone rings as primary interaction seems more likely in the case of **5a** and **5b**.

It is not clear whether compounds **5** and **6** can be formed in Maillard model systems, even if we were not able to detect them in the ethyl acetate extracts of lysine/xylose and glycine/xylose model systems (7), their amounts could be very low or their peaks could be covered by other major components.

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POSTER PRESENTATIONS

A – Proteins and Saccharides

Influence of Irreversible Casein Crosslinking on the Gel Strength of Yoghurt

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Abstract

Irreversible casein crosslinking induced by incubation of skim milk with microbial transglutaminase or by storage of UHT skim milk, respectively, led to increasing breaking strength of yoghurts prepared from such pretreated milks. For yoghurts prepared from transglutaminase treated skim milk intermolecular formation of the isopeptide N^{ε}-(γ-glutamyl)-L-lysine exclusively explain the observed casein oligomerization which was measured by GPC. For caseinates isolated from stored UHT milk, however, the known crosslink amino acids lysinoalanine and histidinoalanine were only in part responsible for the measurable casein oligomerization. Significant amounts of currently unknown crosslinked amino acids must have been formed during storage which significantly influence the functional protein properties.

Keywords: yoghurt; casien crosslinging; UHT milk; cacin oligomerization

INTRODUCTION

Transglutaminase (EC 2.3.2.13) is an enzyme capable of forming inter- and intra-molecular crosslinks in many proteins (1, 2). In dairy products, casein has been shown to be a very good substrate for transglutaminase (3–5). Although the effects of transglutaminase on the functional properties of food proteins are obvious, very little information is available on the relation between casein oligomerization and isopeptide formation on the one hand, and the influence of this reactions on the functional properties on the other hand. Based on this consideration, further questions arose whether non-enzymatic crosslinking occurring during heating or storage of milk might also be relevant for the functional properties of casein. Currently, no information is available on the relationship between the functional properties of food proteins and the chemical and molecular changes occurring during certain posttranslational modifications. To obtain further insights into the corresponding structure-function relationships, the purpose of our study was to correlate the yoghurt/gel-forming properties of caseins with the chemical and molecular changes induced by microbial transglutaminase as well as the irreversible (non-reducing) oligomerization of casein occuring during storage of UHT milk.

EXPERIMENTAL

Chemicals. All chemicals were of highest purity available.

Hydrolysis. Casein samples were hydrolysed either with 6N HCl for 23 h at 110°C or with a set of four enzymes as described previously (6).

Amino acid analysis. This was performed according to (6), using an Alpha Plus amino acid analyser (LKB Biochrom, Cambridge, UK).

Gel-permeation chromatography under dissociating and reducing conditions. This was performed according to (7).

Texture analysis. The yoghurts prepared from fresh or stored skimmed UHT milk as well as from enzymatically treated raw skim milk were investigated by a Stevens-LFRA Texture Analyser (Wiedmayer Meßtechnik, Leonberg, Germany) as described in (7). Breaking strength was evaluated from the force at rupture and expressed as N.

Modification of skim milk with transglutaminase (TG) and preparation of yoghurt. Raw skim milk was incubated with 3.0 U transglutaminase/g protein at 40°C for up to 120 min. After TG-treatment, the milk samples were heated at 80°C for 2 min for inactivation of the enzyme and denaturation of whey proteins (8). One part of the modified milk was used for the production of skim milk yoghurt by adding yoghurt culture (7). From the second part of the TG-treated skim milk, casein was isolated by acid precipitation as described in (9). Skim UHT milk was stored at 37°C for different times. After storage, yoghurt was produced (7) and casein was isolated (9).

RESULTS AND DISCUSSION

In order to obtain information on the degree of enzymatically and non-enzymatically induced casein crosslinking during treatment of skim UHT milk, casein was isolated by acid precipitation from milk after a certain time of incubation resp. storage and fractionated by gel-permeation chromatography (GPC) under reducing and dissociationg condition, thus enabling the measurement of protein oligomerization caused by irreversible (non-reducible) covalent bonds (9). As can be seen from Table 1 casein oligomerization increased during TG-treatment up to 120 min from 10% up to 25%, which was comparable with the casein oligomerization measured for skimmed UHT milk, stored at 37°C up to 27 d. Breaking strength of yoghurt prepared from stored or TG-treated milk increased with increasing time of TG-treatment or storage, correlating with casein oligomerization. Breaking strength of TG-treated skim milk yoghurts increased from 5.5 N up to 9.5 N, whereas yoghurts produced from stored skim UHT milk showed an increase in breaking strength from 2.7 N up to 5.6 N.

Based on the data of casein oligomerization, the minimal concentration of crosslinked amino acids ($[CLAA]_{min}$), necessary to explain the casein oligomerization measured by GPC, could be calculated according to (9). As can be seen from Table 2 $[CLAA_{min}]$ increased with increasing time of enzymic pre-treatment or storage. For TG-treated skim milk, the isopeptide N^{ε}-(γ-glutamyl)-L-lysine, measured via amino acid analysis after enzymic hydrolysis increased in concentration with the extent of casein oligomerization and was in good agreement with $[CLAA_{min}]$. Thus, it can be concluded that isopeptide links

Table 1. Irreversible oligomerization of casein isolated from TG-treated milk or stored UHT-milk and breaking strength of yoghurt prepared therefrom

TG-treated skim milk			UHT-milk, stored at 37°C		
Incubation time [min]	Oligomerized casein [%]	Breaking strength [N]	Storage duration [d]	Oligomerized casein [%]	Breaking strength [N]
0	10	5.5	Fresh UHT milk	14	2.7
10	16	6.2	3	16	3.7
20	18	6.8	7	19	4.6
40	23	7.5	10	21	4.9
60	24	9.1	14	22	5.6
90	25	8.9	20	23	5.7
120	25	9.5	27	25	5.6

Table 2. Comparison between measured amounts of crosslinking amino acids [N^{ε}-(γ-glutamyl)-L-lysine (GluLys), LAL, HAL] and calculated concentration of minimally formed crosslinked amino acids [$CLAA_{min}$]

TG-treated skim milk			UHT-milk, stored at 37°C		
Incubation time [min]	GluLys [mmol/100 g protein]	$[CLAA_{min}]$ [mmol/100 g protein]	Storage duration [d]	LAL plus HAL [mmol/100 g protein]	$[CLAA_{min}]$ [mmol/100 g protein]
0	0.26	0.27	Fresh UHT milk	0.03	0.39
10	0.27	0.38	3	0.06	0.45
20	0.32	0.42	7	0.09	0.57
40	0.53	0.60	10	0.09	0.59
60	0.57	0.61	14	0.12	0.62
90	0.58	0.66	20	0.13	0.66
120	0.57	0.65	27	0.15	0.65

were predominantly formed *intermolecularly*, leading to protein crosslinking and corresponding changes of functional properties.

For caseinates isolated from stored UHT-milk, only lysinoalanine (LAL) and histidinoalanine (HAL) could be detected and quantified via amino acid analysis after acid hydrolysis. An increase of the LAL- and HAL-concentration depending on the storage duration was noticeable (Table 2), correlating with increasing breaking strength and casein oligomerization. Comparing the measured concentration of LAL and HAL with the calculated minimal concentration of crosslinked amino acids, however, it was evident that only 8–27% of the non-reducible casein oligomerization observed after storage of UHT milk could be explained by the intermolecular formation of LAL and HAL. Thus, significant amounts of currently unknown crosslinks must have been formed, which show a remarkable influence on the functional properties of casein.

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Irreversible Casein Oligomerisation and Formation of Fluorescent Crosslink Amino Acids in Dairy Products

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Abstract

Irreversible oligomerisation of casein isolated from commercial dairy products was measured using size-exclusion-chromatography (SEC) with simultaneous UV and fluorescence detection. The dehydroalanine-derived crosslink amino acids lysinoalanine (LAL) and histidinoalanine (HAL) could only in part explain the irreversible casein oligomerisation, which correlated with intensity of heat treatment. Furthermore, in the chromatograms obtained by SEC, an increase in the fluorescence intensity (λ_{ex}= 360 nm, λ_{em}= 410 nm) correlating with heat treatment and carbohydrate concentration could be observed in the oligomeric fraction of the casein samples. Therefore we conclude that fluorescent Maillard reaction products are in part responsible for the casein oligomerisation.

Keywords: casein; crosslinking; lysinoalanine; histidinoalanine; fluorescence

INTRODUCTION

During heating and storage of dairy products, inter- and intramolecular crosslinking of proteins may significantly affect the technological as well as the nutritional properties of the final product. Except for reducible disulphide bridges caused by crosslinking of protein-bound cysteine residues, non-reducible protein oligomerisation occurs by the formation of isopeptides (1), dehydroalanine derivatives like lysinoalanine (LAL) and histidinoalanine (HAL) (2) and Maillard products (3–5). Although a lot of efforts have been made to identify crosslink amino acids in model systems, the nature of protein polymerisation in foods can not be explained satisfactory. The aim of our study was, to investigate the extent of irreversible (non-reducible) casein oligomerisation in dairy products, and to identify and quantify individual protein-bound crosslinking amino acids.

EXPERIMENTAL

Milk samples: All samples were obtained from local retail stores.

Isolation of casein: Casein was prepared by acid precipitation according to (6).

Size-exclusion-chromatography (SEC): This was performed under reducing and dissociating conditions as described in (7). Eluate was monitored first with a UV-detector at 280 nm and subsequently with a fluorescence detector set at λ_{ex}= 360 nm and λ_{em}= 410 nm.

Amino acid analysis: Amino acid analysis was performed after acid hydrolysis as described in (7, 8).

RESULTS AND DISCUSSION

Non-reducible protein oligomerisation of casein samples isolated from dairy products was measured by SEC in the presence of 6M urea and dithiothreitol. The amount of irreversible casein oligomerisation for every sample was calculated from the elution patterns. Casein from raw and pasteurized milk showed no oligomerisation. For more severely heated samples an oligomerisation up to 52% was measured (Table 1). For all samples lysinoalanine and histidinoalanine were quantified using amino acid analysis after acid hydrolysis. The amount of LAL and HAL correlated with heat treatment and casein oligomerisation.

Based on the results of SEC analysis, the minimum concentration of crosslink amino acids $CLAA_{min}$ can be calculated, which is at least necessary to explain the measured casein oligomerisation (9). As can be seen from the comparison of $CLAA_{min}$ with the sum of LAL and HAL, it is evident that the amount of known crosslink amino acids is not sufficient to explain the observed casein oligomerisation of the samples of evaporated milk. For these samples significant amounts of currently unknown crosslink amino acids must be present.

In the chromatograms obtained by SEC, a significant increase in the fluorescence intensity could be observed for the oligomeric fractions (Fig. 1), correlating with heat treatment and carbohydrate concentration.

Table 1. Irreversible casein oligomerisation and crosslink amino acids in dairy products (n.d., not detectable)

Product	Irreversible casein oligomerisation [%]	$CLAA_{min}$ [µmol/100g]	LAL plus HAL [µmol/100g]	Fluorescence-index FI
Raw milk	n.d.	n.d.	n.d.	40–45
Pasteurized milk	n.d.	n.d.	n.d.	50–55
UHT milk, fresh	6–10	180–310	170–245	60–70
Evaporated milk, heat treated	25–33	750–1000	290–630	150–200
Evaporated milk, sterilised	36–52	1100–1700	670–1140	260–350

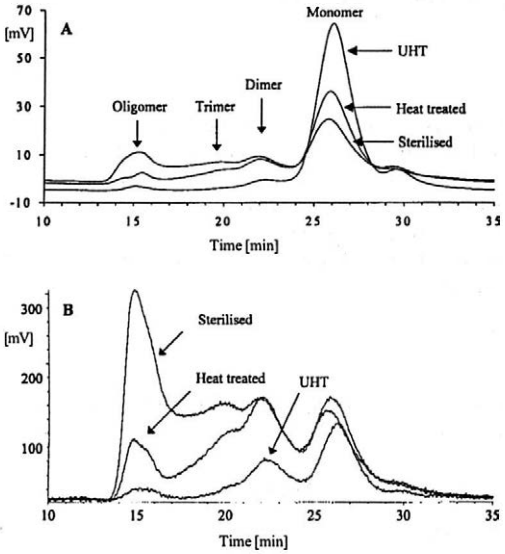

Figure 1. SEC chromatograms of caseins from milk samples (UHT-heated, heat treated and sterilised evaporated milk), obtained via A) UV-detection (λ = 280 nm) and B) fluorescence detection (λ_{ex}= 360 nm, λ_{em}= 410 nm)

Fluorescence intensity as described by the "fluorescence index", which is the ratio of peak areas integrated for identical regions in the fluorescence versus the UV-chromatogram, increased with heating intensity and extent of casein oligomerisation (Table 1). For casein samples heated in the absence of lactose, non-fluorescent oligomers were detected. Based on this observations the formation of fluorescent crosslink amino acids originating from the Maillard reaction must in part be responsible for the casein oligomerisation found in dairy products. Studies on the isolation and identification of this unknown crosslinks are currently in progress.

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Immunochemical Probes for Monitoring Changes in Casein Structure

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Abstract

Simple and sensitive enzyme immunoassay (ELISA) techniques were developed for determination of casein fractions and peptides. Ten different antibodies (against three native and three thermally treated casein fractions, and against four peptides from molecule of α-casein and β-casein, respectively) have been used to recognize changes in immunoreactivity of caseins during different thermal and proteolytic treatment of buffer solution and milk. The explanation of all features which were found is complicated. Generally, below 100°C no significant changes in casein immunoreactivity have been observed. At 100–140°C an increase and a following decrease of immunoreactivity was observed depending on the holding time. Depending on type of proteolytic treatment the casein immunoreactivity can break down, but also can increase even 10 times.

Keywords: ELISA; immunoassay; casein; antibodies; heat-treatment

INTRODUCTION

In view of the impact, that heat and proteolytically-induced changes of caseins have on the final quality of processed milks and dairy products, there is a need to understand the nature of these changes at a molecular level, and to develop effective means of monitoring and controlling these changes during production. To date researchers have made extensive use of electrophoretic (1) and chromatographic procedures (2) to quantify caseins and their peptide parts, in addition to investigating proteolytic degradation of these proteins in dairy products or for determining variation in milk protein composition. Some authors also tried to use these techniques to detecting heat-induced changes in caseins (3).

An obvious alternative technique, gaining in popularity for quality assurance in the food industry, is immunoassay. Although immunoassays (particularly ELISA) with anti-casein antibodies have been developed for the analysis of milk and milk products with a view to monitoring adulteration with cows milk, identification and quantification of caseins after chromatographic or electrophoretic separation (immunoblot), none have been focused on issues of quality. Recently, we have investigated the use of anti-whey-proteins immunoprobes to this purpose. Antibodies have been produced which can detect thermal changes in α-lactalbumin and β-lactoglobulin (4, 5), thus demonstrating the feasibility of applying antibody methods to the analyses of thermally and proteolytically induced changes in dairy proteins. In addition these methods can be used to investigate foods and not just model systems. Here we present similar experiments with antibodies against caseins.

EXPERIMENTAL

Antibodies

Polyclonal rabbit antisera were raised against ten immunogens three native and three thermally treated casein fractions (whole casein, κ-casein, and mixture of α-casein with β-casein), and four nonimmunogenic short peptide parts of α_{s1}-casein and β-casein (EVLN, NENLL, APFPQVF, PFPGPIPNS) coupled to a carrier protein, respectively by subcutaneous injection, intramuscular reinjection and intravenous booster. From each antiserum the IgG fraction was isolated by means of affinity chromatography on protein A-Prosep column.

ELISA

The indirect competitive enzyme immunoassay (ELISA) has been optimised for each of mentioned caseins and peptides. Generally, microplate was coated with casein or peptide-conjugate. Then antibody and standard or sample were added and incubated 1 h. Next, plate was incubated for 1 h with peroxidase-labelled swine anti-rabbit immunoglobulins. Finally, peroxidase activity, bonded to microplate through immunoreactant, was determined by standard *o*-phenylendiamine procedure.

This format shows satisfactorily good results for all antigens tested. The detection limits are enough low, which allows to determine caseins and their fragments in milk at high dilutions.

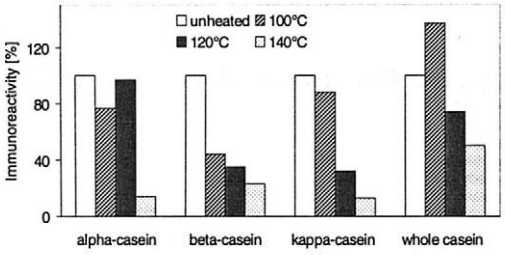

Figure 1. Effect of heat-treatment at different temperatures on the immunoreactivity of caseins with antibodies against native caseins

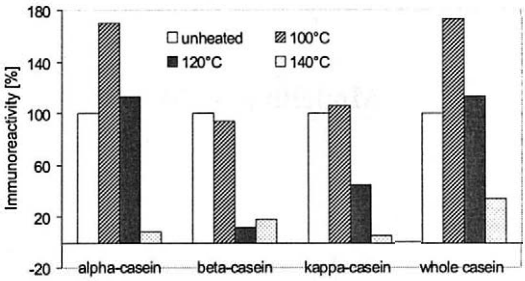

Figure 2. Effect of heat-treatment at different temperatures on the immunoreactivity of caseins with antibodies against heat-treated caseins

RESULTS AND DISCUSSION

The indirect ELISA format was used to follow changes of pure caseins immunoreactivity during heat-treatment. Antisera against both native and pasteurised whole caseins have proportionally low cross-interactions with other caseins (α, β, and κ). Antisera against both native and pasteurised -caseins interact with all other caseins in the approximately same extend with exception of pasteurised α+β-caseins, which had only 6% immunoreactivity. Antiserum against native α+β-caseins interacts much better with whole casein and with pasteurised α+β-caseins than with own immunogen.

During heat-treatment of casein fractions in buffer solution at temperatures below 100°C no significant changes in casein immunoreactivity have been established using any above mentioned antibody. The increase of temperature above 95°C causes not only the decrease of immunoreactivity (Fig. 1 and 2). The changes observed are not the same using antibodies against native caseins in comparison with antibodies against heat-treated caseins (compare Fig. 1 and Fig. 2).The immunoreactivity also significantly depends on the duration of heat treatment. The results showed how complicated are the conformational changes of caseins in respect of time and temperature during thermal denaturation.

One-hour-treatment with proteolytic mixture Pancreatin decreased the immunoreactivity of whole casein with anti α+β-casein antibody and with anti κ-casein antibody 13 times and 75 times, respectively. On the other hand, proteolytic treatment of cultures *Lactobacillus helveticus* and *Lactococcus II* increased the whole casein immunoreactivity with anti α+β-casein antibody 10 times and 6 times, respectively. However, using anti α+β-casein antibody the slight decrease of casein immunoreactivity by 20% and 5% has been observed.

The explanation of all features which were found is complicated. It is necessary remember the complexity of the system used. We used polyclonal antibodies with broad population of immunoglobulins which have different affinities to undefined number of epitopes. The number of actually available epitopes is variable, because during the heat treatment the inner molecule epitopes are manifested on the surface of molecule. Also conformational epitopes change their immunoreactivity.

In milk samples the epitope presentation is influenced by other compounds present in this matrix. Using all prepared antibodies a range of heat-treated milks (pasteurised, UHT and sterilised) and infant milk formulae will be tested to determine a difference in immunoreactivity comparing with raw milk.

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Modelling of Modified Atmosphere Packaging of Meat

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Abstract

A dynamic model of fresh beef packaged under modified atmospheres is presented. This model links the change in meat color with the permeation of gases (O_2, N_2, CO_2) through the packaging film. Its viability is demonstrated with experimental data using muscle semimembranosus with which it was shown that color change of the meat surface was well described by the model. Moreover, the model parameters were obtained independently of the model itself through the literature. A major result of the model showed that surface color is independent of oxygen concentration above 5%. Moreover, under aseptic conditions and ambient atmosphere concentrations, shelf-life of muscle semimembranosus was shown to be 11 days with this value being a minimum.

Keywords: meat color; modeling; MAP

INTRODUCTION

Fresh meat color is defined by the relative amount of three derivatives of myoglobin. Reduced myoglobin is the purple pigment of deep muscle. On exposure to air, myoglobin combines with oxygen to form bright red oxymyoglobin. However, in the presence of oxygen, myoglobin oxidizes to brown metmyoglobin. These color changes are a major concern to the food industry. Consumers associate the quality of a meat product almost exclusively with color. However subjective this criterion is, it is primary judgement by which one meat product is chosen over another.

Improved meat distribution methods may eventually lead to consumer sized portions shipped prepackaged to the retail outlet, eliminating the need for butchering and packaging at the retail outlet itself. Color must therefore be maintained for longer periods of time to allow for shipment. The current popular method for color maintenance is modified atmosphere packaging (MAP). To maximize color duration using MAP, the proper modified atmosphere composition and packaging film for a given muscle type must be chosen. At present these choices are made through long trial and error experimentation.

The overall objective of our study is to predict the surface color of meat packaged under modified atmospheres at any time during storage. The model is deterministic, incorporating the kinetics of myoglobin linked to the dynamics of the headspace gas composition. It is known that at a level of 20% metmyoglobin the ratio of sale of red meat to discolored meat is 2:1 (5) and at 50% the meat is totally unacceptable (8). The model may thus be used to quickly examine a wide variety of MAP schemes to maximize the time before such levels of metmyoglobin occur.

MATERIAL AND METHODS

Model equations: A mathematical model of modified atmosphere packaging of meat describes the changes occurring in the head space above the meat and how those changes impact color of the meat surface.

Myoglobin kinetics are represented by:

$$[\text{MetMb}] \xleftarrow{K3} [\text{Mbo}_2] \xleftarrow{K1,K2} [\text{Mb}] \xrightarrow{K3} [\text{MetMb}]$$

Where Mb, MbO_2 and MetMb denote reduced, oxygenated and oxidised forms of myoglobin, repectively.

Headspace dynamics are presented by an unsteady mass balance:

$$\frac{V}{RT}\frac{dp_i}{dt} = -NiS \qquad \text{and} \qquad N_i = -P_i * \Delta p_i$$

where: V is the head space volume, R is the universal gas constant, T is the temperature, S is the surface area of meat, p_i is partial pressure of component I, t is time and N_i is the flux of component I through the film, P_i is the permeability of component I and Δp_i is partial pressure gradient across the package

When the kinetic equation are written separately for each myoglobin fraction and the headspace dynamics written for each atmospheric component, a set of six ordinary differential equations result. The kinetics and headspace dynamics are coupled through Henry's Law whereby apo_2 is substitued for o_2 in the kinetic equations. Thus we can

write the final form of the model for all gases and fractions of myoglobin considered as:

$$\frac{V}{RT}\frac{dpo_2}{dt} = Po_2 \Delta po_2 \quad (1)$$

$$\frac{V}{RT}\frac{dpco_2}{dt} = Pco_2 \Delta pco_2 \quad (2)$$

$$\frac{V}{RT}\frac{dp_{N2}}{dt} = P_{N2} \Delta p_{N2} \quad (3)$$

$$\frac{d[Mb]}{dt} = -\alpha K_1 [Mb] po_2 + K_2 [Mbo_2] - K_3 [Mb] \quad (4)$$

$$\frac{d[Mbo_2]}{dt} = \alpha K_1 [Mb] po_2 - K_2 [Mbo_2] - K_3 [Mbo_2] \quad (5)$$

$$\frac{d[MetMb]}{dt} = K_3 ([Mb] + [Mbo_2]) \quad (6)$$

The viability of the model was demonstrated with four experiments, in each one type of packaging film and a mixture of gases (%O_2, %CO_2, %N_2) were used. Muscle semimembranosus was used aseptically in al experiments.

Gas analysis: The head space composition was analysed using a 3300 Gas chromatograph (Varian) with a CTR-1 column.

Color measurement: Meat color was determined with Beckman DU spectrophotometer using the method developed by Dean and Ball (3).

RESULTS AND DISCUSSION

1. A model has been developed for following the color determinants of meat packaged under modified atmospheres and its viability demonstrated with experimental data (Figs. 1 and 2).

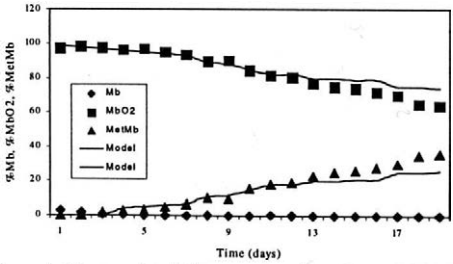

Figure 1. Changes in relative concentration of myoglobin derivatives during aerobic storage 20% O_2 at 2°C (Film: E.V.V.C)

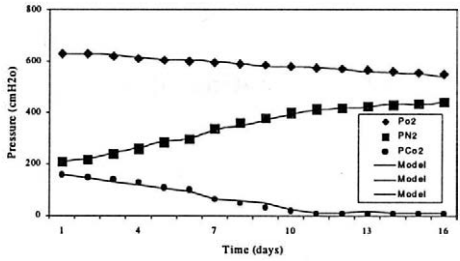

Figure 2. CPressure of O_2, NO_2 and CO_2 inside the package (Film: E.V.V.C)

2. Myoglobin kinetics, as described under MATHEMATICAL MODEL, appear adequate in defining the changes in the relative amounts of the three myoglobin fractions. The model appears to adequately describe headspace dynamics; with assumption made that component loss to the meat was negligible, and the concentration at the meat surface should approach values found in the atmosphere.

3. Myoglobin kinetics indicate that temporal color change is independent of the oxygen concentration. When this is combined with the work done by George and Stratmann (4), oxygen concentrations above 4% do not affect color. This contradicts literature data (1, 2, 6, 7) which indicate a definite color dependency on oxygen concentration.

Meat color deterioration is attribute exclusively to the oxidation of meat pigment. Thus the shelf-life of meat is governed by this.

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Determination of *o*-Tyrosine as a Marker for the Detection of Irradiated Shrimps

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Abstract

o-Tyrosine is proposed as a marker for the identification of irradiated protein-rich food. An HPLC method for qualitative and quantitative determination of non-protein bound *o*-tyrosine in shrimps *(Crangon Crangon)* has been developed. For this purpose the *o*-tyrosine was extracted from non-irradiated as well as irradiated samples with perchloric acid, then separated isocratically (ammoniumformiat buffer, pH 4) on an RP-C18 column and detected by FLD (275/305 nm). The quantification of *o*-tyrosine was based on the use of α-methyl-*p*-tyrosine as internal standard. In non-irradiated shrimps a background level of 28.9 μg/kg was found. The content of *o*-tyrosine in 1 kGy irradiated shrimps was found to be 119.9 μg/kg, which was well 4-fold over the background level. The dependency between radiation dose and the amount of *o*-tyrosine was observed in the range of 0–5 kGy.

Keywords: food irradiation; shrimps; *o*-tyrosine; High performance liquid chromatography; fluorescence detection

INTRODUCTION

The use of radiation for food conservation is in a wide use in 41 countries. For example the highly perishable shrimps can be irradiated up to doses of 5 kGy in France and up to doses of 4.5 kGy in the Netherlands (1). Because of the increasing world-wide acceptance of food irradiation and the latest European Community legislation (2), official food surveillance demands reliable detection methods for irradiated food. Although numerous methods have been published (4–10), none of them has been validated for shrimp meat or its products. If phenylalanine is treated with ionising radiation in the presence of water and oxidants, three tyrosine isomers (*p*-, *m*- and *o*-tyrosine) are formed (3). Based on the assumption that *m*- and *o*-tyrosine are unique radiation products and since *o*-tyrosine is easier to separate from *p*-tyrosine, Karam and Simic (5) proposed the use of *o*-tyrosine as a potential marker for the detection of radiation-processed food. Numerous methods have been published which determined protein-bound *o*-tyrosine in protein-rich foods by means of HPLC/FLD after acid hydrolysis (6–10). Nevertheless the method has not been used in routine applications because of a variety of problems, which occurred. The *o*-tyrosine content of irradiated shrimps (1–3 kGy) was not always significantly different from the background level of unirradiated samples. The acid hydrolysis led to the formation of numerous fluorescent substances that disturbed the selective determination of *o*-tyrosine (10). A method for the determination of *o*-tyrosine, in which the acid hydrolysis can be omitted, was developed. This method is based on the fact that free amino acids, such as phenylalanine, are present in shrimps (11). During irradiation *o*-tyrosine is formed from the free phenylalanine as well as from the phenylalanine bound in proteins. Recently published methods (12, 13) have shown that the determination of the non-protein-bound *o*-tyrosine in irradiated shrimps is possible. The aim of this study was to validate a reliable method for the determination of non-protein-bound *o*-tyrosine in order to distinguish between irradiated and unirradiated shrimps. Additionally the formation of *o*-tyrosine should be investigated when the shrimps were irradiated either in frozen state (–18°C) or in fresh state (ambient temperature).

EXPERIMENTAL

Material

Samples. Fresh common shrimps (*Crangon crangon*) were obtained at a local fish market. They were peeled by hand in the laboratory, vacuum packed in polyethylene pouches and refrigerated until irradiation or analysis.

Irradiation. Irradiation at room temperature with target doses of 1, 3 and 5 kGy was carried out using a blood irradiation plant (137Cs, dose rate 0.7 kGy/h, Beiersdorf, Hamburg, Germany) and irradiation at –10°C up to –18°C using irradiation plant of Gammaster International B.V., Ede, the Netherlands (60Co, dose rate 1.2 kGy/h).

Apparatus. HPLC-FLD-System-Autosampler: Merck-Hitachi, L 655 A; pump: Merck Hitachi, L 6200 A; fluorescence detector: Merck-Hitachi, F-1080; thermostat: LKB Bromma 2155; data were acquired and calculated by Kroma

System 2000 (Bio-Tek Kontron Instruments); Ultra-Turrax: IKA-Labortechnik, T25; pH-Meter: WTW, pH DIGI 520.

Chemicals. DL-*o*-Tyrosine approx. 98% (Sigma), α-methyl-DL-*p*-tyrosine (Sigma), acetonitril gradient grade (Fluka), ammonia solution 25% (Merck), formic acid 98–100% (Merck), perchloric acid 60% (Merck).

METHOD

For the determination of *o*-tyrosine, 1 mL 6 % perchloric acid and 1 mL α-methyl-DL-*p*-tyrosine (435 µg/L) were added to 2 g of shrimps in a centrifuge tube. After homogenisation by an Ultraturrax tissue the mash was centrifuged at 10 000 g and 4°C, for 20 min. The supernatant was filtered through a 0.45 µm membrane filter. Of the filtrate, 20 µL were injected into the chromatographic system and the individual compounds were eluted isocratically from the 250 × 4.0 mm i.d. Multospher AQ 120 5C18 (CS, Langerwehe, Germany). The isocratic elution was achieved using a 0.005M ammoniumformiat buffer, pH 4 without an organic modifier. Before each injection the column was flushed with 0.005M ammoniumformiat buffer/acetonitrile (30:70 v/v). The system was run at 35°C and a flow rate of 0.9 mL/min. *o*-Tyrosine was detected fluorimetrically (α_x = 275 nm, α_{em} = 305 nm). Separation of one sample took 55 min, including flushing and equilibrating of the column. For assessing the dose-yield relationship, shrimps irradiated with 1 to 5 kGy and unirradiated shrimps were used.

RESULTS AND DISCUSSION

The quantification of *o*-tyrosine was based on the use of α-methyl-*p*-tyrosine as internal standard. In non-irradiated shrimps a background level of 28.9 µg *o*-tyrosine/kg (n = 3) and 31.3 µg *o*-tyrosine/kg (n = 3) was determined in accordance to Hein et al. (12) (19.3 µg/kg) and Krach et al. (13) (21.2 µg/kg). The content of *o*-tyrosine in shrimps irradiated with a dose of 1 kGy at room temperature was found to be 119.9 µg/kg (n = 3), which was well 4-fold over the background level and as well as in 1 kGy at frozen state irradiated shrimps (121.2 µg/kg, n = 5). The proportional dependency between irradiation dose and the amount of *o*-tyrosine was observed in shrimps irradiated at room temperature (n = 3) in the range of 0-5 kGy and it could not be observed in shrimps irradiated at frozen state (n = 5) (Figs. 1–3). The *o*-Tyrosine content in the samples irradiated at frozen state varied much more than in the samples irradiated at fresh state.

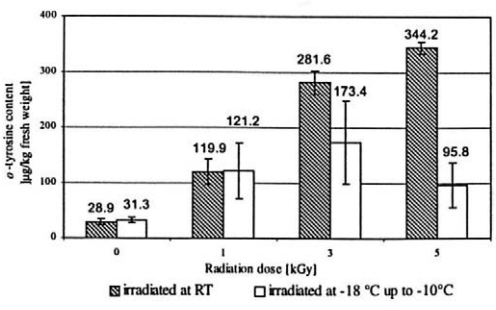

Figure 1. *o*-tyrosine content in shrimps irradiated at RT and at −18°C up to −10°C

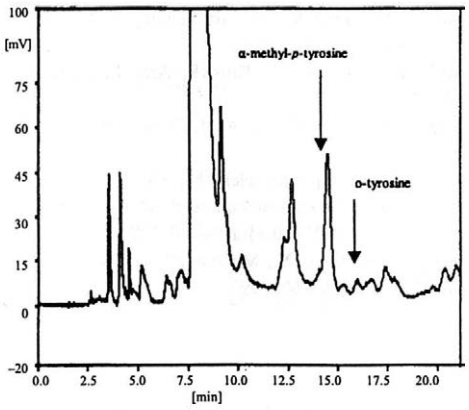

Figure 2. HPLC chromatogram of shrimpextract, unirradiated, FLD at 275/305 nm

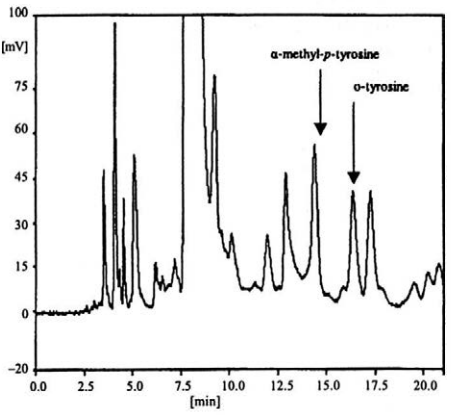

Figure 3. HPLC chromatogram of shrimpextract, irradiated with 5 kGy, FLD at 275/305 nm

An advantage of the developed method is that it is more rapid and simpler than methods previously reported (5–10). To the authors knowledge the formation of *o*-tyrosine in food samples that were frozen during irradiation has not been proved before.

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Changes of Trypsin Inhibitor Activity during Microwave Treatment of Germinated Pea

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Abstract

The method by means synthetic substrate BAPNA (N-α-benzoyl-DL-arginin-p-nitroanilid) was used for trypsin-inhibitor activity (TIA) determination in pea seeds. To the extraction of proteins is used glycine buffer pH 11 containing urea and EDTA, 2 h stirring and centrifugation. Synthetic substrate BAPNA is hydrolysed by trypsin and the absorbance of dislodged yellow *p*-nitroanilid is measured at 410 nm. The changes of TIA during germination and the following microwave treatment and the conventional drying of germinated pea to the final moisture 12–14% were evaluated.

Keywords: trypsin inhibitor activity; pea; germination; microwave treatment

INTRODUCTION

Trypsin-inhibitor activity (TIA) belongs between the most important antinutritional factors in grain legumes since decreases the utilisation of protein content and therefore reduces the possibilities of grain legumes use in human and animal nutrition. Protease inhibitors present in legume seeds belong to the family of Bowman-Birk inhibitors, which show more resistance to heat than the others (1, 2). The level of antinutritional factors can be partially decreased by various processing techniques. Fermentation, germination and the following hydrotermic treatment can reduce antinutritional effect and cause the important positive changes in the biochemical, nutritional and sensory characteristics of the grain legumes seeds (3–5).

EXPERIMENTAL

Material: Samples of pea (*Pisum sativum* ssp. *sativum* L.), nine cultivars (Gotik, Zekon, Merkur, Menhir, Lantra, Grana, Komet, Profi, Sonet) from various breeding farms in Czech Republic, years of harvest 1997–1999. Samples of pea were supplied by Central Institute for Supervising and Testing in Agriculture at Brno.

Methods: Dry pea seeds were finely ground (10–20 μm). 1 g of pea flour sample was suspended in 50 ml of glycine buffer pH 11 with urea and EDTA at ambient temperature under continuous stirring for 2 h. Suspension was then centrifuged 10 min at 3000 rpm. Supernatant was diluted by distilled water in relation 1:1. Solution of sample and individual solutions of reagents were pipetted into the test tubes and incubated 10 min at temperature 37°C according to scheme in Table 1. Enzyme reaction is stopped by adding 1 ml 30% acetic acid. Absorbance of yellow colour of dislodged p-nitroanilid was measured at 410 nm against distilled water. The colour of yellow *p*-nitroanilid is fast some hours.

Calculation of TIA: Values of activity Ac (%) are calculated from measured values of absorbance according the following expression

Table 1. Scheme of pipetting

Number of test tube [μl]	Extract [μl]	Water [μl]	Trypsin [μl]		Substrate [ml]		30% acetic acid [ml]
1	20	80	100	Incubation 10 min.	1	Incubation 10 min.	1
2	30	70	100		1		1
3	40	60	100		1		1
4	60	40	100		1		1
5	80	20	100		1		1
6	–	100	100		1		1

$$Ac_{20} = (A_{20} - A0_{20})/(A_0 - A0_0),$$

where: suffixes 20 and 0 correspond to volume of extract

Theoretical volume of extract (x), which should quite inhibited of 4 μg trypsin activity was determined from the dependence Ac = f (V). This theoretical volume is specified as the point of intersection of axis V and tangent to the curve Ac = f (V) coming through the point Ac = 100.

TIA (trypsin inhibition activity) is defined as amount of pure trypsin (mg), which is inactivated by 1 g of sample.

$$TIA = \frac{V * i}{x}$$

where: V – total volume of extract (μl)
I – amount of trypsin in test (mg)
X – theoretical volume of extract for complete inhibition of i (μl)

Germination: 380 g of pea seeds were put out on the tray (dimensions 34 × 26 cm) into one layer and washed down by 300 ml of distilled water. Time of germination was 72 hours at ambient temperature.

Microwave treatment and drying: Wet germinated pea was heated in microwave oven Whirlpool MT 243/UKM 347, power output 350 W was stopped at 80°C. Time of microwave heating was from 1 to 4 min. Part of microwave treated germinated pea was then dried in laboratory fan assisted dry air oven at 80°C to final moisture content 12–14%.

RESULTS AND DISCUSSION

The goal of this study was the verification of method for determination of TIA in pea by means of synthetic substrate BAPNA and to evaluate the influence of germination and microwave heating on changes of TIA in pea seeds. The method was adapted according to the methodology of Central Institute for Supervising and Testing in Agriculture, Brno.

It was monitored nine cultivars of pea from various breeding farms harvested in 1997–1999 years. The highest average content of TIA was determined for cultivar Gotik (8.68 mg), while the lowest values had cultivar Zekon (3.27 mg). The others cultivars have TIA from 4 to 6 mg, as there are cultivars with medium TIA.

Changes of TIA during germination, microwave treatment and drying are demonstrated on the cultivar Gotik

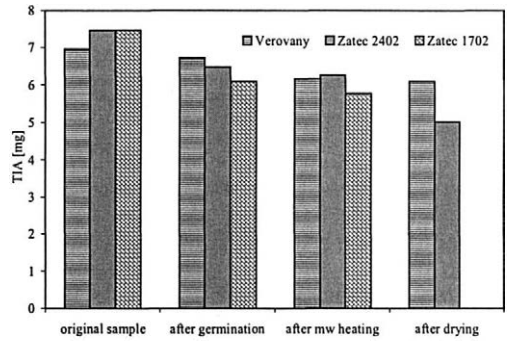

Figure 1. Changes of TIA during germination, microwave heating and drying Cultivar Gotik

(Fig. 1). The highest decrease of TIA was observed after 3 days of germination (13–18%). The single effect of microwave heating up to 80°C was reduction of TIA from 3.4 to 8.4%. By means of combination effect of germination and microwave treatment is possible to improve nutritional quality of pea cultivars with high content of TIA, as is cultivar Gotik.

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Interactions between Gellan Gum and other Polysaccharides

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Abstract

Mixed gelling systems of gellan with other polysaccharides were prepared in order to obtain different textures and to investigate the possible existence of synergistic or incompatibility effects. Among the systems showing synergistic effects, the gellan/ι-carrageenan mixtures seem to be interesting with regards to the gel characteristics obtained in comparison with those of gelatin.

Keywords: gellan; interactions; synergy; polysaccharides; gelatin

INTRODUCTION

Gellan gum produced by *Pseudomonas elodea* is a relatively new polysaccharide developing gel properties in the presence of cations (1).

Using this gum, a wide range of textural properties gels, from fairly soft and non brittle to firm and very brittle, can be obtained with regards to the ionic strength and type of counter ions (2).

Moreover, composite gels are made using gellan with other polymers (3).

In this work, mixed gels of gellan with other polysaccharides were prepared in order to obtain different textures characterised essentially by the hardness, cohesiveness and elasticity and to investigate the possible existence of synergistic or incompatibility effects.

Among the promising applications of gellan or gellan-polysaccharides mixtures is the replacement of gelatin in dessert and confectionary formulations.

Samples developing synergy between the two polysaccharides were characterised with regards to their gel properties.

EXPERIMENTAL

Materials

- Gellan sample (KELCOGEL F) was in a deacylated form and supplied by Nutrasweet Kelco.
- Xanthan (GFS, containing galactomannan) and alginate (Lacticol CFT, Lacticol CMG) Kelco) were from Nutrasweet Kelco. ι-carrageenan (Gelcarin GP 3367) was from FMC.

Methods: Gelling agent solutions were prepared at different concentrations and different proportions. Samples were heated at 85°C in stirred beaker and maintained at this temperature for 5 minutes. $CaCl_2$ was eventually added 20 s just before the end of the temperature upholding.

Texture Profile Analysis (TPA): Texture characteristics were analysed by stable micro system (SMS) TA.XT2 texturometer using a probe of 30 mm diameter with a 3 mm compression depth at compression rate 2.0 mm/sec.

RESULTS AND DISCUSSION

Different gellan based composites were studied with ι-carrageenan, xanthan and alginate as second polysaccharides.

Gellan and ι-carrageenan combination allowed us to obtain a wide variety of gels, as illustrated by the main TPA parameters (Table 1).

Calcium had a big influence on gel hardness as could also be observed with gellan used alone. Indeed, it was previously suggested that calcium helps the formation of stable associations between the flexible gellan coils at high temperatures, gelation process being then induced on cooling by reduction in configurational freedom. Extra amount of calcium ions facilitates formation of chain associations. Gellan is in fact an anionic polysaccharide undergoing a thermally reversible conformational transition involving two disordered coils at high temperature and a left handed, three-fold coaxial helical structures at temperatures below the transition point. Sugars are expected to influence the overall structure of water and the transition temperature (5). Gelation is promoted by cation mediated aggregation of the intertwined helical chains. However, too large quantities of counter ions might induce a disproportionate lateral associations between double-stranded helices and an effective dehydration (6). Hence, partial destruction of the network, syneresis or low-strength precipitated gels might occur.

Table 1. TPA parameters of mixed gels resulting of gellan and ι-carrageenan combination (with or without 0.05% Ca^{++})

	ι-carrageenan 0.2%		0.5%		1.0%		2.0%		
	without Ca^{++}	with Ca^{++}	without Ca^{++}	with Ca^{++}	without Ca^{++}	with Ca^{++}	without Ca^{++}	with Ca^{++}	
Gellan	0.905	0.883	0.818	0.735	0.918	0.866	0.766	0.749	Elasticity
0.5%	0.546	0.295	0.496	0.344	0.517	0.434	0.701	0.726	Cohesiveness
	1.37	39.32	7.13	25.99	13.48	24.68	5.46	5.72	Hardness
Gellan	0.939	0.883	0.706	0.838	0.863	0.854	0.810	0.839	Elasticity
0.6%	0.412	0.285	0.489	0.241	0.517	0.432	0.803	0.665	Cohesiveness
	2.54	50.41	10.75	37.21	6.39	22.24	6.25	8.20	Hardness
Gellan	0.916	0.885	0.875	0.802	0.794	0.762	0.795	0.767	Elasticity
0.7%	0.396	0.297	0.528	0.386	0.536	0.582	0.753	0.643	Cohesiveness
	3.92	70.21	8.70	51.60	4.51	33.57	6.46	8.13	Hardness
Gellan	0.804	0.937	0.889	0.835	0.822	0.607	0.681	0.863	Elasticity
0.8%	0.425	0.414	0.501	0.414	0.629	0.698	0.743	0.605	Cohesiveness
	5.17	87.08	12.26	66.84	3.93	35.75	5.81	9.49	Hardness
Gellan	0.806	0.880	0.862	0.783	0.762	0.573	0.740	0.823	Elasticity
0.9%	0.419	0.430	0.574	0.515	0.699	0.514	0.718	0.682	Cohesiveness
	7.89	101.15	20.60	74.27	3.90	44.25	7.27	9.05	Hardness
Gellan	0.928	0.883	0.840	0.906	0.827	0.921	0.794	0.796	Elasticity
1.0%	0.425	0.452	0.503	0.579	0.547	0.430	0.726	0.691	Cohesiveness
	8.83	114.96	25.99	88.88	7.57	42.89	8.48	9.35	Hardness

ι-Carrageenan is also a linear anionic polysaccharide. It forms clear gels induced by potassium and calcium (7). Its gelation mechanism is yet controversial. Its great hydrophilic character due to the presence of sulphated groups enhances its ability to inhibit syneresis.

Elasticity of the gels from gellan and ι-carrageenan mixture was slightly decreased when calcium was added. In any case, i-carrageenan allowed us to obtain at different concentrations an increase of cohesiveness.

Gellan/xanthan mixtures formed white opaque gels, without calcium addition. The gel strength increased with xanthan concentration (Fig. 1) whilst its elasticity increased with gellan concentration (elasticity superior to 0.9 was observed for gellan concentration of 0.4– 0.5%).

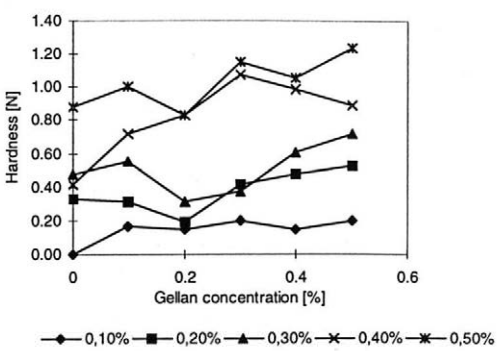

Figure 1. Effect of xanthan concentration on gellan gel hardness

Xanthan is usually considered as a thickener but non-gelling polysaccharide. However, when mixed with other non-gelling polysaccharides of the galactomannan family (this is the case of the commercial preparation used in this study), it leads to gel formation due to synergistic interaction (7). So, it can be assumed that xanthan/galactomannan synergy was responsible for the gel hardness, elasticity being mainly provided by gellan.

Gellane/alginate combination also led to gelation without calcium addition, at 0.1% gellan and 1.6% alginate. The obtained gel was however weak and cloudy. It is worthy to note that at these concentrations, neither gellane nor alginate gelified when used alone.

Composite gels were substantially stronger than the corresponding sum of the two components as previously described (3). In the presence of calcium, phase separation might occur, depending on gellan and alginate concentration. Homogenous gel was obtained at 1.2% lacticol CMG (alginate containing calcium sulfate) and 0.1% gellan.

All gels from gellane/alginate mixtures were very elastic.

CONCLUSION

Our results strengthen claims that gellan can serve as a partial or total gelatin replacement in product applications (4, 8), especially in confectionery.

Gellan composites offer much more textural characteristics than gellan alone and gellan with calcium. In particu-

lar, the compatibility between gellane and ι-carrageenan seems to be interesting because it can lead to various gel hardness, elasticity and cohesiveness.

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Dietary Fibre in Vegetables – The Influence of Origin and Location

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Abstract

The variation of dietary fibre content in eight samples of winter vegetables: lamb's lettuce, white cabbage, potato, endive, cauliflower, chicory, onion and garlic was studied. The samples were purchased from two home sources (Prekmurje and Primorska) and one wholesale trade in Ljubljana, in February 1999. The dietary fibre was determined on the basis of the enzymatic-gravimetric method. It was established that analysed samples contain 0.49–3.39 g/100 g of SDF (soluble dietary fibre), 1.43–2.48 g per 100 g of IDF (insoluble dietary fibre) and 2.06–5.63 g/100 g of TDF (total dietary fibre). Statistical treatment of the results revealed that the studied vegetables differed in the content of the SDF, IDF and TDF content at $\alpha \leq 0{,}001$ and that the differences in the TDF content are statistically significant for four vegetables from different sources (white cabbage, endive, cauliflower, and onion), while the differences for lamb's lettuce, potato, chicory and garlic from different sources are not significant.

Keywords: vegetables; dietary fibre; SDF; IDF; TDF; enzymatic-gravimetric method

INTRODUCTION

Vegetables constitute such a diverse group that it is difficult to generalise about their nutrient content. In general, vegetables are mainly composed of water, especially leafy vegetables, while potatoes are good source of starch and legumes are good source of protein. Nevertheless, because large quantities of vegetables are eaten, they provide beside many vitamins and minerals about half the dietary fibre (NSP) (2). Individual consumption of dietary fibre varies widely between 2 g and 25 g per day. In a healthy diet it is proposed that dietary intakes should be increased to 30 g per day. Recent studies indicate that dietary fibre may be protective against cardiovascular diseases, diabetes, obesity, colon cancer, and other diverticular diseases (2, 3).

This study was undertaken to see the dietary fibre content in vegetables in the winter season and to provide information on whether the origin and location have an influence on the content of SDF, IDF and TDF.

EXPERIMENTAL

Material

Vegetables were purchased in February 1999 from two home sources (Prekmurje and Primorska) and one wholesale trade (Ljubljana). Eight samples of vegetables: lamb's lettuce, white cabbage, potato, endive, cauliflower, chicory, onion and garlic were cleaned all of dirt and analyses were carried out on the edible portion of each vegetable.

METHODS

Dietary fibre analyses: The modified version of the Prosky method (enclosed to a set of BIOQUANT Merck enzymes) was used to determine IDF and SDF. The principle of this enzymatic-gravimetric method is an enzymatic removal of starch and protein and finely gravimetric determination of fibre residues as soluble and as insoluble dietary fibre. The correction for protein and ash in residues were done. The content of TDF was calculated as the sum of soluble and insoluble fractions (1, 4, 5).

Statistical methods: Multifactoral analysis of variance was applied to the data using SAS System with a PC (6).

RESULTS AND DISCUSSION

The results of dietary fibre analyses and the data from Food Composition and Nutrition Tables (7) are summarised in Table 1. The contents of TDF in vegetable investigated ranged from 2.06 g/100 g in potato to 5.63 g/100 g in garlic. While the IDF content varied from 1.43 g/100 g in potato to 2.48 g/100 g in cauliflower, the average content of SDF varied from 0.49 g per 100 g in cauliflower to 3.39 g/100 g in garlic. The statistical treatment showed that the differences in the SDF, IDF and TDF between types of vegetable were significant ($\alpha \leq 0.001$). The comparison our results with the data from Food Composition and Nutrition Tables showed that the averages of TDF content for white cabbage, potato and cauliflower are almost the same, while the results of our analyses for lamb's lettuce, endive, chicory and onion differ. The differences are asigned to the sort and degree of freshness.

Table 1. SDF, IDF and TDF content of vegetables per 100 g of edible portion

Vegetable	Results of analyses (n = 6)			Food Composition and Nutrition Tables (7)		
	SDF $\bar{x}$ ± SD	IDF $\bar{x}$ ± SD	TDF $\bar{x}$ ± SD	SDF $\bar{x}$	IDF $\bar{x}$	TDF $\bar{x}$
Lamb's lettuce	1.02 ± 0.16	2.40 ± 0.28	3.42 ± 0.34	0.15	1.37	1.52
White cabbage	0.58 ± 0.19	2.23 ± 0.42	2.81 ± 0.60	1.33	1.62	2.95
Potato	0.63 ± 0.15	1.43 ± 0.14	2.06 ± 0.17	0.92	1.15	2.07
Endive	0.86 ± 0.25	1.92 ± 0.18	2.78 ± 0.34	0.18	1.04	1.22
Cauliflower	0.49 ± 0.07	2.48 ± 0.29	2.97 ± 0.32	0.49	2.43	2.92
Chicory	1.13 ± 0.18	1.98 ± 0.22	3.11 ± 0.37	0.37	0.89	1.26
Onion	0.85 ± 0.23	1.57 ± 0.29	2.41 ± 0.49	0.29	1.52	1.81
Garlic	3.39 ± 0.46	2.24 ± 0.41	5.63 ± 0.59	/	/	/
Analysis of variance	$\alpha \leq 0.001$	$\alpha \leq 0.001$	$\alpha \leq 0.001$			

The influence of location is demonstrated in Table 2. These results show that the contents of SDF in vegetables from different sources are rather similar. Statistical treatment shows that the differences in SDF content in lamb's lettuce, potato, cauliflower, chicory and garlic from three locations are not significant, while the differences in endive and onion are statistically significant at $P \leq 0.05$ and in white cabbage at $P \leq 0.01$. The data of IDF showed a little different results: the source of vegetables differed significantly at $P \leq 0.001$ in the content of IDF for white cabbage and cauliflower; at $P \leq 0.01$ for chicory and onion; and at $P \leq 0.05$ for potato, endive and garlic; only in lamb's lettuce the differences in IDF between locations are not significant. Analysis of variance showed that there were statistically significant differences among the contents of TDF in vegetables from different sources for white cabbage ($P \leq 0.001$), for cauliflower and onion ($P \leq 0.01$), for endive ($P \leq 0.05$), while the differences for lamb's lettuce, potato, chicory and garlic were not significant.

Table 2. Contents of SDF, IDF and TDF (g/100 g of edible portion) in vegetable from different locations

Vegetable		Prekmurje[a]	Primorska[b]	Ljubljana[c]	P value
Lambcs lettuce	SDF	1.02	1.01	1.05	0.9796
	IDF	2.14[b]	2.72	2.34	0.0502
	TDF	3.16	3.73	3.39	0.3111
White cabbage	SDF	0.51[b,c]	0.82[c]	0.41	0.0017
	IDF	2.35[b,c]	2.63[c]	1.71	0.0004
	TDF	2.86[b,c]	3.45[c]	2.12	0.0001
Potato	SDF	0.79	0.51	0.58	0.1373
	IDF	1.31[c]	1.38[c]	1.60	0.0174
	TDF	2.10	1.89	2.18	0.2131
Endive	SDF	1.16[b,c]	0.71	0.73	0.0499
	IDF	2.01[b]	1.71[c]	2.05	0.0429
	TDF	3.17[b,c]	2.42	2.78	0.0160
Cauliflower	SDF	0.48	0.54	0.45	0.4212
	IDF	2.15[b,c]	2.79[c]	2.52	0.0003
	TDF	2.63[b,c]	3.33[c]	2.97	0.0052
Chicory	SDF	1.08	1.27	1.03	0.4798
	IDF	1.80[b]	2.26[c]	1.90	0.0039
	TDF	2.88	3.53	2.93	0.1041
Onion	SDF	0.61[b]	1.10	0.83	0.0362
	IDF	1.20[b,c]	1.75	1.77	0.0016
	TDF	1.81[b,c]	2.85	2.60	0.0026
Garlic	SDF	3.42	3.10	3.67	0.5718
	IDF	2.75[b,c]	1.98	1.99	0.0228
	TDF	6.17	5.08	5.66	0.1808

$P \leq 0.001$ highly stat. significant; $P \leq 0.01$ stat. significant; $P \leq 0.05$ stat. significant; a, b, c – sources of samples

According the well known effect of soluble dietary fibre, the ratio of SDF in TDF was calculated. The soluble fibre fraction averaged 33.5% of the TDF. In general, the fractions of SDF varried from 16.44% in cauliflower to 36.11% in chicory, the exception is garlic with 60.25 % of SDF.

CONCLUSIONS

The obtained results show that all eight vegetables are quite good source of dietary fibre in the winter season, particularly those we eat in great portion like endive, chicory, lamb's lettuce and potato. Generally our results corresponded to the data in Food Composition and Nutrition Tables.

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Changes of Pea α-galactosides during Processing

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Abstract

The a-galactoside (raffinose, stachyose, verbascose) contents in two cultivars (Lantra and Komet) of Czech grain peas cultivated for three years on three localities and their changes during various culinary processing were determined by HPLC. The average α-galactoside contents were 63.2 and 60.1 g/kg dry matter in Lantra and Komet, respectively. The content of α-galactosides decreased in the average by 20.5% of the original amount during soaking, and by 25.2–36.4 % during combined soaking and cooking. The losses of α-galactosides are caused partially by the extraction in soaking or cooking medium and partially by enzyme cleavage during soaking. It was confirmed by the determination of glucose, fructose, galactose and sucrose in soaking water.

Keywords: peas; saccharides; α-galactosides; changes; soaking; cooking

INTRODUCTION

Peas, similarly to other grain legumes, contain undigestible oligosaccharides, mainly α-galactosides. Their content is rather high in sown peas (*Pisum sativum* L.) in the comparison with other legumes: 3–16 g/kg raffinose, 22–55 g/kg stachyose, 22–42 g/kg verbascose, i.e. 47–113 g/kg total α-galactosides (1). They are not cleaved by digestive enzymes in man (2), but on the contrary, they are cleaved by enzymes of intestinal microflora with formation of lower fatty acids and gases, such as hydrogen, methane and carbon dioxide. These gases cause flatulence, which is the main reason, why the legume consumption is very low in Western and Central European countries (in the Czech Republic about 2 kg per capita per year). In a sociological search (3), the presence of flatulent factors was the objection against legumes in 32% responses. However, other undigestible factors than a-galactosides are also present in legumes, especially resistant starch and hemicelluloses.

Considerable losses of α-galactosides may occur during food preparation, especially, if legumes are consumed fermented or germinated. In Central Europe, they are usually consumed after soaking and cooking. Literature data on losses of α-galactosides during soaking and cooking of legumes show some differences on observed changes. Reduction of α-galactoside content by 45–100% was reported during soaking of lentils (4), high losses occurred in the process of soaking chickpeas, kidney beans and lentils, while cooking reduced the content of sugars only in case of kidney beans (5). The losses of pea α-galactosides during soaking and cooking were not studied.

EXPERIMENTAL

Material: Samples of peas (the cultivars Lantra – green grains, and Komet – yellow grains) were produced in Czech Republic in three different experiment stations – Jaroměřice nad Rokytnou, Čáslav and Žatec.

Analytical Methods: Dry matter was determined by drying at 105°C. Oligosaccharides were extracted by 80% aqueous ethanol by reflux for 1 h, and determined by HPLC (liquid chromatograph Hewlett-Packard with a refractometer detector HP 1047A) with use of a method proposed by Kvasnička et al. (6) with the following modification: steel column 4 × 250 mm packed with Separon SGX NH_2, 5 μm (Tessek Ltd., Prague CZ), mobile phase: acetonitrile:deionised water (35 V/V). Monosaccharides were determined on the steel column 8 × 250 mm packed with OSTION LG-KS 080 Na^+ form (Tessek Ltd., Prague CZ), mobile phase: deionised water

Cooking Procedure: Procedure A – 100 g of peas were soaked in 300 ml of tap water for 12 h at room temperature

Procedure B – 100 g of soaked peas were boiled in 300 ml of fresh water for 35 min

Procedure C – 80 g of soaked peas were boiled in 240 ml of soaking water (the same ratio of peas:boiling water as in procedure B) for 35 min

Procedure D – 100 g of peas were boiled in 300 ml of tap water for 2 min and left to cool for 1 h, 100 g of soaked peas were boiled with 300 ml of fresh water for 50 min

RESULTS AND DISCUSSION

The total α-galactoside and individual a-galactoside contents in grain peas are shown in Tables1 and 2 respectively and losses of total α-galactosides in peas during various type of processing in Table 3. The content of α-galactosides decreased in the average by 20.5 % of the original amount during soaking, and by 25,2–36.4 % during combined soaking and cooking. The decrease of α-galactosides in peas is caused partially by elution to soaking

Table 1. Content of total α-galactosides in grain peas (g/kg of dry matter)

Cultivar	Lantra			Komet		
Locality	1997	1998	1999	1997	1998	1999
Jaroměřice nad Rokytnou	65.5	71.8	53.0	59.7	61.9	55.6
Žatec	71.9	62.4	57.7	66.5	64.7	57.3
Čáslav	69.3	65.9	51.6	57.5	63.3	54.1
Average	68.9	66.7	54.1	61.2	63.3	55.6
Total average		63.2			60.1	

Table 2. Content of individual α-galactosides in grain peas (g/kg of dry matter)

Crop year /α-galactoside	Lantra			Komet		
	Raf.	Stach.	Verbas.	Raf.	Stach.	Verbas.
1997	12.8	27.0	29.1	11.3	29.6	20.3
1998	12.9	21.7	32.1	10.8	25.8	26.7
1999	8.8	20.6	24.7	8.1	26.7	20.8
Average	11.5	23.1	28.6	10.1	27.4	22.6

Raf. = Raffinose; Stach. = Stachyose; Verbas. = Verbascose

Table 3. Losses of total α-galactosides in peas during processing (% of the original amount in dry matter)

Cultivar	Crop year	A	B	C	D
Lantra	1997	19.9	37.1	31.2	40.5
	1998	27.8	39.6	35.7	37.7
	1999	17.6	21.2	17.2	29.1
Average		21.8	32.6	28.0	35.8
Komet	1997	14.3	37.1	31.2	40.5
	1998	19.0	24.9	18.3	39.0
	1999	24.0	25.1	17.6	31.5
Average		19.1	29.0	22.4	37.0
Total average		20.5	30.8	25.2	36.4

stachyose or one molecule of raffinose. Stachyose may yield one molecule of raffinose. The formed individual saccharides can remain in soaked peas or be extracted into soaking water. The soaking is the first stage of germinating, and therefore simple saccharides are a source of metabolic energy. The simple saccharides can be resynthetised to various oligosaccharides as well.

Distribution of individual pea saccharides (100 g cultivar Lantra and Komet from locality Jaroměřice) into soaked peas and soaking water after 12 h and 24 h of soaking is shown in Table 4.

Table 4. Distribution of individual pea saccharides (100 g cultivar Lantra and Komet from locality Jaroměřice) into soaked peas and soaking water after 12 h and 24 h of soaking (mg)

	Saccharide	Dry peas	Soaked peas		Soaking water	
			12 h	24 h	12 h	24 h
Lantra	Glucose	0.0	0.0	62.1	61.9	0.0
	Fructose + galactose	0.0	0.0	113.2	26.3	17.5
	Sucrose	1450.3	1532.5	1518.2	30.0	65.7
	Raffinose	546.6	333.4	548.0	0.0	0.0
	Stachyose	1814.4	1874.3	1551.9	20.6	10.6
	Verbascose	2306.9	2098.0	1860.2	18.8	4.6
Komet	Glucose	0.0	0.0	13.9	55.5	0.0
	Fructose + galactose	0.0	0.0	211.8	66.1	6.1
	Sucrose	1937.3	1722.7	1237.5	37.4	11.9
	Raffinose	845.7	439.4	327.4	0.0	0.0
	Stachyose	2273.2	2184.5	1582.9	0.0	13.5
	Verbascose	1566.4	1570.2	1031.6	0.0	2.0

and boiling medium, partially by hydrolysis. The enzymatic reactions prevail in the case of α-galactosides hydrolysis, because the αform is more resistant to acid hydrolysis than the β-form. Enzymatic reactions proceed during the soaking, during the boiling they are stopped by high temperature. The possibilities of splitting are various. One molecule of verbascose may yield up three molecules of galactose, one molecule of stachyose two molecules of galactose and raffinose one molecule only. All three α-galactosides may yield one molecule of fructose and one of molecule glucose. Verbascose may yield one molecule of

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Superoxide Dismutase Activity of Pea (*Pisum sativum* L.) Hull

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Abstract

In the study the antioxidative properties of pea hull protein were determined. Extracted and partly purified hull proteins were separated by gel chromatography on a Sephacryl S-300 column. Each fraction was analyzed for protein and phenolic compounds contents and SOD activity. All the active protein fractions contained phenolic compounds. No statistically significant correlation between protein and phenolic compounds contents and SOD activity was found. The obtained results indicate that protein and phenolic compounds are responsible for antioxidative properties.

Keywords: *Pisum sativum* L.; seed coat; superoxide dismutase

INTRODUCTION

Legume seeds are a good source of valuable proteins, polysaccharides (dietary fibre, starch), and micronutrients (vitamins, trace minerals). They are also a rich source of a variety of bioactive non-nutrient compounds including antioxidants. With the recently increasing interest in the intake of dietary antioxidants, legume seeds have also been investigated for their antioxidant properties (1, 5). However, little attention has been paid to the antioxidative activity of legume hulls. Generally, the hulls contains numerous bioactive compounds, which play an important role in protection seeds against oxidative stress. Our previous studies showed, that extracted and partly purified (with the use of Sephadex G-75) pea hull protein revealed the activity of superoxide dismutase (SOD) in the xanthine-xanthine oxidase (XOD) system. Electrophoretic pattern of active protein fractions conducted in denaturating condition (SDS-PAGE) indicated the presence of proteins of molecular weights lower than 66 kD, which is characteristic for plant SOD. However in these fractions phenolic compounds were detected (4). It suggested that protein which reveals SOD activity was not separated from phenolic compounds. In present study in order to obtain pure active SOD fractions the Sephacryl S-300 column was employed.

EXPERIMENTAL

The coloured hull from Fidelia var. peas was investigated. Proteins were extracted from hull with Na-phosphate buffer (pH 7.0). Phenolic compounds were removed from the mixture by the addition of PVP (polyvinylpyrrolidone).The supernatant obtained after filtration and centrifugation was fractionated with ammonium sulfate at the saturation of 45 and 75%. After centrifugation the precipitated proteins were dissolved and dialysed overnight against the same Na-phosphate buffer. The dialyzed sample was loaded onto a Sephacryl S-300 column. Fractions were eluted with Na- phosphate buffer and analysed for the UV absorbtion at 280–500 nm, SOD activity and total phenolic content. Protein was assayed by the method of Bradford (2). The superoxide dismutase activity (SOD) was measured using a kit manufactured by Randox Laboratories Ltd Armone, UK. The method is based on the measurement of the concentration of chromophore which is formed in the reaction of superoxide radicals generated in the system of xanthine-(XOD) with 2-(4-iodophenyl)-3-(-4-nitrophenol)-5-phenyltetrazolium chloride. Total content of phenolic compounds was determined according to Julkunen-Tiitto (3).

RESULTS AND DISCUSSION

Figure 1 shows the eluation pattern of dialyzed sample of gel chromatography on a Sephacryl S-300 column. As the result of chromatographic separation 12 fractions absorbing at 280–550 nm were obtained, which contained protein and exhibited SOD activity Protein content in these fractions was varied and ranged from 130 to 1293.8 μg/ml. 3 of the fractions (eluted in volume characteristic for high molecular mass compounds) exhibited the strongest inhibition, approximately 92%. These 3 fractions with the highest SOD activity were characterized by highest protein content. All the active protein fractions contained phenolic compounds ranged from 7.12 to 11mg/g (Table 1). It suggests that phenols were attached to protein to form the phenol-protein complex. Regression analysis showed

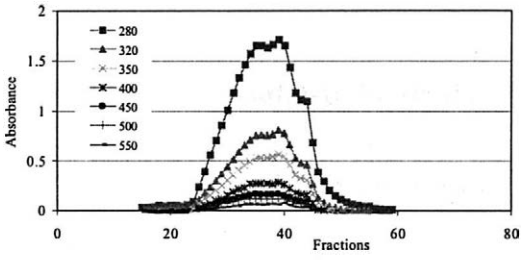

Figure 1. The eluation profils of extract from pea hull by gel chromatography on a Sephakryl S-300 column

no statistically significant correlation between phenolic compounds content and protein content ($R^2 = -19.227$); phenolic compounds content and SOD activity ($R^2 = -0.169$); protein content and SOD activity ($R^2 = 0.047$).The obtained results indicate that phenolic compounds – like SOD protein – can be responsible for antioxidative activity of pea hull.

Table 1. Contents of protein, phenolic compounds and SOD activity of fraction separed by gel chromatography on a Sephacryl S-300 column

Fraction	Protein[x] [g/ml]	Phenolic compounds* mg/g lyophylized fraction	SOD activity [% inhibition]
34	137.50	9.15	86.51
35	130.00	10.14	88.61
36	491.67	11.05	87.61
37	533.33	10.48	89.41
38	950.00	9.71	89.81
39	1085.40	9.19	92.20
40	1293.00	7.91	91.01
41	1220.80	8.45	92.41
42	460.42	8.40	90.51
43	502.08	8.40	90.22
44	418.75	8.42	89.91

[x] BSA was used as the standard; * as (+)-catechin equivalents

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Oxidative Degradation Products of D-Glucose

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Abstract

A study was carried out of oxidative degradation products formed when D-glucose was heated with potassium peroxodisulfate in aqueous solution. Volatile degradation products were isolated from solution by diethyl ether extraction. The extract was then concentrated and analyzed for the content of volatile compounds by GC/FID. Twenty-three volatiles were identified by GC/MS and by comparison with authentic compounds. The majorority of products were derivatives of furane, pyrane and cyclopentene. Also several acids (formic, acetic, 2-furancarboxylic, 4-oxopentanoic) were found. The major products arised were the following: 2-furancarbaldehyde, 2-furancarboxylic acid, acetic acid and 3-hydroxy-2-pyranone.

Keywords: oxidative degradation; peroxodisulfate; glucose; volatile compounds; dehydration; fragmentation; cyclization

INTRODUCTION

Degradation reactions of sugars play an important role during the heat processing of many foods. The result of these reactions is a complicated mixture of products, including very reactive low molecular weight compounds (i.e. formaldehyde, glyoxal, methylglyoxal etc.), products of dehydration and cyclization (furan- and pyran-type compounds), oxidation and condensation products (1–2). In these reactions oxygen free radicals could be generated, which are supposed to take part in oxidative destruction (3). Various oxidative agents such as peroxodisulfates, dichromates, permanganates or Fenton's reaction (decomposition of hydrogen peroxide in the presence of metal ions, mostly Fe^{3+} or Cu^{2+}) are often used for study of the radical mechanism of reactions.

The radicals initiate reactions resulting in oxidative destruction of carbohydrate molecules. The radical mechanism was proposed e.g. as one of the possible ways of the formation of 2-furancarbaldehyde from hexoses (4). Strong support gains the radical theory in beer ageing process (5).

EXPERIMENTAL

The reaction mixture was a solution of 5 mmol D-glucose and 5 mmol potassium peroxodisulfate ($K_2S_2O_8$) in 50 ml of distilled water. The mixture was refluxed for 1 hour and then cooled to room temperature.

Volatile compounds were extracted by diethyl ether (25, 10, 10 and 10 ml). The joint extracts were concentrated, using the Snyder column, to about 5 ml and then evaporated under a stream of nitrogen to 0.2 ml.

The concentrated sample was analyzed by GC/FID using an CP WAX 52 CB fused silica capillary column (30m × 0.25 mm i.d., 0.25 μm film thickness, Chrompack, Middelburg, The Netherlands) and a Hewlett-Packard 6890 Plus gas chromatograph. Oven temperature was set to 60°C and was raised to 220°C at a rate of 5°C per min and kept at that temperature for 45 min. Injection and detector port temperatures were set to 220°C and 250°C, respectively, and the helium carrier gas flow was 0.7 ml/min. Heptadecane was used as the internal standard. GC/MS analyses were carried out on GCD system G1800A (Hewlett-Packard) with the same column and temperature conditions as above, except for the setting of the detector temperature (280°C).

RESULTS AND DISCUSSION

The extract of volatile compounds produced by refluxing D-glucose in the presence of peroxodisulfate was concentrated and after addition of heptadecane analyzed by gas chromatography (Fig. 1).

The components were identified by comparison of their GC retention time data, retention indices (6) and mass spectra with those of authentic compounds or with library data (7). Identified compounds and their amount in the reaction mixture are summarized in Table 1.

It is evident that the oxidation of glucose predominantly yields a 5C fragment (pentose) and formaldehyde. Subsequent reactions of the pentose give rise to 2-furancarbaldehyde. Oxidation of the latter compound yields 2-furancarboxylic and formaldehyde is oxidized to formic acid. In small amount was identified also 3-furancarbaldehyde.

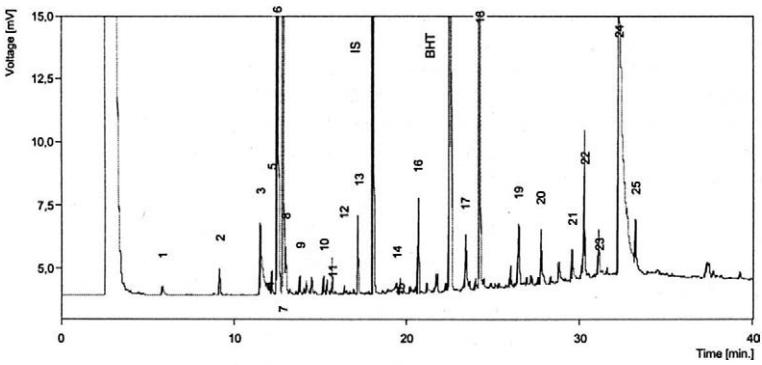

Figure 1. Chromatogram of volatile compounds from D-glucose

Table 1. Identified reaction products of D-glucose

Peak no.	RT [min]	Compound	Amount [μg]	RI	Identification
1	5.92	2-pentanone	0.8	1131	MS
2	9.27	1-hydroxy-2-propanone	1.4	1306	MS
3	11.63	2,5-furandione	7.9	1409	MS
4	12.13	3-furancarbaldehyde	0.5	1432	MS
5	12.30	5-methyl-2(3*H*)-furanone	1.0	1439	MS, RT
6	12.58	acetic acid	25.1	1451	MS, RT
7	12.96	2-furancarbaldehyde	36.9	1467	MS
8	13.09	not identified	3.0	1473	MS
9	13.92	formic acid	a)	1507	MS, RT
10	15.31	dihydro-3-methylen-2*(3H)*-furanone	0.8	1569	MS
11	15.81	4-cyclopenten-1,3-dione	1.5	1590	MS
12	16.48	1,2-ethandiol	0.5	1620	MS, RT
13	17.31	2-hydroxymethylfurane	3.2	1658	MS
14	19.56	2(*5H*)-furanone	1.1	1762	MS
15	19.76	2-hydroxy-2-cyclopenten-1-one	0.7	1771	MS
16	20.78	(*4H*)-pyran-4-one	4.1	1819	MS
17	23.55	2-hydroxy-3-methyl-2-cyclopenten-1-one	2.8	1960	MS
18	24.37	3-hydroxy-2-pyranone	21.6	2000	MS
19	26.63	not identified	4.2	2118	MS
20	27.92	3-hydroxy-4,4-dimethyl-?-butanolactone	3.2	2188	MS
21	29.75	3,5-dihydroxy-2-methyl-(*4H*)-pyran-4-one	2.5	2291	MS
22	30.43	4-oxopentanoic acid	8.4	2331	MS, RT
23	31.28	furylformate	1.9	2382	MS
24	32.41	2-furancarboxylic acid	62.1	2447	MS, RT
25	33.41	5-hydroxymethyl-2-furancarbaldehyde	4.8	2503	MS

a) not quantified

Glucose is also degraded to a small extent as such to 5-hydroxymethyl-2-furancarbaldehyde which subsequently yields levulinic (4-oxopentanoic) acid. Its lactonisation product, α-angelikalacton, was also identified.

2-Furanmethanol, which is mentioned as dehydration product of hexoses, was found in significant amount. Probably, 2-furanmethanol was formed by Cannizzaro reaction from 2-furancarbaldehyde. Small amounts of furylformate can arise by esterification of 2-furanmethanol by formic acid.

The amount of formic and acetic acid, the presence of 1-hydroxy-2-propanone (hydroxyacetone), 2-pentanone, 1,2-ethandiol and scale of cyclised products give evidence of a large range of fragmentation and cyclization reactions. The identified 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) can, for example, originate by the condensation of two molecules of 1-hydroxy-2-propanone (8) or by condensation of biacetyl and acetaldehyde (9). 4-Hydroxy-2-butenic acid lactonised on 2(*5H*)-furanone (α-crotonolactone).

Moreover other cyclic components (derivatives of furane, pyrane and cyclopentene) were identified in the reaction mixture, which can form directly from carbohydrates, via uloses and deoxyuloses (e.g. 1-deoxy-D-*erythro*-2,3-hexodiulose). This suggests other mechanisms can be established regarding the condensation of dicarbonyl and hydroxycarbonyl compounds, the fragmentation products of carbohydrates.

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Creatine and Creatinine Content in Meat Products

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Abstract

The concentration of creatine (Cr) and creatinine (Cn) were determined by newly developed HPLC method in beef meat and chicken breast, after heat treatment at different temperature and duration. Ratio (ψ) of (Cr + Cn)/Cr found in heat-treated meat was compared with the value ψ_m obtained under model conditions. The comparison with ψ and ψ_m for heat treatment at 70°C revealed there are no statistically significant differences between the course of reaction in real samples and under model conditions. The comparison with ψ and ψ_m for heat treatment at 80 or 90°C, showed some influence of pH on the course of reaction. It was shown that the extent of heat treatment of meat product could be estimated from value ψ. Additionally the lean meat content could be determined from the total creatinine after conversion of creatine.

Keywords: creatine; creatinine; meat

INTRODUCTION

Creatine (N-amidinosarcosine) and creatinine (2-amino-1-methyl-2-imidazolin-4-on) are important components in cycle muscular contraction. Creatine and creatinine content in muscle depends on animal kind and on animal age. The creatine content varies from 240–640 mg/100g in pork, 450–560 mg/100g in chicken, and 410–630 mg/100g in beef (1). The highest levels of creatine were found in intensive working muscle (2). Creatinine content in fresh meat is approximately2 % of creatine. It is known that during heating and/or under acidic conditions the creatine converts into creatinine, so that the creatinine content increases. The amount of converted creatine depends on time of treatment, temperature and pH. The conversion of creatine to creatinine is the first order reaction given by well-known equation

$$c_a = c_{a0} * e^{-k*\tau}$$

where c_a is the concentration of creatine after time τ, c_{a0} is the concentration of creatine at $\tau = 0$, and k is the rate constant which depends on temperature according to Arrhenius equation. Witkovska (3) determined under model conditions dependence of the rate constant on pH at temperature 60°C and expressed the relationship by following equation:

$$k * 10^{-2} = 0.3068 + 0.09949 * pH + 0.008158 * (pH)^2$$

Timeout of proper heat treatment increases energy demands on manufacturing of meat products and also downgrades sensory properties, *i.e.*, quality of meat products.

There is a question: How to estimate the extent of heat treatment of anonymous sample? The ratio ψ (sum of creatine + creatinine content divided by creatinine content) could answer such question.

EXPERIMENTAL (4)

List of samples: Beef meat, *Musculus semimembranosus* (HK), Beef meat, *Musculus londissimus dorsi* (HR), Chicken meat – breast (KP), Sausage "Orlický" (OS), Finely minced smoked sausage (TS), Frankfurter (P), Roughly minced smoked sausage (OK), Sausage "Polský" (TP), Lyoner type sausage (K), Lyoner type sausage with cheese (J), Sausage "Myslivecký" (MS).

Conversion of creatine to creatinine under model condition: Solutions of creatine (750 mg/l) in buffer (pH 7.1, 6.2, 5.2, and 4.1) were heated in water bath at 70°C. 3 ml aliquots were taken after 30, 60, 90, 120, 150, 180, 210 and 240 minutes for HPLC analysis. Another model experiment were done with solutions of creatinine (750 mg/l) in buffer about pH 6.2 and temperature 60, 70, 80 and 90°C. Samples were taken after 30, 60, 90, 120, 150, 180, 210 and 240 minutes.

Conversion of creatine to creatinine in meat: Samples of beef meat and chicken breast were cut in cube (2 × 2 × 2 cm), which were singly packaged into PE folia under vacuum. Packaged samples were heated at 70, 80 or 90°C in a water bath for 30, 60, 90, 120, 150, 180, 210 or 240 minutes. The samples were removed at the appropriate time, cooled in running water and frozen at –20°C. Frozen sample was

homogenised in 200 ml of 5% trichloroacetic acid (TCA) for 4 minutes. The extract was filtered through paper filter, ten times diluted with mobile phase and again filtered through 0.45 μm membrane prior to HPLC analysis.

HPLC determination of creatine and creatinine: For the determination of creatine (Cr) and creatinine (Cn) an HPLC method was developed. Separation (ion-exchange principle) was done on Separon SGX CX (5 μm, strong cation exchanger on silica, 250 × 4 mm, Tessek Ltd., Prague) at ambient temperature. Aqueous mixture of 20 mM-KH_2PO_4 + 20 mM-K_2HPO_4, pH 6.8 served as mobile phase at a flow-rate of 1 ml/min. Analysis time was 20 min. A spectrophotometry detection of creatine and creatinine at 210 nm were used. Sample injection volume was 10 μl. An external standard technique (five concentration levels: 5, 10, 20, 50 and 100 mg/l) was used for quantitative analysis.

RESULT AND DISCUSSION

We proved that the developed HPLC method is suitable for simultaneous creatine and creatinine determination in meat and meat products. The chromatogram of Lyoner type sausage extract is given in figure. It is clear that the creatine is pretty nice resolved from creatinine. Unfortunately in some of analysed samples an unknown peak appeared next to creatinine. In such case the creatinine concentration was calculated from peak height rather than peak area. From the results of model experiments coefficients of Arrhenius equation (dependence of rate constant of creatine conversion on temperature) were calculated. We found frequency factor $A = 6.022.10^{-11}$ s^{-1} and activation energy $E_a = 86.250$ kJ/mol. The quadratic equation describing the dependence of rate constant on pH (range from 5.2 to 7.1, constant temperature 60°C) was as follows:

$$k*10^{-2}\ [h^{-1}] = 0.7749 * pH^2 - 12.192 * pH + 50.609$$

Experimentally found rate constant $k = 0.0167$ s^{-1} is in good agreement with literature (3) value 0.0124 s^{-1} (60°C, pH 6.2). Comparison of value ψ_m (model conditions) with value ψ (samples of heat treated meat at 70°C) showed no statistically significant differences ($\alpha = 0.05$). Further good agreement between ψ and ψ_m values at 70°C was found (see Figure 1). It was also found that there are no differences between ψ values for different kind of meat and ψ value is independent on absolute creatine + creatinine content. On the other hand the statistically significant differences ($\alpha = 0.05$) were found for pH 6.2 and 80 and/or 90°C.

Relationship ψ on temperature and time (for all kind of meat) could be expressed by following equation

$$\psi = 45 - 0.045 \times heating\ time\ [min] - 0.41 \times temperature\ [°C]$$

From the coefficients of the equation one can conclude:

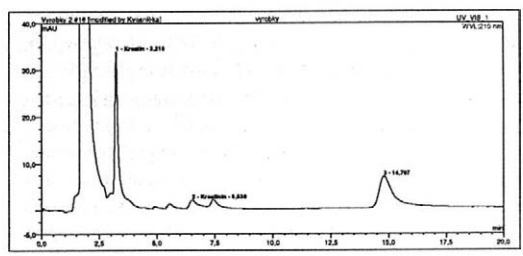
1. Fresh meat contains in average 45times more creatine than creatinine.
2. Prolongation of heating by one minute puts down ψ values of 0.045.
3. Temperature rise by one °C decreases ψ values of 0.41.

Lean meat content could be estimated from creatine and creatinine concentration in meat products. The table summarises such results. It is clear that lean meat determined through creatinine is lower than that determined by indirect method (total protein minus collagen). The value ψ indicates a heat treatment of meat products and through this value the compliance of technology could by checked.

Sample	Cr + Cn* [mg/100g]	ψ [-]	Lean meat content **[g/100g]	***[g/100g]
OS	146.8	8.92	7.3	11.2
TS	132.3	19.39	6.6	10.0
P	123.4	29.88	6.2	10.1
OK	181.1	14.23	9.1	12.2
TP	152.6	10.96	7.6	10.0
K	168.2	14.99	8.4	11.0
J	189.6	7.13	9.5	13.6
MS	189.3	6.93	9.5	10.0

* expressed as creatinine
** 1 g of lean meat/100 g correspond to 20 mg creatinine/100 g (5)
*** determined by indirect method (Kjehldahl nitrogen × 6.25 minus collagen as 4-hydroxyproline × 8.0)

Chromatogram of TCA extract of Lyoner type sausage (conditions see text)



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B – Maillard Reaction

News on the Maillard Reaction of Oligomer Carbohydrates with Glycine

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Abstract

The reaction behaviour of monosaccharides in the Maillard reaction is quite well investigated. Deoxyhexosuloses which are responsible for the formation of aroma, volatile substances and melanoidins are formed for instance from the Amadori compounds. The intimidates of this reaction be quantified as stable quinoxalines by trapping reaction with o-phenylendiamine. However most of the reactions that oligo and polymeric carbohydrates undergo during non-enzymatic browning reaction areun known.

Therefore dextrin (oligomeric α-glucanes) and starch were used as model compounds to investigate the mechanism of the Maillard reaction for oligo and polysaccharides with glycine. In watter free systems oligosaccharides form a α-dicarbonyl compound via a "peeling off" mechanism. It starts at the reducing end of glucane and results in the formation of 1,4-dideoxyhexosulose the main a-dicarbonyl component found in thermally induced degradation of maltodextrines [Hollnagel, Kroh (2)]. This reaction is accompanied by transglycosilation which leads to formation of branched carbohydrate structures and by formation of anhydrocarbohydrates via dehydration [Kroh *et al.* (1)]. The intermediate structure is a glucosylcation. In aqueous model system the degradation of oligomeric carbohydrates undergoes different reaction pathways. The main reaction in absence of an amino compound is hydrothermolytic degradation of a glycosidic bond. It is followed by isomerisation which results in the formation of ketoses at the reducing end of glucanes. Formation of 3-deoxypentosuloses as main α-dicarbonyl compound could be proved by trapping reactions. Other α-dicarbonyls play only marginal roles in the Maillard reaction α-glucanes (Hollnagel, Kroh [3]).

Keywords: Maillard reaction; carbohydrates; glycine; deoxyosuloses; dicarbonyls; glucanes

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Infant Formulas Indicators of Maillard Reaction

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Abstract

The progress of the Maillard reaction (MR) in adapted infant milk formulas was studied for a 12 month period at 20 y 37°C. The following four indices of the MR were analysed: available lysine and AGEs (fluorimetric methods), browning pigments (spectrophotometric) and colour (Hunter colorimeter values). After a 12-month storage period the total protein and the available lysine contents (g/100g sample) were lower than at zero-point (11.02 vs 12.61 and 0.86 vs 0.94, respectively). The AGEs and browning index values changed in a similar way and the total higher than the free values. An increase in colour, at two temperatures (20 and 37°C) was observed during storage period.

Keywords: infant formula; storage; Maillard reaction

INTRODUCTION

The composition of milk (high lactose and protein contents), the thermal treatments applied to preserve it (pasteurisation, sterilization, evaporation ...) and in the case of powdered milks the long storage periods, often in adverse humidity and temperature conditions, are factors that favour the development of the Maillard reaction (MR). The early stage of MR can be evaluated through the loss of available lysine (1). In the advanced and final MR stages fluorescent and/or coloured compounds are formed by routes not yet well understood and most of them are still unidentified. The structures and the relevance of these compounds in milk remain also to be established. In the final stage Amadori products can form cross-links between adjacent proteins or with other amino groups, thus giving rise to polymeric aggregates, or the so-called advanced glycation end products (AGEs) (2). The aim of our work was to evaluate of the usefulness of available lysine, AGEs, and browning pigment contents together with colour values as indicators of the protein quality of infant formulas during the storage period.

MATERIALS AND METHODS

Samples: One adapted milk-based powdered infant formula was analyzed. The composition as given on the label was: proteins 11.6%, carbohydrates (lactose) 55%, lipids 28%. The samples were stored at 20 and 37°C in a storage chamber (< 10% RH and temperature controlled) and analyzed just after manufacture (at zero time) and at 3, 6, 9 and 12 months of storage. Samples were maintained in their airtight containers until analysis.

ANALYTICAL METHODS

Available lysine: A fluorometric method of Goodno *et al.* (3) adapted in our laboratory was applied. 1 mL SDS 12% was added to 950mL of water and 50 mL of liquid sample (reconstituted at 13%w/v) (65 to 130 μg of proteins). It was let to cool at 4°C for 12 hours and then sonicated for 15 minutes at 25°C. A 100 mL aliquot was taken and 3 mL OPA was added, and the mixture was shaken for 2 minutes at 25°C. The fluorescence (fluorimeter Shimadzu RF-5000) at $\lambda_{excitation}$ 340nm and $\lambda_{emission}$ 455nm was measured. The interferences originated by non protein components were analyzed in the supernatant obtained after 10% TCA precipitation. A standard of casein bovine milk was used to prepare a calibration curve (7.65 to 76.5 mg lysine/mL). The casein to lysine conversion factor was calculated considering that the α-casein to β-casein ratio was 1:1, with 7 and 5.2% residues of lysine, respectively (4). Determinations were carried out in quadruplicate.

AGEs and browning index- Free AGEs and colored compounds were measured as follows: 2ml of liquid sample (reconstituted at 20% w/v) was deproteinized with 2ml of TCA (24% w/v). To measure total AGEs and colored compounds a modification of the enzymatic hydrolysis proposed by Palombo et al. (5) was applied. 1.5 ml of liquid sample (reconstituted at 20% w/v) was digested in a stoppered test tube with 0.4 ml of pronase solution (27 U/ml), and the mixture was shaken for 30 minutes at 25°C. Both solutions (free and total) were centrifuged at 13 000 g for 10 min at room temperature and the supernatants were diluted in 1M sodium-borate buffer (pH 8.2) (0.1/5). AGEs were measured by fluorescence (fluorimeter Shimadzu RF-5000) at lexcitation 347nm and lemission 415nm. A quinine sulfate

solution (0.2 μg/ml) was used as a standard for calibration of the instrument at 100% relative fluorescence. The browning index (Perkin Elmer Lambda 2 spectrophotometer) was measured at 420 nm. A methyl orange solution (0.7 and 2μg/ml) was used as a standard for calibration of the instrument at 100% absorbance. The analyses were carried out in triplicate.

Color. Color was measured by using a Hunter Labscan II colorimeter. The results being expressed according to the CIELAB system with reference to illuminant D65 and a visual angle of 10°. The measurements were carried out in triplicate.

Total protein determination. The Kjeldahl method (Kjeltec system 1026 Distilling Unit) was used to measure total nitrogen (6). The 6.25 conversion factor was applied. The analyses were carried out in triplicate.

RESULTS AND DISCUSSION

The total protein and available lysine contents together with the color values (CIE Lab scale) of the mentioned adapted milk-based infant formula at different periods of storage (3, 6 9 and 12 months) and temperatures (20 and 37°C) are reported in Table 1. AGEs and browning index values at 20 and 37°C in the ninth and twelve month are indicated in Table 2.

A statistically significant difference ($p < 0.05$) was found between the total protein contents of the formulas stored at 20° and 37°C throughout the storage period.

After a 12-month storage period the available lysine content (g/100g sample) was lower than the one obtained at zero point, although the difference was not statistically significant ($p < 0.05$). When the available lysine content is calculated per 100g of protein instead of 100 g sample, no decrease is observed because of the reduction in the total protein content during this time. Rossi and Pompei (7) report the evolution of the available lysine content of liquid infant formulas during an 18-month storage at 4°, 20° and 38°C. They found a regular decrease in this parameter during the first 1.5–2 storage months, and an increase from the 18th month on. The initial available lysine contents of their formulas were lower (5.44- 6.02 g/100g proteins) than in our study (7.47± 0.19 g/100g proteins).

In each of the storage periods the DE values of formulas stored at 37°C are higher than those corresponding to samples stored at 20°C, although at both storage temperatures an increase in color (ΔE) with time is observed. Analysis of each parameter conforming the equation ΔE (L*, a*, b*) shows that the difference is due to the b* (yellow component) values, which are higher in samples stored at 37°C than in those at 20°C. In their study of ΔE evolution in liquid infant formulas subjected to different thermal treatments and stored for 18 months at 4, 20 and 38°C, Rossi and Pompei (7) observed an increase in ΔE during the whole storage period, and report that at the 12th month of storage the values for samples stored at 4 and 20°C were similar to or slightly higher than those of our study, while in samples stored at 38°C they were much higher (5 to 7 times). The discrepancies in the results can be ascribed to differences in the technological processes applied in manufacturing the liquid formulas (UHT at 140°C for 24 s, in-containers sterilized at 107°C for 120s), and also to the fact that the shelf life of liquid formulas is lower than that of powdered ones. The values of AGEs and the browning index are higher for total than for free and both are similar during the two trimesters of storage and at the two considered temperatures, except for the total browning index values at 20°C.

Table 1. Available lysine contents and color values of the adapted milk-based infant formula stored at different temperatures and times.

Sample T [°C]	month	Total protein [%]	Available lysine g/100g sample	Available lysine g/100g protein	Color L*	Color a*	Color b*	DE[1]
–	0	12.61±0.27^{a}	0.94±0.02^{a}	7.47±0.19^{a,b}	92.34	-0.25	12.31	-
20	3	12.42±0.59$	0.91±0.03$	7.35±0.26^{a,b}	92.06	-0.36	12.67	0.47
	6	12.14±0.20^{b}	0.88±0.13^{a,b}	7.25±1.08^{a,b}	92.54	-0.56	12.78	0.60
	9	11.49±0.18^{b,c}	0.84±0.03^{b}	7.30±0.26$	92.37	-0.42	12.82	0.54
	12	11.43±0.27^{c}	0.87±0.04^{a,b}	7.63±0.39^{b}	92.36	-0.43	12.99	0.70
37	3	12.47±0.19$	0.88±0.02$	7.08±0.20^{a,b}	92.08	-0.39	13.29	1.02
	6	11.84±0.18^{b}	0.83±0.04^{a,b}	7.04±0.36^{a,b}	92.55	-0.60	13.44	1.20
	9	11.61±0.10^{b,c}	0.77±0.06^{b}	6.59±0.49$	92.35	-0.37	13.57	1.27
	12	11.02±0.20^{c}	0.86±0.04^{a,b}	7.79±0.32^{b}	92.09	-0.27	13.86	1.58

1: $\Delta E = (\Delta L^* + \Delta a^* + \Delta b^*)^{0.5}$

No coincidence in the letters in a single row indicates significant differences ($p<0.05$)

Table 2. AGEs (% relative fluorescence/g sample) and browning index (% methyl orange) values of the adapted milk-based infant formula stored at different temperatures and times

	AGEs[1]			
T[°C]	Free		Total	
	9	12	9	12
20	1252.22±78.90	1284.08±62.44	7508.43±219.05	7869.83±852.72
37	1216.09±112.07	1225.57±16.72	7643.38±348.89	7928.89±1287.10

	Browning index 420 nm			
T[°C]	Free[2]		Total[3]	
	9	12	9	12
20	51.19±2.06	57.47±7.96	88.10±2.38	134.68±2.06
37	47.61±4.12	48.28±13.80	101.19±5.46	102.25±6.10

1: %standard quinine sulfate 0.2 μg/ml; 2: % methyl orange 0.7 μg/ml; 3: % methyl orange 2 μg/ml

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Preliminary Studies on the Renal Handling of Lactuloselysine from Milk Products

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Abstract

Studies with healthy volunteers revealed that urinary excretion of Amadori products is significantly affected by daily food and can be decreased by diets free of Maillard compounds. After consumption of a certain amount of milk with controlled content of lactuloselysine, it could be shown that excretion of Amadori product is within the first 24-h-urine after food consumption. Only about 2% of applied Amadori product can be found in the urine. Plasma levels of Amadori products were not affected by food consumption. This results point out to the fact that the healthy kidney is capable to remove food-derived Maillard compounds efficiently. For patients with impaired kidney function, however, dietary Maillard compounds or derivatives resulting from currently unknown transformation processes might contribute significantly to the total "AGE-load" of the body.

Keywords: Amadori products; urine; Maillard compounds

INTRODUCTION

It is generally accepted that amino acid derivatives formed during various stages of the Maillard reaction (i.e. advanced glycation endproducts, AGEs) contribute to the pathogenesis of diabetes, uraemia and aging (for reviews see e.g. [1–2]). Several individual AGEs have been identified and quantified in tissues, plasma and urine. From the quantitive point of view, however, the amount of AGEs ingested from certain heated foods can be more than ten times higher than the total amount of "endogenously" formed AGEs in the body (3), but information on absorption and excretion of chemically defined Maillard compounds and related physiological consequences is lacking. Based on reports published recently (4), the purpose of the present study was to study daily urinary excretion of Amadori products by healthy volunteers and to investigate whether this excretion is affected by daily food consumption.

MATERIALS AND METHODS

Study design: The test and the control group consisted each of 4 volunteers. Starting at the end of day 2, each group was fed for four days (day 3 to day 7) a diet mostly free of Maillard compounds (i.e. no cooked or fried food, only water, no smoking etc.). In the morning of day 4, the test group was asked to drink one 500 ml-portion of reconstituted milk, made from roller-dried milk powder, containing a certain amount of the protein-bound Amadori product lactuloselysine ("AGE-milk"; 906 mg furosine per 100 g milk powder hydrolysate; milk was prepared by dissolving 50 g milk powder in 450 ml of water, corresponding to 458 mg furosine per 500 ml AGE-milk). From end of day 7, dietary control was finished and the volunteers were allowed to eat and drink what they want. For each volunteer, heparin plasma samples were drawn daily during the whole study in the morning and evening. For the test group receiving the AGE-milk, additional samples were drawn at the start (i.e. ~ 10 min before drinking) and for 6 hours every hour after drinking. 24h-urine and plasma samples were collected. All samples were stored frozen until analysis.

Analysis: Furosine, the degradation product of lactuloselysine formed during acid hydrolysis, was quantified by cation-exchange chromatography after prior hydrolysis with 6 M HCl. Amino acid analysis with direct UV-detection and subsequent derivatization with ninhydrin was performed as described previously (5).

RESULTS AND DISCUSSION

Urinary excretion of Amadori products (furosine as a paradigm of early-stage reaction products) is significantly affected by the composition of the daily food, ranging from 2 to 12 mg furosine per 24 h urine (Fig. 1a, day 1 and 2). For test persons put on a strictly "AGE-free" diet, furosine values decreased within two days to rates less than 1

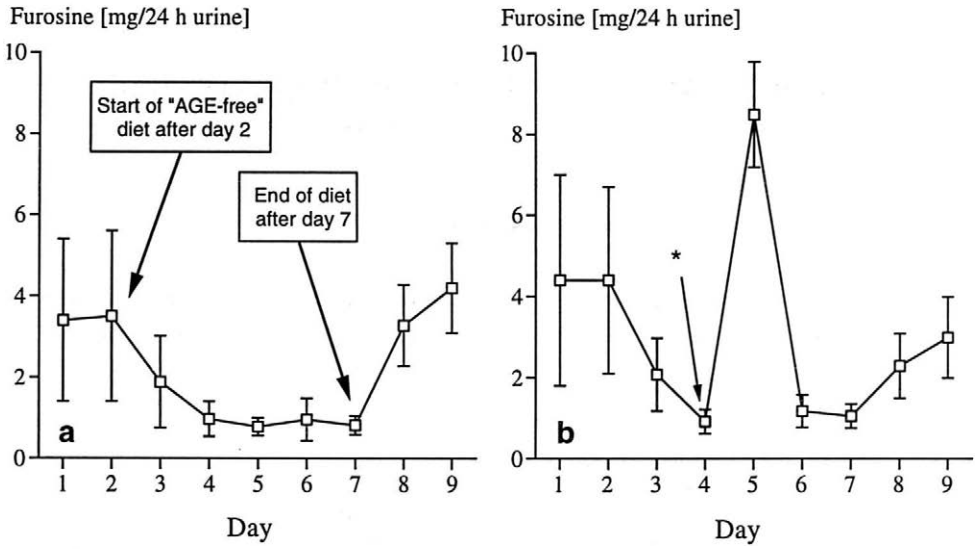

Figure 1. Furosine as a measure for Amadori compounds in 24 h-urine of healthy volunteers. An "AGE-free" diet was given from end of day 2 until end of day 7. a: Control group (n = 4) b: Test group (n = 4; * single meal of 500 ml of "AGE-milk")

mg (Fig. 1a, days 3 to 7), followed by a significant increase after ending of the dietary control (Fig. 1a, day 8 and 9). For volunteers which were asked to drink one portion of lactuloselysine-containing "AGE-milk", a significant increase in furosine excretion could be found after ingestion of the AGE diet (Fig. 1b, day 5), whereas plasma levels of furosine were not affected by the diet and remained constant (20 mg ± 3 mg/100 g of protein) for all volunteers throughout the whole study. For healthy volunteers, excretion of the Maillard compound was very fast and was completed within the first 24 h-urine after ingestion.

It is noteworthy, that only about 2% of the Amadori product applied with the milk sample could be found in the urine. As only minor amounts of lactuloselysine could be found in the faeces (4), the fate of more than 90% of the ingested lactuloselysine remains unexplained. This data are in agreement with reports published (4) and point to currently unknown mechanisms of degradation of Amadori products, e.g. transformation by the intestinal flora.

Our results indicate that the healthy kidney is capable to remove food-derived Maillard compounds efficiently. For patients with impaired kidney function, however, diet-AGEs or derivatives resulting from currently unknown transformation processes might contribute significantly to the total AGE-load of the body and thus might have to be considered as exogenous risk factor in uraemia or diabetes (6). Corresponding studies are currently in progress.

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The Antioxidant Character of Melanoidins

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Abstract

The melanoidins are obtained by interactions of amino acids, peptides or proteins with reducing sugars in terms of thermal treatment and storage (Maillard reaction). The melanoidins play an important part in the physico-chemical transformations of food and the biochemical changes inside the human body. There is already numerous data concerning the antioxidant character of the volatile compounds. Despite the fact there is little knowledge about the structure and the composition of the melanoidins, there are some works trying to demonstrate their antioxidative activity without actually separating these compounds from the reaction system. In this study there will be displayed the results regarding the antioxidative properties of the melanoidins from the glucose-lysine model system. The basic concept we started from is the idea that melanoidins are macromolecular compounds with molecular masses of up to 100 000 Da; they results from the heating of a sugar amino acid model system, during a long period of time. In order to obtain melanoidins a Likens-Nickerson apparatus was used. Volatile compounds, which are continuously extracted, and a brown reaction matter are formed by heating a glucose-lysine water solution. Parts of this reaction matter were extracted with ethyl acetate at determinate periods of time. Than, the ethyl acetate was removed and the remaining material was solved with methanol. Were used in analyzing the resulted methanol solution two methods (1, 2). The first one is based on the comparison between the antioxidant character of TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the antioxidant character of melanoidins. Simultaneously, there was studied the influence of the melanoidins and the one of Trolox on the interaction between the ABAP radicals and the unsaturated chain of the crocin – 8,8 diapocarotendioic-bis-(6-O-D-glucopyranozil-D-glucopyranozil) ester. The outcomes emphasize that melanoidins have the feature of inhibiting the bleaching of the crocin under of ABAP (2,2,-azobis-2-amidinopropane dihydrochloride). This quality belonging to the melanoidins is comparable to that of Trolox. In the process of mixing the oil with well-defined amounts of methanol solution containing melanoidins, it can be observed a slowing down of the oil oxidation (an extended induction time). The oxidation curves obtained from the Rancimat test describe the resistance of the oil to oxidation. The results show that the resistance to oxidation depends on the length of the thermal treatment. Also, the oil oxidation is influenced by the amount of melanoidins. Consequently, the study of the antioxidant character of melanoidins obtained in the model systems or in real system (food) is essential because the melanoidins does take a decisive part in the biochemical processes inside the human body.

Keywords: glucose-lysine model system; melanoidins; antioxidant character; Rancimat test; Trolox; crocin bleaching

INTRODUCTION

The antioxidant activity of Maillard browning mixtures has been reported by many workers and has been generally attributed to the amino or non amino reductones formed in the system models(1–6). Also the activity of the melanoidin pigments has been reported (7). Although the relationship between the color and antioxidant activity of Maillard reaction was reported (8, 9).

EXPERIMENTAL

***Preparation of melanoidins*:** Glucose and lysine are heated in water solution 1M(0,1 mol glucose and 0,1 mol lysine in 100 mL water). The non buffer mixture was treated at 100–110°C for 2, 4, 6, 8, 10, 12 hours. The system was thermal treated in Likens-Nickerson apparatus. The volatile compounds, was extracted with ethyl ether – pentane. In the Likens-Nickerson apparatus was formed a brown reaction matter One part of this reaction matter was extracted with ethyl acetate at determinate periods of time. Than, the ethyl acetate was removed and the remaining material was solved with methanol (the part A). Another part of this brown reaction matter (the part B) was dissoluted in water and the solution was submitted at absorbance measurement (420 and 280nm).

The evaluation of antioxidant action of melanoidins: The part A of brown reaction matter was submitted at two methods for determination of the antioxidant character of melanoidins: (a) *chain-breaking activity and (b) the Rancimat test.*

a) The procedure for determinating the chain - breaking activity is in according with the method described by Tubaro *et al.* (1).

According to the procedure adopted, the ability of a compound or a mixture of compounds to quench peroxyl radicals is measured by analysing the first order rates of crocin bleaching due to the presence of peroxyl radicals. The presence of an antioxidant slowed down the rate of bleaching. The rate bleaching of crocin was followed at 443 nm using a spectrophotometer (Kontron Instruments, Uvikon 860 Milano, Italy).

The competition kinetics follow the equation (3):

$$\Delta A_o/\Delta A = V_o/V = (Kc[C] + Ka[A])/Kc[C] = 1 + (Ka/Kc) * ([A]/[C])$$

where ΔA_o and ΔA are the absorbance variations in the absence or presence of antioxidants, respectively; V_o and V are the bleaching rates in the absence or presence of antioxidants; *kc* and *ka* are the rate constants of the crocin bleaching in the absence or presence of antioxidants; and [*A*] and [*C*] are the concentrations of antioxidant and crocin.

The following reactants were used: ABAP, 2,2'-azobis (2-amidinopropane) dihydrochloride (Wako Chemicals Co., Osaka, Japan) as the peroxyl radical generator; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Aldrich Chemical Co., Germany) as the reference antioxidant (vitamin E hydrosoluble analogue); potassium dihydrate phosphate and potassium mono-hydrate phosphate (Carlo Erba, Milano, Italy); saffron (Sigma Chemical Co., Sant Louis, MO, U.S.A.). Crocin was isolated from saffron by methanol extraction after repeated washing with ethyl ether. The crocin solution was diluted with methanol in order to obtain a 10 mmol/L crocin concentration (the absorption coefficient of crocin is 1.33×10^5 L/(mol cm) at 443 nm). Because of the low solubility of Trolox in water, the Trolox solution was obtained by dissolving 39.7 mg in a minimal volume of absolute ethanol and diluting to 100 mL with distilled water. The Trolox concentration was 1.59 mmol/L.

Analyses were carried out at 40°C in 2 mL incubation medium containing 0.1 mol/L phosphate buffer pH 7.0, 9.5 µmol/L crocin and increasing amounts of Trolox or sample extracts. The reaction was started by adding 40 mL of a 97.7 mmol/L ABAP aqueous solution. Triplicate measurements were made for each sample.

The ability of the sample extracts to slow down the crocin bleaching rate was measured in terms of Trolox molar concentration. Thus, as reported by Tubaro *et al.* (1) and Bressa *et al.* (3), all the extracts were assumed to have antioxidant properties and, in order to calculate the antioxidant molar concentrations, the molecular weight of Trolox (MW = *250.29)* was used.

b) The Rancimat test was carried out according to the method described by Hasenhuettl and Wan (2). The aliquots or 3 g soy bean oil with the determinate amounts of melanoidins mater were put in the reaction tubes and heated at 95°C in the presence of air flow (20 L/h). As a reference was tested a soy bean oil – water sample.

RESULTS AND DISCUSSION

The results of the absorbance at 280 nm and 420 nm for melanoidins extracted with ethyl acetate are presented in the Fig. 1, by lg(AxDil) were A is absorbance of melanoidins mater(part A) and Dil is dilution of melanoidins solution(1:1000).

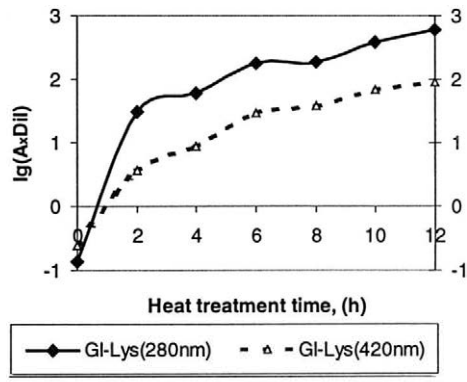

Figure 1. The variation of absorbance (AxDil) for melanoidins in the Glucose-Lysine model system with the time head treatment (h)

The kinetic of bleaching crocin due to Trolox oxidantand and the crocin bleaching due to melanoidins mater extracted with ethyl acetate are presented in the Fig. 2, respectively Fig. 3.

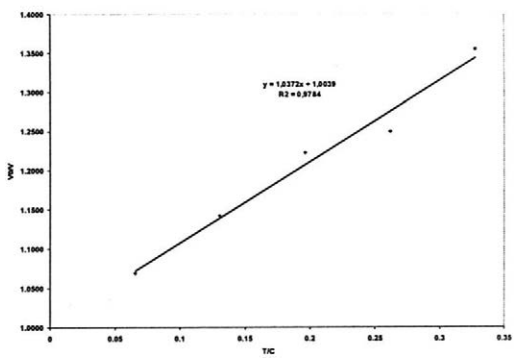

Figure 2. The kinetic of bleaching crocin due to Trolox oxidant

The assessment of antioxidative activity using chain breaking show an increase of the antioxidative character at the beginning and a slowly decrease of it at the end (Fig. 4).

The results obtained by Rancimat test there are in the Table 1.

The two methods show an antioxidative effect of the melanoidins in the glucose-lysine model system.

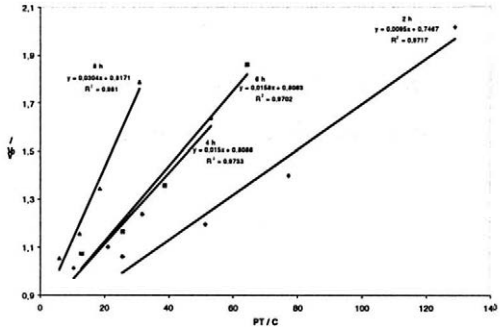

Figure 3. The crocin bleaching due to melanoidins mater extracted with ethyl acetate

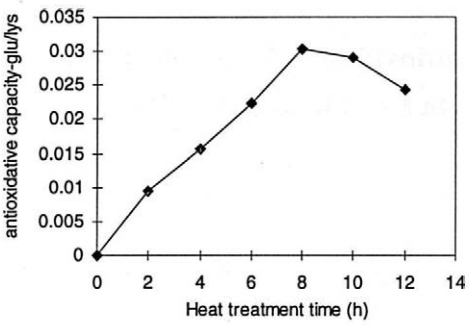

Figure 4. The antioxidative capacity versus heat treatment time

Table 1, The induction time values of samples (soy bean oil+part A of mater) obtained with Rancimat test

No.	The sample	Part A [g]	The induction time to oxidation reaction [h]
1.	Soy bean oil	–	10, 9
2.	Soy bean oil + part A (4h TT)	0, 03	19, 8
3.	Soy bean oil + part A (4h TT)	0, 09	25, 9
4.	Soy bean oil + part A (6h TT)	0,03	24, 3
5.	Soy bean oil + part A (6h TT)	0,09	28,7
6.	Soy bean oil + part A (8h TT)	0,03	27, 1
7.	Soy bean oil + part A (8h TT)	0,09	30, 3
8.	Soy bean oil + part A (12h TT)	0,03	21, 4
9.	Soy bean oil + part A (12h TT)	0, 09	24,7

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Antioxidative Maillard Reaction Products in Heated Pork Meat Juice from two Phenotypes (RN^- and rn^+rn^+) from Hampshire Crossbred Pigs

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Abstract

The addition of heated meat juice from pork from Hampshire crossbred pigs of the RN^- phenotype to pork patties significantly reduced the formation of lipid oxidation products during cooking and chill-storage, compared to samples without added meat juice or with added meat juice from the rn^+rn^+ phenotype. The strong antioxidative effect of heated meat juice from the RN^- phenotype may be explained by the high glucose content of the meat, as no differences in the content of non-protein nitrogen were found.

Keywords: pork; meat quality; Maillard reaction; lipid oxidation; WOF; antioxidants

INTRODUCTION

The Maillard reaction is a very important one in many industrial food processes, including the processing of meat. The significance of the Maillard reaction to food is manifold: the production of colour, the production of flavour or off-flavour, the reduction of nutritional value, toxicity and antioxidative properties. The Maillard reaction consists of a very complex network of many different chemical reactions, in which, during the early stages, amino acids combine with sugars. The content of reducing sugars and free amino acids in meat varies with intrinsic factors such as age, species, genotype, muscle type, ageing, condition before slaughter (e.g. stress), and extrinsic factors such as slaughtering and chilling methods. During post-mortem glycolysis, reducing sugars and lactic acid are formed from glycogen. Pork from the Hampshire breed may contain very high levels of reducing sugars. A major dominant gene, called RN^- (Rendement Napole), may be present in pork from the Hampshire breed and crossbreeds. Meat from carriers of the RN^- gene (RN^- phenotype) has a high glycogen content in the muscles, while non-carriers (rn^+rn^+ phenotype) have a normal glycogen content (1–3).

An off-flavour associated with lipid oxidation, warmed-over flavour (WOF), is formed during the chill-storage of cooked meat products. Many Maillard reaction products (MRPs) are known to have an antioxidative effect, which may be used to prevent WOF. The inhibition of WOF is an important quality issue enabling the meat processing industry to produce high quality products which meet exacting consumer preferences. The objective of this investigation was to study whether antioxidative MRPs, inhibiting the formation of WOF during the storage of cooked meat, were formed in heated pork meat juice from two phenotypes (RN^- and rn^+rn^+) from Hampshire crossbred pigs.

EXPERIMENTAL

Materials and methods: Lyophilized and rehydrated (concentrated 7 times) meat juices from pork from Hampshire crossbred pigs of the RN^- and rn^+rn^+ phenotypes, respectively, were heated in an autoclave (1 h, 121°C) in order to produce MRPs.

The antioxidative effect of the heated meat juices was evaluated in cooked and chill-stored minced pork patties. Fresh pork (*M. bicep femoris*) was ground and standardised to a fat content of 10%. Heated meat juices were added to the minced meat (3% v/w). As a control, water (3% v/w) was added to the meat. Patties (80 ± 1 g) were cooked in an oven at 180°C to a final internal temperature of 80°C. The samples were covered with aluminium foil to avoid browning during heating. The patties were stored at 4°C for 0–2 days in oxygen permeable plastic bags, then vacuum packed, and kept frozen (–80°C) until analysed for chemical composition and lipid oxidation.

Chemical analysis: The phenotypes (RN^- and rn^+rn^+, respectively) were deduced by determining the glycogen levels in the raw meat as the sum of glycogen, glucose and glucose-6-phosphate (4). Animals with a glycogen concentration $\geq$40 μmole/g meat were regarded as carriers of the RN^- allele. Non-protein nitrogen was analysed in the raw meat, according to Kjeldahl, after precipiting the proteins with sulfosalicylic acid (5). The cooked pork patties were analysed for hexanal and pentanal content, using HS-GC-MS, and TBA-value (2-thiobarbituric acid) before and after 2 days of chill-storage (6).

For the analysis of hexanal and pentanal, pork samples (20 g) were homogenised and equilibrated for 15 min at 25°C in an absorption tube. Volatile compounds were absorbed on a Tenax trap (Tenax TA, 60-80 mesh) by passing helium through the absorption tube for 15 min (flow rate 60 ml/min). The volatile compounds were desorbed at 250°C for 30 min with a helium flow of 60 ml/min in a Perkin-Elmer ATD400 automatic thermal desorption system and retrapped on a Tenax-packed cold trap maintained at –30°C. The volatile compounds were injected into the GC-column by thermal desorption of the trap at 300°C for 2 min with a split 1:15. A GC 8000 gas chromatograph (Fisons) connected to a Trio-1000 mass spectrometer (VG Masslab) was used for the GC-MS analysis. The following chromatographic conditions were used: HP-1701 capillary column, 0.25 mm × 30 m, film thickness 1.0 μm; oven temperature 50°C for 2 min, 50°C to 200°C with a slope of 5°C/min, 200°C to 220°C with a slope of 20°C/min and finally 220°C for 6 min; helium flow 1.3 ml/min. Electron impact mass spectra were recorded with an ionisation energy of 70 eV. 1-Chlorononane was used as the internal standard (ISTD).

Statistics: Analysis of variance (ANOVA), using the model y = constant + phenotype, followed by Tukeys' pairwise comparision test, was performed using SYSTAT (Wilkinson, Leland, version 7.0).

RESULTS AND DISCUSSION

The heated meat juice from pork of the RN^- phenotype had a much darker colour than the meat juice from the rn^+rn^+ phenotype, indicating that more melanoidines had been formed. The hexanal content was significantly lower in patties with added meat juice from the RN^- phenotype, compared to samples with added meat juice from the rn^+rn^+ phenotype or samples without added meat juice (43 % reduction) on day 0 (Table 1). After chill-storage for 2 days, the hexanal and pentanal levels and TBA values were significantly lower in the pork patties with added meat juice from RN^- phenotypes, compared to the rn^+rn^+ phenotypes or the control. The relative inhibition in the formation of lipid oxidation products in pork patties with added meat juice, compared to control samples without added meat juice, is shown in the Table (Table 1).

Meat juice from the RN^- phenotype had a high glucose content (703 mg/100 g raw meat), while meat juice from the rn^+rn^+ phenotype had a low glucose content (132 mg/100 g raw meat). The strong antioxidative effect of heated meat juice from the RN^- phenotype may be explained by the high glucose content, as no differences in the content of non-protein nitrogen were found (34 mg/100 g raw meat).

The addition of antioxidative MRPs during the production of cooked meat products for chill-storage may be one way of inhibiting the formation of WOF and producing high quality meat products. The effect on the flavour characteristics of pork patties with added meat juice needs to be studied, since no sensory analysis was performed in the present investigation. Moreover, the safety of heated meat juice needs to be further evaluated, due to the possible formation of carcinogenic heterocylic amines (7).

Acknowledgement: The study has been carried out with the financial support of the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT96-1080, "Optimisation of the Maillard reaction. A way to improve quality and safety of thermally processed foods. "This study does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

Table 1. LS-means of the hexanal and pentanal levels (%/ISTD) and TBA values (mg MDA/kg) in cooked chill-stored pork patties with added meat juice. The relative inhibition (%), compared to control samples, is shown

		Hexanal[1]		Pentanal		TBA value	
Sample/Storage (days)	*n*	0	2	0	2	0	2
Control	4	527[a]	660[a]	123[a]	222[a]	1.5[a]	4.9[a]
RN^-	4	302[b]	398[b]	77[a]	125[b]	0.9[a]	2.9[b]
Inhibition (%)		43	40	37	43	38	40
rn^+rn^+	4	469[a]	573[a]	147[a]	202[a]	1.6[a]	4.8[a]
Inhibition (%)		11	13	0	9	0	3

[1]LS-means having a different index within a column differ significantly according to $p \leq 0.05$

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Melanoidins in Brown Alcoholic Beverages

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Abstract

The presence of melanoidins in sweet wines and beers and their contribution to the colour of this product was studied. In addition, to the analysis of different beers and sweet wines, some brandies and rums were analysed in order to compare the brown pigment present in every of them. Melanoidins were isolated by dialysis. The results showed that melanoidins are present in sweet wines; these compounds have never been described before in wines. In addition, it could be observed that high molecular weight melanoidins of beers and sweet wines have an important contribution in the final colour of both sort of beverages, as a result of the presence of both melanoidins and phenolic compounds.

Key words: beers; sweet wines; brandies; rums; colour; melanoidins; polyphenol polymers

INTRODUCTION

Melanoidins are substances which structures are not well known. During heat treatment of foods, amino groups, usually of protein aminoacids, react with the aldehydic moiety of sugars, thus leading to a complex series of compounds accounting for browning. These compounds are called Maillard reaction products, and melanoidins are one of them. According to the fact that melanoidins are mainly formed in foods that have carbonyls and amino group, and are favoured by the presence of polyphenols and caramelization reaction, their presence in some alcoholic beverages is expected, and they should contribute to sensorial properties of them.

Papers related to beers show that in dark wort and beer melanoidins with lower molecular weight (1500-30000) are the most abundant (3). These compounds are particularly sensitive to oxidation and during beer manufacture they are broken down to form volatile compounds increasing the flavour (1), although not all of them have a positive effect on beer flavour (2). In addition, colour, other important quality parameter of beer, is influenced by the Maillard non-enzymatic browning reactions, which form the yellow or brown coloured compounds (melanoidins) (5).

Although no papers about melanoidins in wines were found, according to the winemaking process of sweet wines, the formation of these compounds is very probably and then they could influence the colour and flavour of these wines. It is important to note that some of the most famous Spanish sweet wines are elaborated by addition of boiled grape must which is one of the responsible of the dark colour and the flavour of these product. It is expected that the sort of melanoidins presents in both beverages will be similar, at least in solubility and may be in molecular weight.

In this work, we study the presence of melanoidins in sweet wines and beers and their contribution to sensorial characteristics of these products, especially over the colour. Brown pigments isolated of sweet wines and beers were compared to brown pigments formed during aged of other alcoholic beverages (brandies and rums), which are mainly phenolic polymers.

EXPERIMENTAL

Developing a method for the quantitative isolation of melanoidins in food is a rather difficult problem due to the complex composition and specificity of each food product, their low content and the absence of objective indices for the assessment of their purity. So, melanoidins were evaluated, using as standard melanoidin the product obtained by reaction between glucose and glycine, following the guide of COST Action 919. This product was employed to obtain a curve of calibrate (absorbance at 345 nm versus concentration) for the quantification.

Different beers, sweet wines, brandies and rums were used in this study. Melanoidins and brown phenolic polymers were isolated by dialysis. Spectral data (200-600nm) were measured on standard melanoidin solution, original products, and on the dialysed products. In addition, colour of the different products and dialysed fractions was measured by the absorbance at 420 nm, using a 10-mm quartz cuvette, and melanoidin content was evaluated on global products and dialysed fractions. All processes and parameters were measured in duplicate.

RESULTS AND DISCUSSION

UV-spectra: It was observed that the majority of analysed beers showed similar UV-spectra, with a maximum absorption at 280 nm and a shoulder in the region from 310 to 340 nm. Their dialysed fractions also showed similar spectra, but their absorbances at 280 nm (maximum) were lower. Therefore, the retained fractions were free of other compounds with have a great absorption in this region, like polyphenols. The absence of polyphenols was confirmed by LC-MS analysis of this fraction (4). Then, it could be said that this fraction mainly has high molecular weight melanoidins (> 12000 Da, HM fraction), which probably have an important influence in the colour.

Spectra of sweet wines and beers were similar, as it could be expected. However, a spectrum of brandies and rums, and the ones of their dialysed fractions, were so different. The most important difference is the absence of the shoulder in the region from 310 to 340 nm, although the absorbance in this region is not zero, because phenolic compounds have some absorption in it.

Relation between colour and concentration of melanoidins: The colour of the all studied beverages (Tcolour) showed a good positive correlation with the colour of the dialysed fraction (HM colour) (Fig. 1).

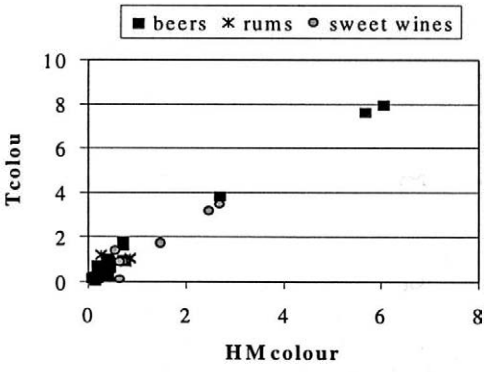

Figure 1. Plot of high molecular weight polymers colour vs. beverages colour

Good correlation between levels of high molecular weight melanoidins (HM), estimated by the absorbance values at 345 nm, and colour of the HM fraction (Fig. 2a) were observed. Similar results were obtained between levels of high molecular weight melanoidins and colour of beverages. However, the relation between levels of total melanoidins (TM), measured on the original products, and colour of the beverages was not so good (Fig. 2b). Rums and brandies showed a special distribution, which could be explained according to the different nature of their brown pigments. Then, the effect of phenolic polymers must be considered, because these compounds could be interference the measurement of total melanoidins.

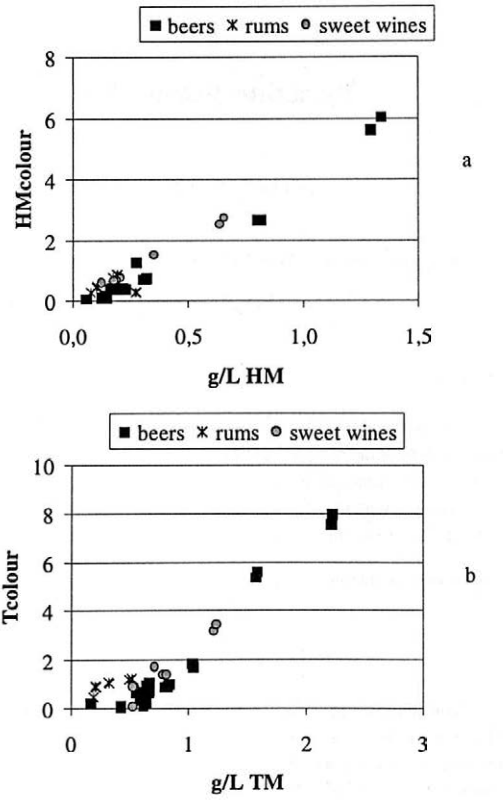

Figure 2. (a) Plot of concentration of high molecular weight melanoidins vs. colour of HM; (b) Plot of total concentration of melanoidins vs. colour of beverages

CONCLUSIONS

Yellow-brown compounds of sweet wines showed similar characteristics that compounds present in beers. Some of them could be melanoidins and they have an important contribution of the colour of these beverages, especially high molecular weight melanoidins.

These are the first results about presence of melanoidins in wines and obviously more studies are necessary in order to improve the knowledge about the type of melanoidins, levels and their contribution to sensorial properties.

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Pyrazine Formation in Course of Extrusion Cooking

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Abstract

Wheat semolina was extruded after an addition of sugars and glucose and amino acids or proteins. Heterocyclic oxygen and nitrogen containing derivatives belonged to the most important roasted flavour compounds. The formation of furans, maltol and pyrazines was enhanced by addition of reducing sugars and by addition of amine derivatives. Free amino acids were more efficient in producing pyrazines than proteins, as reactive Strecker precursors were available, while mostly unreactive 6-amino groups of bound lysine were only available in proteins.

Key words: extrusion cooking; flavour; pyrazines; furans; maltol; sugars; amino acids; protein

INTRODUCTION

Extrusion cooking is a modern, progressive technology, which is more advantageous than the traditional baking because of lower cooking temperature, shorter time and lower costs. A disadvantage is, however, weaker aroma and flavour intensity, caused by mild processing conditions.

The aroma of bread crust and of other baked cereal products is caused mainly by browning reaction products, particularly furan, pyran, pyrazine and pyrrole derivatives (1). The formation of bread crust flavour products may be enhanced under extrusion conditions by addition of browning reaction precursors, mainly reducing sugars and amino acids (2, 3). Therefore, we have studied the effect of amino acids and proteins added to the extruded mixture of wheat flour and glucose.

EXPERIMENTAL

Material. Wheat grits (semolina), 5% D-glucose, D-fructose or sucrose, 2% pure amino acids (L-alanine, L-lysine, L-threonine, L-leucine) and 2–30% edible proteins or protein concentrates (casein, skim milk powder, egg albumin, gluten, full-fat or extracted soybean flour) were mixed, and extruded (without any additional water addition) in a single-screw collet extruder VUMPP 83 (Research Institute of Milling and Baking Industry, Prague), performed at Goldim Ltd. (courtesy by Mrs. H. Poskočilová, M.Sc.). The maximum processing temperature was 140°C, the residence time 30 s.

Methods. Volatiles were extracted by means of Solid Phase Micro Extraction (SPME), using a 65 μm Carbowax™ divinylbenzene fibre, extraction for 1 h at 85°C. The gas chromatograph GC 8000 (Fisons Instruments, Milan, Italy) was equipped with a 60 m ´ 0.32 mm column coated with Supelcowax 10 (Supelco Bellafonte, USA). The Fisons MSD 8000 mass spectrometer was used as a detector.

RESULTS AND DISCUSSION

Between 65–140 compounds were identified in the volatile fraction of extruded products. They consisted of aldehydes, ketones, alcohols, carboxylic acids and hydrocarbons, but the most interesting volatiles were heterocyclic compounds because of their pronounced effect on the sensory value. Furans and pyrans (maltol) were the main oxygen-containing heterocycles (Table 1), and pyrazines and pyrroles the main nitrogen-containing heterocycles. The total peak area of furans and maltol represented the most important fraction of total heterocycles, however, pyrazines were more important than oxygen heterocycles because of their lower perception thresholds.

Table 1. Formation of furans and maltol during the extrusion cooking of mixtures of wheat semolina with glucose, amino acids and proteins

Extruded mixture	Number of compounds	Peak area of furans	Peak area of maltol
Wheat semolina	5	13.2	0
Wheat semolina + 5% glucose	14	28.2	15.2
Semolina + 5% glucose + 2% amino acids	9–13	16.3–31.9	5.5–35.3
Semolina + 5% glucose + 10% protein	11–13	5.2–25.5	10.6–28.3

The content of oxygen-containing heterocycles is given above in Table 1. It is substantially lehigher in extruded mixtures containing sugars than in original semolina. Maltol was formed only in mixtures containing sugars. An addition of amino acids and proteins mostly increased the concentration of furan derivatives, but the same is not valid for the formation of maltol.

The content of pyrazines in the volatile fraction was lower than in model experiments, performed under roasting conditions (4). Substituted pyrroles and pyridines were formed only in small amounts as they are mostly formed at higher, roasting temperatures, which are less favourable to the formation of pyrazines (5). The pyrazine content was relatively low in extruded semolina (Table 2), but it has substantially increased by addition of sugars, and still more, by addition of free amino acids to a mixture of semolina with 5% D-glucose. The reason is that dicarbonylic degradation products of D-glucose decompose amino acids after the Strecker degradation mechanism, and ammoniac or volatile amines produced in this reaction form easily various pyrazines with other dicarbonylic degradation products of sugars. Not only the number of pyrazines, but also the relative peak area of pyrazines increased in this way.

Table 2. Influence of sugars, aminoacids and proteins on the formation of pyrazines in extruded samples

Extruded mixture	Number of pyrazines	Relative peak area of pyrazines
Wheat semolina	11	2.7
Semolina + 5% sugars	16–17	4.8–13.3
Semolina + 5% glucose + 2% amino acids	23–40	8.1–25.8
Semolina + 5% glucose + 5% proteins	19–22	2.5–3.3

Pure amino acids are rather expensive so that an addition of commercial plant-scale produced proteins or protein concentrates would be preferable. Results are summarized in Table 3. The number of pyrazines produced in course of the extrusion process was about the same as after addition of D-glucose to semolina. Mostly methyl and ethyl substituted pyrazines were detected, but vinyl and acetyl substituted pyrazines were also present in small amounts. Pyrrolo, cycloalka and furano pyrazines were present only in traces, if at all, as they are formed easily only at higher reaction temperatures, corresponding to roasting (6).

The relative peak area of pyrazines was smaller in mixtures containing proteins than in mixtures containing free amino acids. The reason is that most free amino groups available in protein molecules are ε-amino groups of bound lysine, which cannot participate in the Strecker degradation, and thus, do not contribute to the formation of pyrazines. Nevertheless, extrusion products obtained from mixtures of semolina with proteins possess still agreeable bread crust flavour. An addition of proteins to the extruded sample thus seems to be justified for the production of suitable extrusion products.

Table 3. Effect of proteins on the pyrazine formation in semolina-glucose-protein mixtures during extrusion

Protein added	Amount of protein [%]	Number of pyrazines	Relative peak area of pyrazines
Casein	10	17	1.77
Skim milk powder	10	18	3.18
Gluten	10	19	3.32
Egg albumin	5	19	2.48
	10	19	1.68
Full-fat soya flour	10	15	0.97
Extracted (defatted) soya flour	5	22	3.32
	10	22	3.11
	20	22	4.85
	30	22	6.43

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The Organoleptic Evaluation by Sniffing Method on Volatile Compounds in Glucose-Lysine

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Abstract

Besides the color and the taste, the flavor is an important factor in evaluating the quality of the thermal processed food. It has been proved it is possible to identify hundreds of compounds, which contribute to the proportion of the flavor in each boiled, baked or roasted food. In this study, there are set forth the outcomes from the separation, identification and organoleptic evaluation of some volatile compounds, obtained in the glucose-lysine model system. The system was thermal treated at 100°C in Likens-Nickerson apparatus. The volatile compounds, extracted with ethyl ether-pentane, were separated by means of Gas-Chromatography method; then, they were identified by means of GC-MS method. The system revealed a decreasing of pH during the heat treatment at the same time with the formation of the volatile compounds. In these circumstances, there are prevalently formed the furan and furan derivatives, all having caramel flavor. Compounds from pyrazines class result at pH 7–6.5, but not at pH 5–4. The evaluation of the flavor features has been made using a *sniffing* installation attached to the evacuation extremity of the capillary column belonging to the GC instrument. Thus, it has been obtained the "aromagrama" containing more than 40 volatile compounds, extracted from the glucose-lysine model system. The sniffing analysis coupled with GC-MS can be successfully applied to the selection of the volatile compounds, which selection depends on the specific flavors.

Keywords: glucose; lysine; model system; Likens-Nickerson apparatus; sniffing; CG-SM

INTRODUCTION

The heterocyclic compounds play an important role in food flavors and particularly in heated food products. They are formed during thermal interaction of aldehydes and ammonia, which result from degradation components of lipids, amino acids and sugars. Amino acids can react with reducing sugars very rapidly; in the course of reaction latter are decomposed forming highly reactive compounds (1, 2).

Many of the heterocyclic compounds have interesting organoleptic properties and relatively low flavor threshold values, and they deserve a particular attention (3, 4).

The aroma and color of heated foods are very important for consumers. In heated foods, the Mallard reaction is responsible for the development of aroma compounds (5, 6).

In this paper there is studied the reaction of glucose with lysine in order to separate, and to evaluate by *sniffing* method on volatile compounds.

The flavor products generated from Maillard reaction in the glucose-lysine model system has been studied. Aqueous solutions of glucose (1M) and lysine (1M) (initial pH 7) were refluxed without control of the pH and time of heating and temperature (100 °C).

MATERIALS AND METHODS

Lysine monochloride (Sigma Italy), D(+)-glucose anhidrous (RPE-ACS reagent, Carlo Erba, Milano, Italy), penthane, ether (BAKER ANALISED-REAGENT, Holland).

Making the extract of volatile compounds. Solutions of glucose (0.5M) and lysine mono chloride (0.5M) in bidistilled water have been refluxed in a distiller /extractor of type LICHENS-NICKERSON. The extraction solvent was a mixture of pentane: etylic ether of ration 9:1. The whole system was heated to 100 degrees without pH control. The extracts thus obtained have been concentrated into a volume of 0.5–1mL by using a Vigreux column up to a final volume of 0.02–0.25mL with the apparatus Kuderna Danish.

The chromatographic analysis of gases and the mass spectrometry (CG-SM). The CG-MS analysis was made by means of a gas chromatograph Varian, model 3400 connected to a mass spectrometer VARIAN SATURN (IT DMS).

The volatile compounds separation has been achieved by using a capillary column DB-5. The conveying gas was He of 1.5 mL/min flow. The instrument was fitted with a personal computer Filer Karnak and a printer EPSON LX-400. The working conditions of the mass spectrometer were: the ionizing technique: electron impact; electron energy: 70 eV; ion collector temperature: 180°C; the transfer line temperature: 200°C; ionizing current: 10 mA; acceleration voltage: 4kV; scanning time: 0.1 sec; mass range: 30–2000; time between two scanning: 0.5 sec; resolution: 1000; injection technique: splitless; injector temperature: 210°C.

The Program of temperatures form the capillary column: 40°C (5 min.), 20°C/min., 80°C (0 min.), 80°C/min, 200°C (20 min.). The components have been identified by means of standards and the data bank (WILEY 5 MS) belonging to the mass spectrometer.

Marks of flavors have been attributed using the sniffing technique.

RESULTS AND DISCUSSION

From Fig. 1 it can be noted that with higher heat treatments, a higher acidity of the model system investigated is reported.

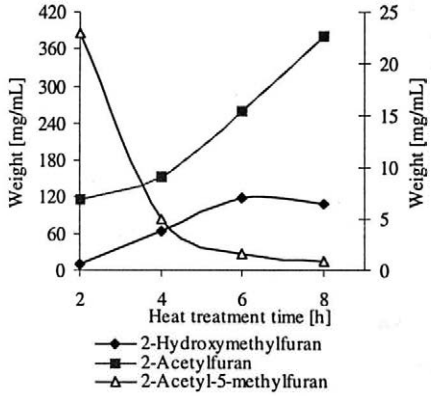

Figure 1. The pH values of model system glucose-lysine versus heat treatment time

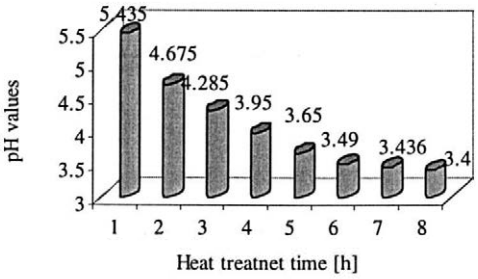

Figure 2. The quantitative evolution of some furan derivates in the glucose-lysine thermic treated system (μg/mL)

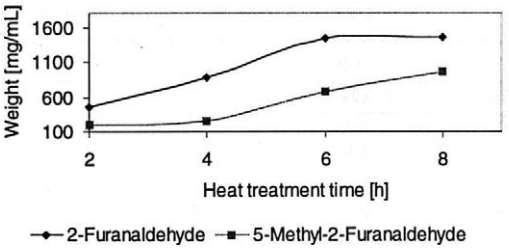

Figure 3. The quantitative evolution of 2-furanaldehyde and 5-methyl-2-furanaldehyde in the glucose-lysine thermic treated system (mg/mL)

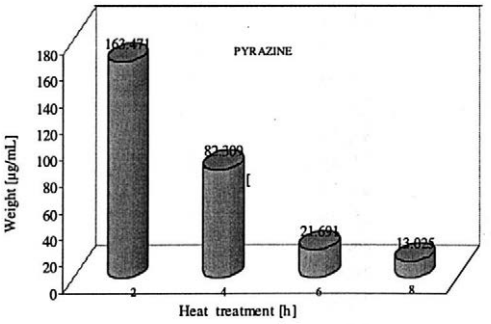

Figure 4. The quantitative evolution of pyrazines versus heat treatment time

Using GC and GC-MS analysis, more than 40 major components of flavors have been identified. Furan and furans derivatives were obtained and the amounts have been correlated with pH and time of heating model system (Fig. 2, 3).

The pyrazines (Fig. 4) were detected at pH 7–6.5, but not at pH 5–4. Our results are in good agreement with hypothesis that at a basic pH, flavours pyrazines is formating in the Maillard reaction. Generally the furan derivates accumulate in the system, while the concentration of nitrogen compounds (pyrazines and pyrrol derivates) decreases.

The evaluation of the flavor features (Table 1) has been made using a *sniffing* installation attached to the evacuation extremity of the capillary column belonging to the GC instrument. Thus, it has been obtained the "aromagrama" of more than 40 volatile compounds, extracted from the glucose-lysine model system.

The sniffing analysis coupled with GC-MS can be successfully applied to the selection of the volatile compounds, which selection depends on the specific flavors.

Acknowledgement: We are greatly indebted to Professor CARLO R. LERICI and his work group from Food Chemistry Department, University UDINE–ITALY for the substantial support.

Table 1. The organoleptic evaluation by sniffing method on volatile compounds in the glucose-lysine model system

No.	T.R.	Symbol	The odour	The identified compounds
(1)	(2)	(3)	(4)	(5)
1.	3, 85	A	caramel	2,3-Pentandione
2.	4, 62	B	caramel, burnt, pungent	Pyrazine
3.	8, 20	C	sweet, caramel	2- Methyltetrahydrofuran-3-ona
4.	11, 27	D	caramel	2-Formylfurane
5.	12, 51	E	burnt sugar	–
6.	12, 64	F	unidentified	–
7.	13, 41	G	sweet, caramel	5-methyl-2(3H)-Furanone
8.	14, 47	H	burnt caramel	Furfuryl alcohol
9.	15, 55	I	sweet caramel	2-Pyranone
10.	16, 06	J	burnt sugar	2-Acetylfurane
11.	16, 58	K	etherized	–
12.	18, 31	L	easily vinegary	–
13.	19, 09	M	bitterish	2-Ciclohexen-1-ona
14.	20, 33	N	almonds	5 – Methylfurfurale
15.	21, 25	O	etherated	2,3,4-trimethyl-2,3-dihydro-Furane
16.	21, 85	P	etherized	–
17.	22, 50	Q	caramel, burnt	–
18.	24, 70	R	sweet caramel	2-Acetyl-5-methylfurane
19.	25, 59	S	cigar smoke	–
20.	26, 11	T	unidentified	–
21	26,88	U	burnt, caramel	–
22.	27, 25	V	sweet, caramel	–
23.	27, 72	X	burnt rubber	–
24.	28, 72	Y	etherized	2-Acetylpyrole
25.	29, 07	Z	unpleasant	–
26.	29, 17	W	burnt caramel	–
27.	30, 39	A'	burnt oil	–
28.	30, 64	B'	burnt rubber	–
29.	31, 81	C'	etherized, pungent	2-Formyl-N-methyilpyrole
30.	32, 62	D'	unpleasant, pungent	1H-1,2,4-Triazol-3-phenyl
31.	32, 79	E'	burnt rubber	–
32.	32, 93	F'	mushrooms	–
33.	33, 89	G'	burnt oil	–
34.	34, 20	H'	unidentified	–
35.	34, 38	I'	unidentified	–
36.	35, 87	J'	almonds	–
37.	37, 59	K'	burnt, sweet	Bis-(5-methyl-2-furyl)-methane
38.	39, 62	L'	burnt, sweet	–
39.	42, 59	M'	burnt	2-Hydroxy-5-methyl-acetofenona
40.	44, 54	N'	burnt rubber	Eicosane

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Metabolic Transit of Amadori Products in Rats and Humans

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Abstract

In several studies the absorption and urinary excretion of free and protein bound Amadori products were measured in rats and humans. Both, in vitro tests with everted intestinal sac preparations and in vivo experiments, show that there is no active intestinal transport of these compounds but an absorption by diffusion. Trials with tissue slices have shown that there was an uptake into the cells of the liver, kidneys and muscles. Metabolism of Amadori products, if it exists in animals, tends to be extremely low. Micoorganisms in the large intestines decompose the Amadori products almost completely. The profile of urinal excretion of Amadori products after the ingestion of test meals showed a rapid elimination of the absorbed part, while the fecal output, although low because of the hind gut fermentation, persisted up to 3 days. Only 1–3% of the ingested amounts of protein bound Amadori products were recovered in the urine, which suggests a low absorption rate. Protein, rich in Amadori products, but not the isolated compounds, exhibited cytomegalic effects in the proximal tubules of the outer medullary zone of the kidneys similar to the well known lesions caused by lysinoalanine. Some other adverse effects, e.g. interference with the transport of some amino acids and increased renal losses of zinc and copper, were observed.

Keywords: Amadori products; lysinoalanine

In vitro and *in vivo* Studies on the Metabolic Transit of N^{ε}-Carboxymethyllysine

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Keywords: carboxymethyllysine; CML; casein; xenobiotics; dietary glycation

N^{ε}-Carboxymethyllysine (CML) has been identified as an advanced glycation end product (AGE) in both severely heat treated, browned foods and in living organisms. The consequences of the lifelong dietary intake of these browning products, termed as melanoidins, and of their precursors were viewed neither in the context of their bioreactivity nor in relation to specific organ functions, until it was demonstrated in two recent studies that orally absorbed glycation products may exhibit toxic effects to different organs, mainly the kidney (2, 3).

Specific *in vivo* effects of dietary glycation-products have not been identified so far because of their vastly heterogeneous classes. Among them, only two known structures, the highly reactive dialdehyde methylglyoxal and the putatively non-reactive AGE CML were analysed both by using specific antibodies (5) or by HPLC-analysis (1).

In the present transit studies, casein-bound CML was administered to rats for 10 days at two different doses (100 and 300 mg CML/kg body weight/d). Metabolic transit data were followed by faeces, urine and tissue samples and the effects on xenobiotic phase-I and phase-II enzymes were investigated in the small intestines, the liver and kidney. *In vitro* experiments on intestinal Caco-2 cells were performed to study CML mediated cellular mechanisms of enzyme induction and signal transduction pathways, the latter putatively mediated by binding to the receptor for advanced glycation end products (RAGE).

As a result, the transit data suggest that the ingested protein-bound CML is absorbed as the pure compound. Urinary and faecal excretion as well as tissue levels of CML reflected the ingested dose, whereas plasma levels of CML increased 10-times after a 3-fold increase of orally administered casein-bound CML. Phase-II glutathione-S-transferase (GST) activity was markedly increased in the kidney (4).

Results obtained from the *in vitro* experiments, using CML as the pure compound, confirmed the inductive effect on GST activity. For the casein-bound CML it was shown that signal transduction pathways were induced supporting the hypothesis that protein-bound CML may act as a ligand of RAGE.

In conclusion, dietary glycation products containing CML are shown to enhance the endogenous burden of AGEs, namely CML, and to cause specific biologic effects both at the tissue and cellular level. With respect to the lifelong exposition of dietary glycation-products, future studies are needed to characterise the chemical structure of further compounds and their bioreactivity.

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Markers of Oxidative Stress in Enteral Solution

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Keywords: oxidative stress; antioxidant activity; melanoidins

INTRODUCTION

Formula diets for enteral nutrition (EN) must be a complete and balanced source of proteins, carbohydrate fats, mineral and vitamins. These products are widely used for the nutritional treatment of patients who cannot be orally fed properly and they often represent the only source of nutrients (1). Formula diets contain animal (milk) or from vegetal (soy) protein, macronutrients, mineral and vitamins. Carbohydrates are prevalently maltodextrins with variable amounts of oligosaccharides. These products are subjected to severe thermal treatments to ensure sterilisation and long shelf life, the simultaneous presence at high amount of reducing carbohydrates and proteins greatly speed up Maillard Reaction (MR) leading to the decrease of nutritional value and to the formation of potentially harmful compounds. Indeed the intake of advanced glycation end products (AGEs) represents an additive risk factor in diabetes (2) and diabetic nephropathy (3). The occurrence in enteral formulas of antioxidant molecules is also of interest. It is important from a technological point of view (to preserve fatty acid oxidation) but also for improving nutritional quality of products. Antioxidants, mostly present in these products, are Ascorbic acid, vitamin E and also MR products.

In this work we have considered five different enteral formulas looking for the presence of some indicators of MR. In particular we measure the formation of melanoidins and the presence of protein bound carbonyl compounds. Beside MR also the antioxidative properties of these products were investigated to ascertain if there is a correlation between antioxidants and formation of MR products.

EPERIMENTAL

Materials: Chemicals of the highest purity, purchased from Serva (Heidelberg, Germany). Enteral products were obtained from Nutricia, Novartis and Abbot, Pasteurized (numbered from 1 to 5), (P) UHT (U) and Sterilized (S) milks were purchased from local supermarkets

Methods

Hydrophilic Antioxidant Activity (DMPD method). The total hydrophilic antioxidant activity (HAA) was determined accordingly to a published procedure (4). A dose-response curve was derived for AscH solutions by plotting the absorbance at 505 nm as percentage of the absorbance of the uninhibited radical cation solution (blank) according to the equation: (Inhibition of A_{505} (%) = $(1 - A_f/A_0) \times 100$). The A_0 is the absorbance of uninhibited radical cation and A_f is the absorbance value 10 minutes after the addiction of the sample. Protein-free samples were prepared by isoelectric precipitation (5) in order to eliminate interference. HAA was expressed as AscH equivalent (mg/ml solution).

Lipophylic Antioxidant Activity (ABTS assay). Lipophilic antioxidant activity (LAA) of enteral samples was measured in accordance with a published protocol (6). The antioxidant activity was expressed as Trolox Equivalent Antioxidant Capacity (TEAC) on lipophylic material was extracted from 1 ml aliquots of enteral solution by using an isopropanol-chloroform mixture (5:3.5 v:v) (7).

Size Exclusion Chromatography. The amount of brown polymers (melanoidins) was determined by High Performance Liquid Chromatography (HPLC). Casein fraction were isolated, from both enteral solution **P**, **U** and **S** milk samples, by isoelectric precipitation (5) and then lyophilized. Protein aliquots (200 mg) were carefully weighed and dissolved in 9 ml of 0.2 M NH_4HCO_3 (pH 8) containing 0.1 μM DTT. Aliquots (0.1 ml) of the protein solution was fractionated by a size exclusion column (TSK-gel G 3000 PWXL) (TosoHaas, Japan). Stationary phase was equilibrated and eluted with 0.2 M NH_4HCO_3 (pH 8) at 1 ml/min flow rate. The elution of macromolecular protein aggregates (melanoidins) was monitored at 420 nm and their levels expressed as Absorbance Unit (AU)/mg of protein.

Carbonyl Assay. The level of protein-bound carbonyls (PC) was measured in enteral samples and in milk samples (**P**, **U** and **S**; N = 12), by indirect ELISA essentially according to a published protocol (8), but oxidized and reduced casein were prepared and used for standard curve construction. PC level in standard curve point was determined spectrophotometrically (A_{370}) after reaction with 2,4- Dinitrophenylhydrazine (DNPH) and the PC amount was calculated using the molar extinction coefficient of 22,000 M^{-1} cm^{-1} (9). PC content was finally expressed as nmoles/mg of total proteins.

RESULTS AND DISCUSSION

PC accumulation in enteral formula depends upon their carbohydrate and lipid content. Carbonyl groups are early intermediates during the Maillard reaction (MR) and their level was used to evaluate the extent of oxidative stress in enteral formulas. Values of calibration curve ranged between 0.2 and 30 nmol/mg and increased linearly both in spectrometric (A_{370}) and ELISA (A_{490}) assay (r^2 = 0.998 and 0.970, respectively). PC content in enteral solutions (Table 1) was higher than that measured in thermal-treated milk samples (ranging from 1.02 to 8.84 nmol/mg). The correlation between PC accumulation and the heat treatment intensity cannot be verified in enteral formula because no details about the thermal treatment of these products are available. In enteral formula PC level are negatively correlated with the protein content (r = –0.91) and it is positively correlated with and carbohydrate or lipid amount (r = 0.74 and 0.87, respectively). This result is in accordance with previous data, indicating a positive correlation between MR yield with the sugar/protein ratio and the contribution of oxidized sugar and lipid to PC accumulation onto proteins.

Enteral products differ for their melanoidin content. During the late phase of the MR, brown high molecular weight polymers called melanoidins are produced. The amount of melanoidin formed depends on the composition and on the intensity of the thermal treatment. Since glycoxidative pathway is also involved in polymer formation, the presence of antioxidants can interfere with melanoidin formation. Melanoidin are eluted with the void volume of the column (MW > 100 kDa) with a retention of time of 5.5–5.6 min and the measured amounts expresses as peak area/mg of protein are in (Table 2). Melanoidin level in milk samples is positively correlated with the thermal treatment intensity, whereas, in enteral solutions they range between samples (from 1.6 to 5.1 × 10^6 AU/mg).

Table 1

Samp	PC* nmol/mg	Protein g/100mL	Sugars g/100mL	Lipids g/100mL
P	1.02 ± 0.18	3.2	4.8	3.5
U	4.81 ± 0.98	3.2	4.8	1.5
S	7.84 ± 1.00	3.2	4.8	1.5
1	11.52 ± 0.02	6.2	10.6	9.2
2	10.01 ± 0.06	5.6	13.4	2.8
3	15.55 ± 0.02	4.0	12.3	3.9
4	13.41 ± 0.03	4.2	13.4	3.4
5	18.61 ± 0.05	2.9	25.6	9.6

* mean ± SD

Table 2

Sampl	Melanoindin level	Sampl	Melanoindin level
P	7.2 × 10^5	2	3.1 × 10^6
U	1.2 × 10^6	3	5.1 × 10^6
S	1.4 × 10^6	4	1.5 × 10^6
1	3.4 × 10^6	5	1.6 × 10^6

A negative correlation between melanoidin and PC levels (r = –0.76) was found suggesting that the extensive cross-linking may lead to a decrease of exposed carbonyls on the protein surface. This explanation is consistent with literature data referring PC accumulation during the initial stages and the formation of insoluble aggregates during the late phase of the MR.

Melanoidin yield is affected by the antioxidant content. Antioxidants may adversely affect the MR extent, by contrast, the oxidation of AscH may leads to the formation of compounds capable of reacting with protein amino groups via MR. Antioxidative ability (HAA and LAA) of enteral products was evaluated by colorimetric methods. Results are summarised in Table 3 where the amount in µg × 100ml of AscH and Vitamin E (TH) was reported. The positive correlation between LAA and the TH concentrations (r = 0.89; $p < 0.05$) indicated that this compound was the mayor contributor to the LAA. By contrast HAA was not strictly related to the amount of AscH. This is consistent with the well-known role of Maillard reaction products in determining the HAA (10). The correlation HAA, LAA and melanoidin yield was also evaluated. A negative correlation between LAA and melanoidins (r = –0.79) was found whereas no correlation between HAA and melanoidins was observed.

Table 3

AscH	TH	AAL	AAH
42.3	4.8	70	4.8
13.3	1.3	43	8.8
9.5	1.1	38	1.5
12.4	3.2	85	3.1
22.4	6.0	65	1.7

CONCLUSION

In this study, the oxidative status of enteral solution is reported. Oxidative stress markers, HAA and LAA were

determined in enteral solution and in differently heated milk samples. PC and melanoidin are positively correlated with the heating intensity in milk products whereas in enteral products they widely varied, being affected by macronutrient composition and especially by the oligosaccharides/proteins ratio. In this case, unfortunately, there were no enough information to assess the relationship between the thermal process parameters and the MR extent. The composition of the analyzed products was not homogeneous and also it was not possible to get information about processing conditions of each sample.

Further experiments are required for better understanding the relationship between chemical composition and oxidative susceptibility of enteral products.

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Maillard reaction indicators in commercial tomato products

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Abstract

The quantitative analysis of 2-furoylmethyl amino acids (2-FM-AA) in commercial tomato products was carried out in order to evaluate their utility as indicators of the damage caused by Maillard reaction during processing. The presence of 2-FM-AA was observed in all types of commercial tomato products. Differences between samples may be attributed to the variety of processing technologies employed to manufacture the different commercial tomato products. In general, 2-furoylmethyl-GABA (2-FMGABA) was present in larger amount than furosine and can be considered an adequate indicator for early detection of Maillard reaction in these kind of foods. The presence of high amounts of 2-FM-AA in tomato pulp and juice samples may be attributed to the use of tomato concentrate in their manufacture.

Keywords: 2-Furoylmethyl amino acids; processed tomato products; Maillard reaction

INTRODUCTION

The heating conditions applied in the tomato industry may cause chemical and physical changes, which affect the quality and nutritional properties of the final products. Changes are due to complex reactions including Maillard reaction that has been considered predominant. Reaction progress can be followed through out the measure of 2-furoylmethyl amino acids (2-FM-AA) produced by acid hydrolysis of the Amadori compounds (first stable products of the Maillard reaction) (1, 2).

This study was undertaken to evaluate whether the quantitative determination 2-FM-AA would allow to detect the Maillard reaction extent in different types of commercial tomato products.

EXPERIMENTAL

Samples. 24 commercial samples of different tomato products were purchased in local markets: five samples of tomato pulp, six ketchups, five juices, seven sauces, and one sample of double-concentrated tomato paste (28%).

Analytical Methods. Dry matter content of samples (g per 100 g of product) was determined following the AOAC Official Gravimetric Method n. 964.22 (3).

°Brix were determined using a refractometer ATAGO type 500.

2-FM-AA analysis. An ion-pair RP-HPLC method (5) was used to analyze 2-FM-AA. Tomato samples were hydrolyzed according to the procedure of Hidalgo *et al.* (1).

All analysis were performed in triplicate.

RESULTS AND DISCUSSION

2-FMGABA and 2-furoylmethyl-lysine (furosine) were found in commercial tomato products. Figure 1 shows the chromatographic profiles corresponding to tomato pulp (A) and double-concentrated tomato paste (B).

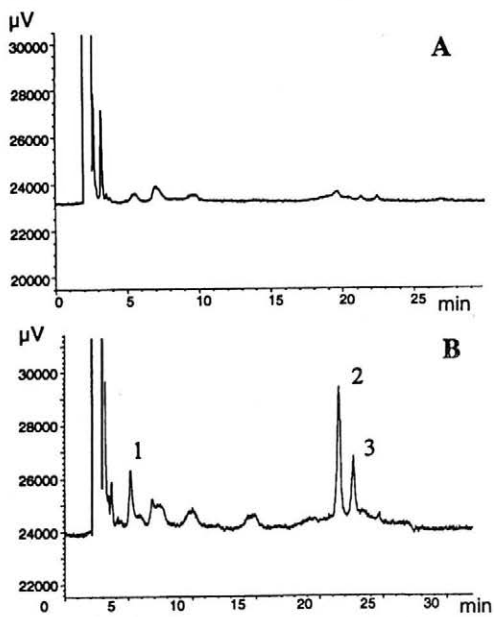

Figure 1. HPLC profiles of 2-FM derivatives of (1) pyroglutamic acid, (2) GABA and (3) lysine (furosine) from tomato pulp (A) and double-concentrated tomato paste (B)

Low amounts of 2-FMGABA and furosine were detected in tomato pulp samples whereas double concentrated tomato sample showed the maximum 2-FM-AA content. Besides 2-FMGABA and furosine, a third compound assigned as 2-furoylmethyl-pyroglutamic acid (2-FMPCA) was also detected in double-concentrated tomato sample.

Hidalgo *et al.* (1) reported that furosine appears to be a good heat damage index of tomato products. Present results show that 2-FMGABA is formed in larger amounts than furosine which confirm the potential utility of this compound as indicator of the Maillard reaction development in this kind of foods.

The presence of others 2-FM-AA different than furosine could be explained taking into account the results previously reported by Eichner *et al.* (6) on the chemical changes during the tomato processing. These authors detected the presence of fructose-pyrrolidone-carboxylic acid (Fru-PCA), fructose γ-aminobutyric acid (Fru-GABA) and fructose-glutamic acid (Fru-Glu) in tomato paste and they were proposed as early chemical markers for undesirable changes during the heat processing. On the other hand , the possible conversion of Fru-Glu to Fru-PCA by cyclization in acid medium has also been referred by these authors (6). The absence of 2-furoyl methyl-glutamic acid (2-FMGlu) in the present study may be due to the possible conversion of Fru-Glu to 2-FMPCA during the acid hydrolysis.

Figure 2 shows the plot of 2-FMGABA against furosine in the studied samples. Variations in 2-FM-AA content between samples of the same type of products may be related to the different conditions applied during their manufacture. Tomato pulp ($n = 3$) and juice ($n = 2$) samples obtained from fresh tomato showed negligible amounts of 2-FM-AA, whereas higher amounts of these compounds were found in tomato pulps ($n = 2$) and juices ($n = 3$) produced from concentrates. Traces amounts of 2FM-AA were also detected in two of the seven sauces samples. These results indicate that tomato juices, pulps and sauces may be manufactured without appreciable changes due to Maillard reaction. In all ketchup samples the presence of 2-FMGABA and furosine were detected. Double-concentrated tomato paste showed the highest 2-FM-AA content. The presence of 2-FM-AA in these products may be caused by severe heat treatment or the use of tomato concentrate in their manufacture.

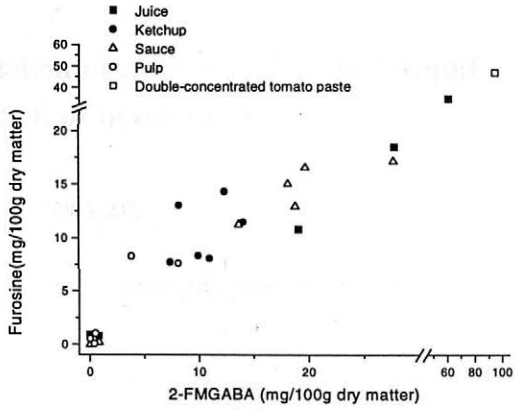

Figure 2. Graphical representation of 2-FMGABA against furosine contents (mg/100 g dry matter) in commercial tomato product

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Improvement of β-Casein Functionality by Non-Enzymatic Glycation in Relation to its Structural Changes

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Abstract

The functionality of β-casein (βCN) can be altered by glycation. The degree of glycation was estimated to be approximately six glucose units bound to one molecule of βCN. In contrast to stabilizing properties, the foam-forming properties of glycosylated βCN were better when compared with the intact one. Increases in emulsion-forming and -stabilising properties were observed. These properties were worse at higher pH and changes in ionic strength did not affect them. The surface excess was greater for glycosylated βCN than for the intact one and increased with decreasing pH value. It appeared that the incorporation of sugar moieties resulted in a type β-turn formation after adsorption onto the interface. The changes in secondary structure content were more indicative at lower pH value.

Keywords: β-casein; non-enzymatic glycation; functionality; structural properties

INTRODUCTION

Bovine β-casein is a flexible, approximately random coil protein. It can be used for its functional properties (1). A number of molecular parameters such as mass, conformation, net charge and hydrophobicity of proteins have been shown to play important roles in the definition of their functional properties (2). A number of attempts have been made to improve the functional properties of milk proteins by chemical modifications (3). Chemical modifications are useful tools in the study of structure-function relationship of proteins. One of such modifications, is non-enzymatic glycation as reported in this study. The preparation of glycoproteins has been done to immobilize enzymes; to increase the heat stability of enzymes and inhibitors; to improve the functional properties of proteins (4–7). In our earlier studies we concluded that glycosylation of βCN proceeded in stages. We found that isoelectric point and solubility increased upon glycation. Potential structural changes within βCN were confirmed by different UV adsorption spectra and better heat stability after glycation (7–9).

The present work was carried out to determine the the effect of covalent binding of glucose molecules to βCN on its emulsion, foam and structural properties. The influences of pH and ionic strength on the above-mentioned properties were also investigated.

EXPERIMENTAL

Materials. Bovine βCN, containing mainly the genetic variants A^1 and A^2, was purchased from Eurial (France). All other chemicals were purchased from Merk (Germany) or Sigma (England).

Methods. *Glycosylation* was carried out according to the method by Nessar and Furth (10). Intact bCN (0.5 mg/cm^3) was incubated in 0.05 M sodium phosphate buffer (pH 7.4) containing 0.5 M glucose and 0.003 M sodium azide for 24 h, at 37°C. The amount of glucose being bound was checked up by analysis of matrix-assisted laser-desorption/ionisation time-of-flight (MALDI-TOF) mass spectra (11). *The foaming and emulsifying properties* of intact and glycosylated βCN were studied in a small-scale screening tests at pH 4.0; 6.7 and 9.0 and ionic strength 20–75 mM (11). The emulsion-forming property was determined by measuring the droplet-size distribution (d_{32}). The emulsion instability was estimated by measuring a decrease in turbidity at 500 nm. The surface excess was determined with a depletion method. The foams were made by whipping for 60s at 2500 rpm and monitored for 1 h. *Far-UV CD wavelenght spectra* of intact and glycosylated βCN as well as their dispersions covered teflon particles were recorded as averages of 10 spectra on Jasco J-715 spectropolarimeter. The secodary structure content of the proteins was calculated using a non-linear regression-procedure (11).

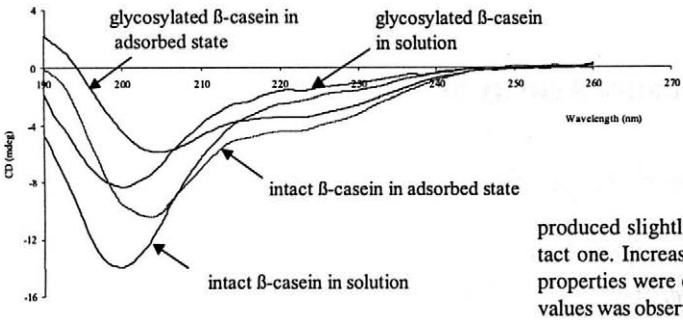

Figure 1. Far-ultraviolet CD spectra of intact and glycosylated β-casein recorded at pH 6.7 and I = 20 mM free in solution and adsorbed at teflon

Table 1. Structural, emulsion and foam properties of intact and glycosylated β-casein[a]

Structural/emulsion/foam		β-casein Intact	β-casein Glycosylated
Γ [mg/m²][a]	pH 6.7	3.1	3.3
	pH 9.0	2.8	3.1
α-Helix[b] [%]	pH 6.7	10/19	10/16
	pH 9.0	7/13	7/15
β-Turn[b] [%]	pH 6.7	2/3	19/23
	pH 9.0	2/3	18/21
Emulsion	pH 6.7	1.7	1.5
Formation[c]	pH 9.0	1.9	1.7
Emulsion	pH 6.7	90	100
Stabilization[d]	pH 9.0	80	90
Foam	pH 6.7	++	+++
Formation[e]	pH 9.0	++	+++
Foam	pH 6.7	++	++
Stabilization[e]	pH 9.0	++	++

[a] Determined at I = 20mM and 75mM
[b] Estimated value calculated from CD spectra of samples in solution/adsorbed samples
[c] Average dropley-size (mm) immediately after homogenising
[d] % of turbidity left; turbidity at 0 hrs is set up to 100%
[e] – and + indicate the extent of foam formation and stabilization

RESULTS

It was reported that susceptibility of individual lysine residues for saccharide attack was extremely different with Lys 28/29, Lys-32, Lys-99 and Lys-107 being preferably glycated (7, 8). It appeared that the N-terminal part of βCN with six potential reaction sites is the most favourite place for glycosylation. The results obtained via MALDI-TOF MS support that point of view. From the data obtained via mass spectrometry the degree of glycation was estimated to be approximately six glucose units bound to one molecule of βCN. In Table 1 we present the foam and emulsion screening test results and structural properties of βCN. In contrast to stabilizing properties (they were not significantly changed), the foam-forming properties of glycosylated βCN were better when compared with intact one.

Generally, our results show that neither pH nor ionic strength had a significant effect on foam-forming and -stabilising properties of glycosylated βCN. Glycosylated βCN produced slightly smaller emulsion droplets than the intact one. Increases in emulsion-forming and -stabilising properties were observed. Moreover, the influence of pH values was observed. These properties were worse at higher pH. Despite the differences in droplets size and recoalescence of the emulsion during emulsification at all pH's used, a change in ionic strength did not affect the properties that were investigated.

A prerequiste of proteins to form emulsions is their adsorption onto the oil/water interface. Therefore, the secondary structure of intact and glycosylated βCN both in solution and adsorbed onto a hydrophobic teflon/water interface were studied by far-ultra violet circular dichroism. The CD measurements of teflon-adsorbed intact and glycosylated βCN were performed at the saturation coverage only. The surface excess was greater for glycosylated βCN than for the intact one. Furthermore, the surface load increased with decreasing pH value. The ionic strength had no influence on it. Hydrophobic teflon surface layer favored the transformation of the loop fragments of βCN into α-helix. On the other hand, it appeared that the incorporation of sugar moieties resulted, with high probability, in a type β-turn formation after adsorption onto the interface. The changes in secondary structure content were more indicative at lower pH value.

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Antioxidative Activity of Melanoidins

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Abstract

Melanoidins may deterioration reactions in food and induce preventive effects against biological damage. Several mechanisms can be reponsible for autooxidative activity of melanoidins. The possibility of melanoidins to scavenge free oxygen was detected with the help of an oxygen sensor. To measure the radical scavenging properties a stable radical (DPPH) was used and the combination with the redox system Fe^{2+}/Fe^{3+} made it possible to indicate electron transfer reactions. We investigated melanoidins formed under different reaction conditions (temperature, reaction time, water activity) and different starting materials (animo acids, carbonyl compounds). Result of each detection method were compared for various melanoidins and the influence of formations conditions were described. In general results of all methods show a comparable tendency. However it was not always a proportionality. With higher reaction temperature, longer reaction time or higher water activity of melanoidins was detectable. Mostly melanoidins with high colour intensity possessed the highest antioxidative activity. Nevertheless, in contrast the high molecular weight (nodialyzable) fraction of melanoidins dit not show such a strong antioxidative effect than the non dialyzed Maillard reaction mixture. Antioxidative activity of mealnoidins decrease with increasing dp of carbonyl compound used as starting material. Compared with glycine the use of arginine or alanine as an amino acid for melanoidin formation induced a higher possibility to scavenge free oxygen and radicals as well as a higher reducing power. The investigations show that melanoidin formation conditions influenced not only the strength of antioxidative effect but also the antioxidative mechanism a melanoidin worked. Therefore it seems possible to form melanoidins possess very specific antioxidative activity.

Keywords: melanoidins; antioxidative activity; redox system

C Lipids and Antioxidants

Resistance of High-Oleic Acid Peanut Oil against Autoxidation under Storage and Deep Frying Conditions

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Abstract

Oil from high-oleic SunOleic peanuts was compared with a conventional peanut oil from Virginia peanuts. In all methods used (Schaal Oven Test, Oxipres method, Active Oxygen Method (AOM), storage in emulsions, frying tests), the stability of SunOleic oil against oxidation was found substantially superior to that of Virginia peanut oil. Tocopherols were decomposed at much slower rate in SunOleic oil than in Virginia oil. Natural tocopherol content and differences in phospholipid composition could not explain better stability of SunOleic oil. The chief reason of its better stability was much lower content of polyunsaturated triacylglycerols, compared to conventional peanut oil.

Keywords: oxidation; peanut oil; phospholipids; tocopherols; triacylglycerols

INTRODUCTION

Most refined edible oils have low resistance against oxidation on storage and at frying temperatures because of high content of polyunsaturated fatty acids. The only exception is olive oil, where oleic acid prevails, and the content of linoleic acid is low. Therefore, new oilseed cultivars were developed with high oleic acid content and low linoleic and linolenic acid contents (1), suitable for various edible uses and even for frying (2). High oleic sunflower, soybean, linseed and peanut oils became available. High oleic peanut oil was studied in our laboratories, and some results were recently presented at a FECS meeting in Budapest (3). New results will be given here.

EXPERIMENTAL

Material. High oleic peanuts from the cultivar SunOleic (4) and conventional Virginia peanuts were extracted with hexane after Soxhlet. The linoleic acid content was 29.7% in Virginia oil and 3.1% in SunOleic oil.

Methods. The Schaal Oven Test was carried out at 40°C, and oxidative changes were monitored by weighing; the same test at 60°C was monitored by peroxide value determination. The procedure in the Oxipres apparatus was carried out at 100°C (5). The AOM oxidative stability was assayed at 97°C. Peanut oil emulsions were prepared using soybean lecithin as an emulsifier, and their oxidation was determined at 40°C in the presence of copper ions as a catalyst (6). In the frying test, french fries were fried at 177°C for 9 days, 3 fryings each day. Tocopherols were determined by HPLC using an electrochemical detector (7). Triacylglycerols were determined by HPLC using a Separon SGX-C18 (5 μm) column, and acetone, acetonitrile and methanol (4:2:1 v/v) as a mobile phase. Phospholipids were determined by HPLC after IUPAC 5.302 in extracts obtained after Folch. Polymers formed during the frying process were determined by high-performance size-exclusion chromatography (HP-SEC) with tetrahydrofuran as a mobile phase.

RESULTS AND DISCUSSION

An example of changes under conditions of the Schaal Oven Test at 40°C is shown in Fig. 1; SunOleic oil was at least 4 times more stable than Virginia oil. Similar differences in oxidative stabilities were obtained at 60°C. At 100°C and at the oxygen pressure of 0.5 MPa, as determined in the Oxipres apparatus, the induction period of Virginia oil was 11.95 h, and the induction period of SunOleic oil was 79.78 h, respectively. The ratio of induction periods was similar to results obtained using the AOM. The difference was moderately smaller in the frying experiment. The stability of peanut oils in an emulsion was equal to 2.0 h and to 3.3 h in cases of Virginia oil and SunOleic oil, respectively.

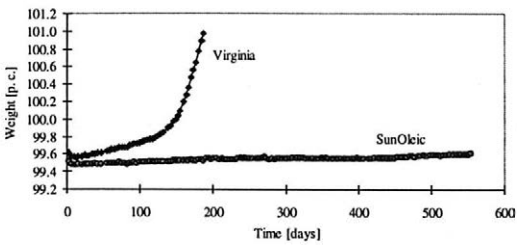

Figure 1. Oxidative stability of peanut oils

The contents of total tocopherols were 303 mg/kg in Virginia oil and 353 mg/kg in SunOleic oil, respectively. Ratios of a:g:d tocopherols were rather similar in the two oils. The observed differences in oxidative stabilities cannot thus be due to tocopherols. It was shown that the decomposition of a-tocopherol in the concentration of 5 g per kg of emulsion was much slower in SunOleic oil emulsion than in Virginia oil emulsion (Fig. 2).

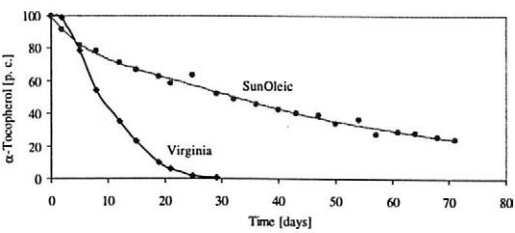

Figure 2. Degradation of α-tocopherol in emulsions of peanut oils

Differences in the phospholipid composition (means of 2 samples) were pronounced (Table 1) as SunOleic oil contained more acidic phospholipids and less phosphatidylcholine than Virginia oil. Metal traces could thus be better chelated in SunOleic oil. These differences could moderately affect the oxidative stability, but only in a moderate degree.

Table 1. Phospholipid composition of SunOleic and Virginia peanut oils

Phospholipid class	Virginia oil [p.c.]	SunOleic oil [p.c.]
Phosphatidylethanolamine	4.0	5.0
Phosphatidic acids	8.7	15.6
Phosphatidylinositol	13.0	23.4
Phosphatidylserine	9.2	9.9
Phosphatidylcholine	65.2	46.2

The only important difference between the two oils was in the triacylglycerol composition (Table 2), where the content of polyunsaturated triacylglycerols (ECN 40-46) was much lower in SunOleic oil than in Virginia oil. On the contrary, the content of low unsaturated triacylglycerols (ECN 48-56) was lower in Virginia oil than in SunOleic oil. The triacylglycerol composition of SunOleic oil is similar to that of olive oil, which is sufficient to explain its better oxidative stability.

Table 2. Triacylglycerol composition of SunOleic and Virginia peanut oils

Triacylglycerol classes	Virginia oil [p. c.]	SunOleic oil [p. c.]
ECN 40-46	55.73	8.59
ECN 48-56	44.29	86.71

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Degradation Effect of Microwave Heating on Fats

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Abstract

The oxidation stability of various substrates (lard, rapeseed and sunflower oils) was investigated during 10 min microwave and conventional heating experiments. It was observed that the microwave heating accelerates their oxidation two or three times faster than conventional heating. This effect starts when the temperature of samples rises over 100°C. Below this temperature the rate of oxidation was relatively slow. In directed microwave heating, when the temperature of samples was kept below 100°C by breaking the heating, only slight oxidation was observed. Some attempts were made to minimize this undesirable effect with the addition of antioxidants (0.1% of BHT or rosemary extract). The best results were observed for lard, moderate for sunflower and rapeseed oils.

Keywords: microwave heating; oxidation of edible oils; rosemary extract

INTRODUCTION

At present many households have a microwave oven. These ovens are widely used for cooking and food processing for its advantages, which include speed and convenience. There is a significant difference between microwave and conventional heating of foods (1). The heating of food when exposed to microwaves is produced by interaction of an electromagnetic field with the chemical constituents of foods. These interactions generate heat because of molecular friction. Some studies have been published on the effects of microwaves on food constituents (2) and on the nutrient retention in food. Several publications dealing with certain aspects related to edible fats (2–7) and its constituents (3) also appeared. The objective of this study was to determine the effects of microwave heating on lard, sunflower and rapeseed oils with and without addition of antioxidants.

EXPERIMENTAL

Materials

- Three common edible fats were used in this study (Table 1). Rapeseed (RO) and sunflower (SO) oils were obtained from Palma-Tumys a.s. Lard was purchased at the local market.
- Dry rosemary plant material was extracted in Soxhlet apparatus with 95 % ethanol, obtained solution was evaporated to dryness and resulting powder was used without further purification.
- BHT was commercial product (Aldrich).

Table 1. Chemical parameters of samples

Parameter		Lard	Oil	
			rapeseed	sunflower
Acid value	(mg KOH/g)	0.8	0.2	0.13
Saponification value	(mg KOH/g)	199	210.95	213.94
Iodine value	(g I_2/100 g)	59.8	11.6	125.4
Peroxide value	(0.5 mmol O_2/kg)	2.9	0.28	0.35
Tocopherols	(mg/100 g)	–	52.3	28.05

Heating experiments

The samples were treated in a microwave or conventional oven as follows:

- The five samples (5 g) + antioxidant (0,1% wt.) in open 100 ml Erlenmeyer flasks (approx. of the same diameter) was heated in Electrolux EME 1960 (750 W, 2.45 GHz) microwave oven. This run was a direct experiment. After each 2 min of heating one flask was taken off for analysis (temperature, peroxide and acid value) and replaced with the new one to keep the mass in the oven constant. This run was so called "parallel" experiment.
- In directed microwave heating experiments the conditions were the same but the microwave radiation was interrupted after each 2 min allowing the sample to dissipate the absorbed microwave energy (2 or 5 min).
- The silicon oil bath at the constant temperature 155°C was used for conventional heating experiments. The other experimental conditions were the same as above.
- Each reported value is a mean of two measurements.

RESULTS AND DISCUSSION

The oxidation stability experiments of all samples were run at the equal conditions (reaction flasks, contact surface with the air). Since the maximum temperature of samples after 10 min of microwave heating was about 155°C, conventional heating experiments were run at this temperature. The results are summarized in Tables 2.

Table 2. Peroxide values (0.5.mmol O_2/kg)) of lard, rapeseed oi and sunflower oil during heating experiments

Sample	Time of heating [min]	Conventional heating at 155°C	Microwave heating		
			Control (no antioxidants)	with 0.1% of BHT	with 0.1% of rosemary extract
Lard	0	2.51	1.34	1	1.36
	2	2.59	1.98	1.38	1.88
	4	4.67	4.76	1.55	2.36
	6	6.39	9.13	2.04	2.4
	8	7.8	13.96	2.41	2.55
	10	11.06	19.75	3.11	3.74
Rapeseed oil	0	1.26	1.82	1.15	0.83
	2	2.38	3.82	1.84	1.49
	4	4.57	8.56	2.5	1.83
	6	4.89	15.89	5.97	2.15
	8	5.45	17.26	10.45	5.3
	10	5.62	28.72	24.69	7.69
Sunfower oil	0	1.16	1.5	1.51	1.51
	2	1.63	2.17	1.99	1.65
	4	2.31	3.13	2.81	2.47
	6	3.91	6.15	5.02	6.68
	8	5.29	25.58	10.94	11.87
	10	6.51	41.9	15.48	14.98

Lard was the most stable and sunflower oil was the most sensitive in microwave heating experiments. In conventional heating experiments both plant oils were more stable than lard. The addition of suitable antioxidant (0.1% of BHT or rosemary extract) was capable to retard the oxidation of samples during microwave heating to the level of conventional heating one (Table 2). The best effect of antioxidants was observed for lard, moderate effect for plant oils. The microwave heating accelerates the oxidation of lard, rapeseed and sunflower oils (expressed by peroxide value) two or three times faster than conventional heating. This effect begins after 4 min of heating when the temperature of the sample rise over 100°C. In directed microwave heating (with 2 or 5 min breaks after each 2 min of exposition to keep the temperature of the sample below 100°C) the oxidation stability was in the range of conventional heating one (Table 3).

Table 3. Directed microwave heating of sunflower oil (with 2 and 5 min breaks)

Exposition [min]	Control (no breaks)		Breaks 2 min		Breaks 5 min	
	t (°C)	PV*	t (°C)	PV*	t (°C)	PV*
0	25	0.41	25	0.41	25	0.41
2	98	0.88	86	1.01	80	1.27
4	113	1.34	109	1.4	90	1.4
6	125	5.88	110	2.04	91	1.52
8	133	16.22	116	2.8	98	1.87
10	135	18.99	117	6.24	98	2.05

*Peroxide values (0.5.mmol O_2/kg)

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Effect of Interesterification on the Structure and Physical Properties of Rapeseed Oil – Milk Fat Mixtures

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Abstract

The interesterification of rapeseed oil – milk fat mixtures, in different proportions, by interesterification was carried out. In the work, two raw materials – with a different characteristic of fatty acids, rapeseed oil and milk fat, obtained from domestic butter by fractionation, were used. The rapeseed oil contains about 90% unsaturated fatty acids (mono- and polienic) and milk fat about 70% saturated fatty acids. The effect of these processes was restructurization of triacylglycerol and in consequence changes of rheological properties of the obtained fats. Decreased of solid phase content (by NMR determined) of all mixtures in a nonlinear fashion in the temperature range 10°C to 40°C except the milk fat were observed. The results of the work revealed a partial elimination saturated fatty acids from internal position *sn*-2 TAG MF and introducion, in this place, of unsaturated C 18 FA, from rapeseed oil, non destructing these fatty acids. The new TAGs were characterized by a new physical behaviour and a lower hipercholesterolemic effect as compared to TAG from milk fat.

Key words: interesterification; triacylglycerols; rapeseed oil; milk fat; TAG structure

INTRODUCTION

The rapeseed oil is widely accepted as a highly nutritious dietary fat source. The another different fat material is butter. The triacylglycerols (TAG) of milk fat consist of about 70% saturated fatty acids and oppositely the TAG of rapeseed oil consist of about 90% unsaturated fatty acids (mono-, di- and polienoic). Butterfat is a mixture of more than 100 000 different TAG with a melting range –40 to 40°C. Noted presence of about 400 different fatty acids (FA) in butterfat, 25% of which were short-chained and 45% were long-chained saturates (1, 2). The very important nutritional fact is that the milkfat is a source of conjugated linoleic acid – CLA (3). Contrary to margarine, butter has a limited plastic range (4, 5). Nutritionally, butterfat contains a high persentage of hypercholesterolemic fatty acids (mid-lengh saturated FA) located predominantly at the *sn*-2 position of TAG (6, 7). The hypercholesterolemic effect of saturated fats is largely due to 12-, 14-, 16-carbon fatty acids, which represent about 40% to 50% of total fatty acids in milkfat (8, 9, 10). The rapeseed oil , a highly nutritious dietery fat source, mainly is composed of unsaturated C 18 fatty acids carbon (~60% oleic, ~20% linoleic, ~10% linolenic) (11). Modification (blending, interesterification) rapeseed oil with butterfat can lead to spreads that harmonize nutrition and offer desireble organoleptic attributes (12, 13, 14). Chemical modification causes a statistical randomization of fatty acids distributions that leads to modifications in TAG composition and, cosequently in physical behaviour (15, 16, 17). The replacement of saturated FA at the

sn-2 position of butterfat TAG by hypocholesterolemic FA, derived from vegetable oils is an important nutritional consequence of interesterification (7).

The aim of work was to investigate on obtaining of new type of fats with a new triacylglycerols structure (TAG) on the way of chemical and phisycal modification and rheological behaviour determination.

EXPERIMENTAL

Materials and methods: The two materials - low erucic rapeseed oil and butter - were used. The following processes were applied: dry fractionation of milk fat, "blending", the chemical interesterification using sodium methylate as acatalyst, filtration and drying under vacuum.

The following methods were applied:

- solid phase content determination by NMR technique using a Bruker "Minispec PC-120",
- fatty acids composition analysis by gas chromatograph, Hewlett-Packard 6890,
- melting point determination in open sealed capillary tube,
- triacylglycerols (TAG) structure determination using pancreatic lipase, TLC and GC.

In the first stage of this work, the anhydrous milkfat (AMF) from domestic butter was obtained. The next, high melting fraction – HMF, melting point 42°C and middle

melting fraction – MMF, melting point 33°C by dry fractionation were obtained. These fats were used as the raw materials in interesterification processes with and without rapeseed oil blends in different proportions – milkfat 100%, MF or HMF and RO (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80).

RESULTS AND DISCUSSION

The melting profiles of milkfat before and after interesterification were different (Fig. 1). The interesterified fat, characterized the lower solid phase content in temperature range between 10°C to 18°C and the higher solid phase content in 18°C to 35°C, comparing with MF before interesterification. In the case the milkfat – rapeseed oil mixtures interesterification, in different mass proportions, the result indicated lowering melting profiles, in all areas of temperatures (Fig.1). The next investigations of interesterification processes the HMF (mp. 42°C) and rapeseed oil mixture, indicated in general lowering of solid phase content and melting point. The very interesting were the samples with proportion (HMF-RO) 6:4 and 7:3.These can be for preparation margarine blends used. The determination of triacylglycerols structure (TAG) by hydrolysis using pancreatic lipase, indicated partially elimination of saturated fatty acids from *sn-2* position and introducing in this place unsaturated fatty acids C 18-number (oleic and linoleic) from *sn-1,3* position of rapeseed oil.

The fatty acids composition, in *sn-2* position of TAG, (Table1) shown the higher content of unsaturated (mainly oleic) and lower content of saturated acids, past interesterification. The modification processes of rapeseed oil and milkfat (MF), high melting fraction (HMF) mixtures, allows to obtain a new type of fats with different TAG structure and melting behaviour. Some the obtained interesterified fats allow for preparation fat blends, margarine bases and spreads with very good spreadability at refrigerator temperature.

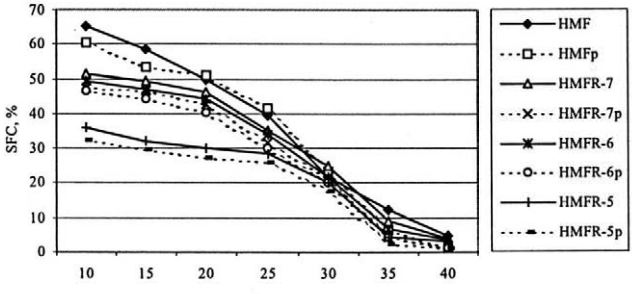

Figure 1. Changes of the solid fat content (SFC) before and after interesterification selected mixtures. High melting fraction (HMF) – rapeseed oil (R)

Table 1. Fatty Acids Composition of TAG and in *sn-2*, *sn-1,3* positions TAG (AMF-RO)

FA	Before interesterification			After interesterification		
	TAG	*Sn-2*	*Sn-1,3*	TAG	*Sn-2*	*Sn-1,3*
10:0	1.5	3.0	0.7	1.7	1.5	1.9
12:0	2.1	4.5	0.9	2.0	2.1	1.9
14:0	6.8	15.1	2.6	6.6	7.3	6.2
16:0	18.7	22.7	16.6	18.4	19.4	18.0
18:0	7.8	4.0	9.7	7.9	7.8	8.0
18:1 Σ iso (p+g)	37.8	22.9	45.1	37.7	36.8	38.1
18:2 Σ iso (p+g)	10.9	10.2	11.1	10.8	10.3	11.0
18:3 Σ iso (p+g)	4.0	4.0	4.0	4.3	4.1	4.4

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Kinetics of Triacylglycerol Species Degradation at High Temperature

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Abstract

Only little information is known about degradation of different triacylglycerols in real oils. In this study, changes in composition of two rapeseed oils (with different peroxide value) were followed during heating at temperatures from 150°C to 200°C and during frying. In all experiments, contents of triacylglycerols with equivalent carbon number 42, 44 and 46 decreased. For oil without hydroperoxides, reaction rate constants of these triacylglycerols degradations (as a first-order reaction) strongly depended on the degree of unsaturation. However, during experiments with oil contained hydroperoxides, the reaction rate constants for triacylglycerols with ECN 42 and 44 were about equivalent.

Keywords: degradation; oil; rapeseed; triacylglycerol; kinetic

INTRODUCTION

Kinetics of individual triacylglycerols (TAGs) degradations at different conditions are usually studied in model systems contained only one triacylglycerol (1). However, real plant oils are more complex mixtures with many triacylglycerols, which each other affect their degradations (2, 3). In our laboratory, degradations of individual triacylglycerols in different plant oils were followed during autoxidation at middle temperature (40 and 60°C) (4). In this study, kinetics of triacylglycerol species (according integer equivalent carbon number – ECN (5)) degradations at high temperature (during heating and during frying) are studied in rapeseed oil and compared with total degradation of these systems (followed as polymers and total polar compounds contents).

EXPERIMENTAL

Material. Refined rapeseed oils I (peroxide value 0 meq/kg) and II (peroxide value 0.65 meq/kg) were produced in Setuza a.s., Ústí nad Labem, Czech Republic. Pre-cooked snack foods prepared by extrusion from potatoes were given by Canto s.r.o., Týništì nad Orlicí, Czech Republic.

Procedure. Rapeseed oil I was heated at 150, 160, 180 and 200°C for 6 hours. Rapeseed oil II was heated at 170°C for 8 hours. Identical oil was used for 2 frying experiments without adding of fresh oil (experiments A and B). In these experiments, oil was heated at 170°C in the same way as during heating without frying. During this heating (without oil cooling between frying), snack foods were fried (1 portion during 1 hour in experiment A and 3 portions during 1 hour in experiment B). In all cases, polymerised TAGs, total polar compounds and triacylglycerol groups (according integer ECN) were determined in the optimum time intervals.

Analytical methods. The total polar compounds (6) and triacylglycerol groups were determined together using reversed phase HPLC: pump: LCP 4000.2, Ecom Prague; mobile phase: acetone–acetonitrile–methanol (4:2:1, v/v); flow rate: 0.8 ml/min; injection port: autosampler HP1050, Hewlett Packard; sample preparation: drying by Na_2SO_4 and dissolving in the mobile phase (50 µl of oil to 1.5 ml of mobile phase); injected volume: 50 µl; column: Separon SGX C18, 5 µm, 4 mm × 250 mm, Tessek Prague; column temperature: 40°C; detection: refractometric, HP1047A, Hewlett Packard; signal treatment: chromatography station CSW 1.6, DataApex, Czech Republic; quantification: internal normalisation without any correction factors. As polar compounds, substances with lower retention times than TAG with ECN 40 were calculated. TAG groups were identified by GLC analyses of fatty acids presented in the individual HPLC fractions (4). The polymerised triacylglycerols were determined by HP-SEC using the same apparatus as previous: mobile phase: tetrahydrofuran; flow rate: 0.6 ml/min; sample preparation: drying by Na_2SO_4 and dissolving in the mobile phase (100 µl of oil to 1.5 ml of mobile phase); injected volume: 5 µl; column: PL-gel Mixed E, 3 µm, 7.5 mm × 300 mm, Hewlett Packard; quantification: internal normalisation without any correction factors.

RESULTS AND DISCUSSION

The polymer determination is the best method for the following of high temperature degradations of lipids (7). In all experiments in this work, a linear dependence of polymers content on the heating time was found. The same results were obtained in many similar experiments (8–10). However, during heating of trioleoylglycerol (1), the rate of polymer formation increased during heating time (according the higher stability of the oleic acid hydroperoxides) and during frying with adding of fresh oil (11), the rate of polymer formation decreased (according the tocopherols with antioxidant activity adding).

Table 1. Kinetics of triacylglycerol species degradation during heating of rapeseed oil

Temperature [°C]	Triacylglycerols groups ECN 42	ECN 44	ECN 46
	Reaction rate constants (s^{-1})		
150	-2.78×10^{-6}	-2.06×10^{-6}	-0.87×10^{-6}
160	-3.13×10^{-6}	-2.45×10^{-6}	-1.02×10^{-6}
180	-4.64×10^{-6}	-3.74×10^{-6}	-1.79×10^{-6}
200	-4.67×10^{-6}	-3.94×10^{-6}	-1.94×10^{-6}
Activation energies (J/mol)	18.9×10^{3}	22.9×10^{3}	28.8×10^{3}

During heating of oil I, the rate of polymers formation increased with temperature from 0.40% per hour (at temperature 150°C) to 1.05% per hour (at temperature 200°C). In this temperature interval, the dependence of rate of polymer formation on the heating temperature was linear (with probability higher than 99%). The rate of polymers formation measured during heating of oil II at 170°C (0.67 % per hour) corresponded to previous rates. During frying experiments, the rates of polymers formation (0.90 and 0.91% per hour) were higher than rate measured during heating at the same oil temperature. However, quantity of fried foods did not affect this rate.

In all experiments, total polar compounds content increased and contents of triacylglycerol groups with ECN 42 (content in fresh oil about 9%; the main triacylglycerol oleoyllinoleoyllinolenoylglycerol – OLLn (4)), ECN 44 (21%; oleoyldilinoleoylglycerol and palmitoyloleoyllinolenoylglycerol – OLL and POLn) and ECN 46 (25%; dioleoyllinoleoylglycerol - OOL) decreased, all in the same dependence on heating time as polymers. The rates of polar compounds formation correlated with rates of polymers formation (correlation coefficient R was 0.967) and were about 2 times lower than the second ones. In all cases, the rate of degradation of triacylglycerol group with ECN 44 was the highest and occurred in the interval from 0.17% per hour (for heating at 150°C) to 0.32% per hour (for heating at 200°C). The rates of degradations of triacylglycerols groups with ECN 42 and 46 were about like each other and about 2 times lower than the previous one. The sums of rates of degradations of all triacylglycerols with ECN from 42 to 46 correlated with rates of polar compounds formation ($R = 0.872$) and slightly also with rates of polymers formation ($R = 0.775$).

The respective reaction rate constants for degradation of individual triacylglycerols groups (as a first-order reaction (1)) were than calculated. For heating of oil I, these constants together with activation energies (calculated according Arrhenius' equation) are in Table 1. From data in this table, it is evident that in fresh oil (without hydroperoxides), kinetic parameters of individual triacylglycerols groups are strongly depended on the degree of unsaturation. However, during experiments with oil II (which contained some hydroperoxides), the reaction rate constants for degradation of triacylglycerol groups with ECN 42 and ECN 44 were about equivalent, during heating and during frying too. Reaction rate constants for degradation of triacylglycerol group with ECN 46 were in these experiments about 2 times lower than previous ones alike as during experiments with oil I. Similar results were obtained also during autoxidation at middle temperature during induction periods (4).

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Dicarboxylic Acids as Molecular Markers of Thermal Degradation of Fish Oils

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Abstract

The effect of heating on oils rich in polyunsaturated fatty acids (PUFA) of the ω-3 series was investigated by considering the formation of polar low molecular weight compounds and the decrease of polyunsaturated compounds. Samples of fish oils were heated both in air and under nitrogen at temperatures between 140 and 200°C. Fatty acids and oxidation products were determined by GC/MS, after derivatization with acid methanol under mild conditions. A relevant degradation of PUFA occurred after heating over 160°C, and was much greater in air than under nitrogen. The main polar compounds detected in oils after heating were dicarboxylic acids. Nonanedioic acid (azelaic acid) was the more abundant and could be considered a potential molecular marker of thermal degradation in air.

Keywords: fish; dicarboxylic acids; PUFA; azelaic acid

INTRODUCTION

Chemical modifications of lipids during technological processes and storage affect primarily polyunsaturated fatty acids(1). Oxidation leads to hydroperoxides and secondary compounds such as aldehydes, ketones, oxiranes and hydrocarbons (2, 3). Cyclic acids and polymerization products have also been detected especially under deep frying conditions (4). As modifications occurring during refining, storage and cooking reduce the nutritional value of oils, it is considered useful the availability of analytical methods in order to detect secondary compounds as molecular markers for assessing quality and safety of oils.

Previously, we presented a fast GC-MS method to detect oxiranes, squalene and linolenic acid isomers in virgin and refined olive oil (5). Now we report the results on the formation of short-chain carboxylic acids during heating of fish oils from fresh and sea water(6), to be considered as molecular markers of thermal degradation. The decrease of PUFA was also determined, both in air and under nitrogen.

MATERIALS AND METHODS

Fresh oils for experiments were extracted from samples of the commercial fishes mackerel and salmon, having different contents of PUFAs.

Recovery, derivatization and analysis . Samples of freshly extracted fish oil (0.20g) were disposed on silica gel thin strips and kept in air at the desired temperature (room temp., 140, 160, 180, 200°C), in an oven. Samples under nitrogen were kept into special vials and conditioned before heating by flowing nitrogen continuosly through capillary channels.

After thermal treatment, the oily material was recovered from strips by washing with ethyl acetate (20 ml), evaporated and weighed. The residue was dissolved in 1 ml methanol, treated with 0.15 ml thionyl chloride as acid catalyst and kept at 50°C for 1 hour for exhaustive methylation . The methanolic sample was evaporated, and the residue was dissolved in 1 ml dichloromethane for GC/MS analysis.

GC/MS was performed on a HP-6890 Series II gas chromatograph coupled to a HP-5973 mass selective detector (Hewlett-Packard, Palo Alto, CA, USA). The gas chromatograph was equipped with DB-5 capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness, JW Scientific, USA). The GC oven program was: isotherm at 50°C for 2 min, increase of 20°C/min to 190°C, isotherm at 190° for 14 min, further increase of 20° min to 230°, isotherm at 230° for 10 min, last increase of 20°C to 270° and final isotherm at 270°C for 8 min. Injector and transfer line were kept at 280°C, the helium flow was 1.0 ml/min. The injection mode was splitless (0.2 min). MS conditions were as follows: ion source 230°C, the quadrupole was at 150°C, electron impact fixed at 70 eV, scan rate of the mass spectrometer 2.14 scan/s in the range of *m/z* 50–400.

RESULTS AND DISCUSSION

Relevant degradation of PUFA occurs during thermal treating of fish oils with formation of significant amounts of short chain mono- and dicarboxylic acids. The total ion

chromatograms of a methylated sample of mackerel oil, before and after heating in air at 200°C for 30 min, are reported in Fig. 1a, b and show a dramatic decrease of the ω-3 series fatty acids. Lower temperatures and nitrogen atmosphere affect less the degradation. The decrease of PUFAs for salmon and mackerel oil samples, referred to the total content of saturated fatty acids at various temperatures in air and under nitrogen, is summarized in the graphics (Fig. 2 a–d). PUFA content was referred to that of saturated fatty acids as these were not appreciably affected by heating, even in air. The inertness of the saturated fraction was demonstrated by comparing GC profiles of treated and untreated samples additioned with internal standards.

As expected, the presence of oxygen accelerates the degradation of PUFA and causes the complete disappearance in samples treated in air over **180°C**. A detailed analysis was done to determine low molecular weight compounds formed during oxidative degradation: methyl esters of short-chain mono- and dicarboxylic acids were identified by their GC retention times and by mass fragmentation. Typical GC/MS profiles recorded for carboxylic acids identification are reported in Fig3(a,b).

The amounts of these acids, determined at various temperatures, are reported as percentages of the total oil in Table 1 (mackerel) and Table 2 (salmon).

Azelaic acid (C9di) and decanoic acid (C10) are the compounds more abundant in the degraded oils. As azelaic acid lacks in the fresh oil, while is abundant in the treated samples it could be considered a molecular marker of thermal degradation.

High molecular weight compounds are certainly produced during heating of oils and work is in progress to separate this fraction by gel permeation and to characterize by spectroscopic techniques.

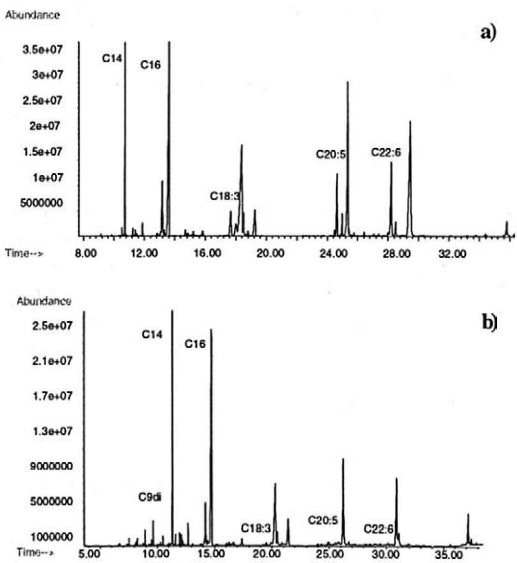

Fig.1. GC/MS profiles with evidences of the ω-3-fatty acid composition detected in the mackerel oil samples, after esterification with acid methanol: a) fresh sample; b) sample after heating in air at 200°C for 30 min

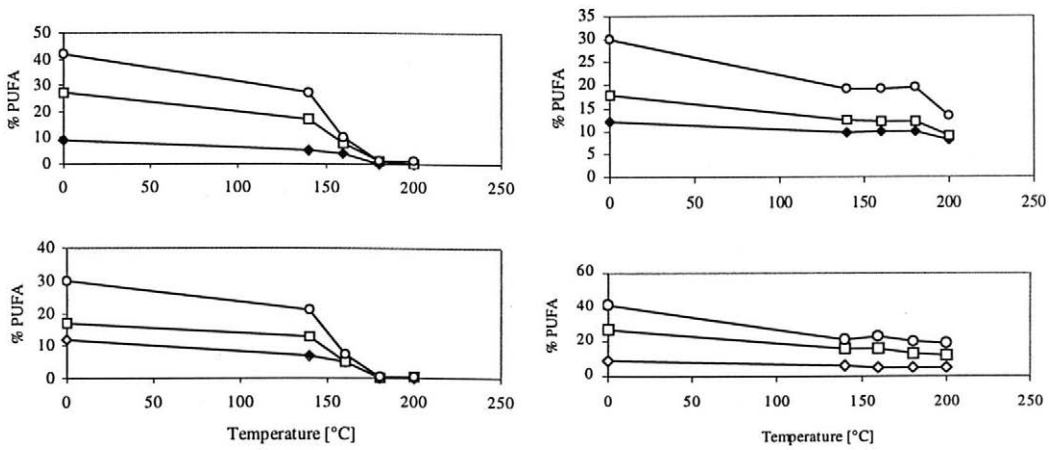

Fig.2 Percentage of C22:6 (O), C20:5(□), C18:3(◆) in mackerel oil (**a**, **c**) and salmon oil(**b**, **d**) heated at various temperatures in air(**a**, **b**) and under nitrogen (**c**,**d**), referred to the content of saturated fatty acids

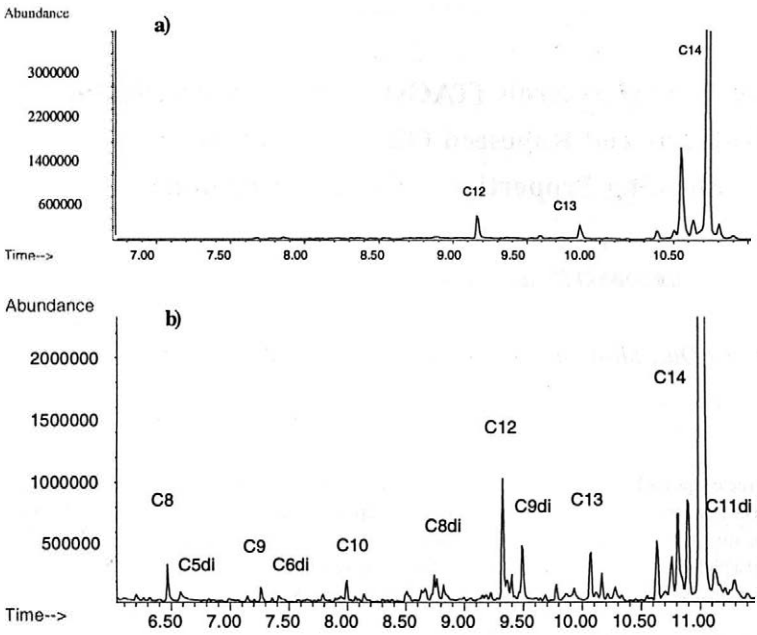

Figure 3. GC/MS typical profiles with evidences of the short-chain carboxylic acids detected in the mackerel oil samples, after esterification with acid methanol: a) untreated sample; b) sample kept in air at 200°C for 30 min.

Table 1. Percentage content of mono- and dicarboxylic acids recorded in mackerel oil heated in air at various temperatures (mean of two experiments with analysis in triplicate) (r.t. = room temperature)

	Monocarboxylic acids			Dicarboxylic acids						
Temperature [°C]	C8	C9	C10	C5di	C6di	C7di	C8di	C9di	C10di	C11di
200	0.022	0.054	0.055	0.057	0.045	0.096	0.201	0.366	0.195	0.291
180	0.007	0.04	0.042	0.045	0.036	0.093	0.162	0.333	0.165	0.249
160	–	0.004	0.011	0.012	0.007	0.003	0.022	0.036	0.007	0.022
140	–	0.009	0.011	–	–	–	–	0.003	–	–
r.t.	–	–	0.012	–	–	–	–	–	–	–

Table 2. Percentage content of mono- and dicarboxylic acids recorded in salmon oil heated in air at various temperatures

	Monocarboxylic acids			Dicarboxylic acids						
Temperature [°C]	C8	C9	C10	C5di	C6di	C7di	C8di	C9di	C10di	C11di
200	0.037	0.052	0.022	0.032	0.032	0.071	0.203	0.502	–	–
180	0.021	0.018	0.016	0.038	0.043	0.055	0.139	0.393	–	–
160	0.003	0.004	0.006	0.012	0.002	–	0.048	0.048	–	–
140	–	0.004	0.004	–	–	0.004	–	0.048	–	–
r.t.	–	–	0.001	–	–	–	–	–	–	–

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The Modification of Triacylglycerols (TAGs) in a Interesterification Process of Milk Fat and Rapeseed Oil and its Influence on Structure-Forming Properties of Obtained Product

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Abstract

Anhydrous milk fat (AMF) and low erucic rapeseed oil (RO) from industrial production were interesterified on the chemical way using sodium methylate as catalyst. The new type of fats with a wide spectrum of fatty acids at a long chain from C4 to C22 was obtained. The change of polymorphic structures of milk fat in the direction of creating crystal form ß-type occurred. Emulsion with new fat, type w/o (50:50) showed a high degree of dispersion of water phase. Steady dispersion of water phase, spreadability after cooling and good plasticity, these are the practically effects of influence of modified TAGs.

Keywords: anhydrous milk fat; rapeseed oil; interesterification; emulsion

INTRODUCTION

Milk fat is an important animal fat, one of the most complex natural fats. Its chemical and physical properties depend on contents and structure of triacylglycerols (TAGs). TAGs milk fat shows wide range of melting temperatures and unfavourable high content saturated fatty acids and cholesterol (1). To improve rheological features and nutritional value various experiments on modification of milk fat were carried out, including its fractionation (2,3) also SFE technique (4) or reesterification. The interesterification process can be used here, too (5).

The aim of this work was to obtain fat, interesterificated on the chemical way from milk fat and rapeseed oil, and to describe the usable characteristics and structure-forming properties of created TAGs.

EXPERIMENTAL

Materials and Methods

Anhydrous milk fat and low erucic rapeseed oil obtained from industrial production were used. The following processes and methods were applied:

- the interesterification process were carried out at the following parameters: catalyst – sodium methylate, inert gas – argon, temperature of reaction – 110°C, vacuum – 5 mm Hg, time – 30 minutes,
- blending and emulsification of fat and water phases,
- fatty acids composition analysis by gas chromatographic method (Hewlett-Packard),
- solid phase content determination by NMR technique using a Bruker "Minispec PC-120",
- microscope analysis of emulsion and computer analysis of an image.

The work included the analytical valuation of raw materials – rapeseed oil (RO) and milk fat (AMF), execution of interesterification process of mixture of these raw materials at following proportions: 4:6, 5:5, 6:4, analytical examination of obtained products, obtaining of experimental emulsions type w/o (20:80, 50:50) and their microscope analysis.

RESULTS AND DISCUSSION

The results of analysis of rapeseed oil and anhydrous milk fat are shown in Table 1.

Table 1. Characteristic of raw materials for interesterification process

Determination	Rapeseed oil (RO)	Anhydrous milk fat AMF)
Fat content [%]	99.90	99.90
Moisture content (H_2O) [%]	0.10	0.10
Acid value – AV [mg KOH/1g]	0.05	0.20
Peroxide value – PV [milieq.ox/kg]	0.10	0.20

The fatty acids composition (FA) AMF and fats obtained after interesterification processes are presented in Table 2. The new type of fats was obtained with a wide spectrum of fatty acids at a long chain from C4 to C22. The

Table 2. Comparison of content and composition of fatty acids of anhydrous milk fat and interesterified fats

FA	Content [%]			
	AMF	AMF/RO		
		4:6	5:5	6:4
C4:0	1.5	0.9	0.6	1.0
8:0	1.0	0.7	0.6	0.7
10:0	2.4	1.4	1.3	1.6
10:1	0.3	0.2	0.1	0.2
11:0	0.1	< 0.1	< 0.1	< 0.1
12:0	3.1	1.6	1.5	1.9
12:1	0.2	0.1	0.1	0.1
13:0 (iso)	0.2	0.2	0.1	0.1
13:0	0.1	0.1	0.1	0.1
14:0 (iso)	< 0.1	0.2	0.2	0.2
14:0	10.8	5.3	5.3	6.4
14:1	1.0	0.5	0.5	0.6
15:0 (iso)	0.7	0.3	0.3	0.4
15:0	1.3	0.7	0.6	0.8
15:1	0.1	0.1	< 0.1	< 0.1
16:0 (iso)	< 0.1	0.1	0.1	0.2
16:0	28.8	17.1	17.0	18.6
16:1 (Σ)	0.5	0.3	0.3	0.4
17:0 (iso)	1.9	1.0	1.0	1.2
17:0	0.7	0.3	0.3	0.4
17:1	1.0	0.5	0.5	0.6
18:0 (iso)	0.3	0.2	0.2	0.2
18:0	11.2	6.6	6.6	7.1
18:1 (t8)	0.5	–	–	–
18:1 (t9)	3.2	1.5	1.4	1.8
18:1 (c9)	22.1	37.8	38.7	35.6
18:1 (c11)	0.5	1.8	1.8	1.6
18:1 (c12)	0.2	0.1	0.1	0.2
18:1 (c13)	0.1	0.1	0.1	0.1
18:1 (c14)	0.5	0.3	0.3	0.3
18:1 (c15)	0.4	0.3	0.3	0.3
18:2 (ct/tc)	0.5	0.2	0.3	0.4
18: 2 (cc)	1.4	12.4	12.2	10.3
18:3 (iso)	0.2	0.3	0.3	0.4
18:3 (ccc)	0.8	4.3	4.3	3.7
20:0	1.5	0.9	0.8	1.0
20:1 (Σ)	0.3	0.8	0.9	0.8
20:2	0.1	0.1	0.1	0.1
20:3	0.2	–	–	–
20:4	0.1	–	–	–
22:0	0.1	0.2	0.2	0.2
22:1(Σ)	0.1	0.5	0.5	0.4
24:0	< 0.1	0.1	0.1	0.1
24:1	0.1	0.1	0.1	0.1

changes of solid fat content (SFC) measuring at temperature 10°C for samples taken during the interesterification process for series: 1 (AMF/RO 6:4), 2 (AMF/RO 5:5) and 3 (AMF/RO 4:6) are shown on Figure 1.

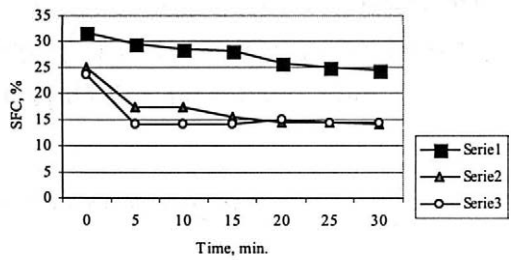

Figure1. Kinetics of interesterification process of anhydrous milk fat and rapeseed oil

As a result of this process the change of polymorphic structures of milk fat in the direction of creating crystal form ß-type occurred. Emulsion type w/o (50:50) showed a high degree of dispersion of water phase. The Gander classification of emulsion showed a high degree of dispersion of dispersed phase, the balls, diameter 3–6 μm – 95%, 6–12 μm – 5%. Steady dispersion of water phase, spreadability after cooling in a cooler (5–8°C) and good plasticity, these are the practical effects of influence of modified TAGs.

CONCLUSIONS

1. The new type of fat was obtained with a wide spectrum of fatty acids both at a chain length (from C4 to C22) and at a unsaturation degree and good structure-forming properties.
2. Fat, with modified TAGs enables profitable influence on fatty compositions and creation new recipes of shortenings and margarine bases of desired rheological properties.

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Determination of Fatty Acids in Ordinary and Omega-Enriched Eggs, Prepared in Four Different Ways: Raw, Boiled For 3 and 8 Minutes and Fried, Old 3–7 Days and After 4 Weeks of Storage

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Abstract

The aim of our research was to determine the fatty acids composition in ordinary and omega-enriched eggs, both commercialy available. Analyses were made from lipid extracts with gas liquid chromatography in four various prepared samples, four repetition of each. Analysed were raw eggs, soft boiled eggs (cooked for 3 minutes), hard boiled eggs (cooked for 8 minutes) and fried eggs, old 3–7 days. The same treatment were repeated after 4 weeks of storage at refrigerator temperature 4°C. Eggs were random sampled. Experimental data were collected in five groups: methyl esters of saturated fatty acids (SFA), methyl esters of monounsaturated fatty acids (MUFA), methyl esters of polyunsaturated fatty acids (PUFA), methyl esters of omega-3 fatty acids (ω-3 FA) and methyl esters of omega-6 fatty acids (ω-6 FA). With statistical experimental design Split-split-plot different factors were observed: sort of eggs, age of eggs and kind of preparation. Significant differences were noted between SFA and kind of preparation ($\alpha \leq 0{,}05$), MUFA and age of eggs ($\alpha \leq 0.001$), MUFA and kind of preparation ($\alpha \leq 0.05$), PUFA and sort of eggs ($\alpha \leq 0.01$), PUFA and age of eggs ($\alpha \leq 0.01$), PUFA and kind of preparations ($\alpha \leq 0.05$), ω-3 FA and sort of eggs ($\alpha \leq 0.001$), ω-3 FA and age of eggs ($\alpha \leq 0.01$), ω-3 FA and kind of preparation ($\alpha \leq 0.001$), ω-6 FA and sort of eggs ($\alpha \leq 0.001$) and ω-6 FA and age of eggs ($\alpha \leq 0.05$).

Keywords: eggs; omega-enriched eggs; fatty acids; gas liquid chromatography method

INTRODUCTION

Health benefits of dietary omega–3 polyunsaturated fatty acids were discussed in several studies and various are quoted to support relationship between omega–3 fatty acid consumption and relatively low risks from coronary heart disease. A good source of this acids are fish oils and "designer" eggs – omega enriched eggs.

EXPERIMENTAL

Material. Eggs – ordinary and omega–enriched – commercially available from the same producer.

Methods. Lipid extracts were derivatizated with BF_3. Gas liquid chromatography method (Varian 3400, SP–2380 Fused Silica Capillary Column) was used to determine fatty acids composition.

Statistical methods. Experimental design Split–split–plot.

RESULTS AND DISCUSSION

The results of fatty-acids composition are summarised in Tables 1–5. Samples display non-homogenous fatty acid composition. The influence of sort of eggs (P), age of eggs (R) and kind of preparation (T) are demonstrated in Table 6. The results speak in favour of eggs, enriched with omega-3 fatty acids, which confirms our expectations and data, published with other authors.

Table 1. Methyl esters of saturated fatty acids (SFA) in eggs [%]

EGGS (n = 4)	Mean ± s.e.	Minimum	Maximum	KV [%]
Fresh raw	38.90±0.692	37.50	40.80	3.556
Fresh soft boiled	37.00±0.147	36.60	37.30	0.796
Fresh hard boiled	36.90±0.610	35.40	38.30	3.304
Fresh fried	38.42±0.771	36.80	40.50	4.011
Old raw	39.17±0.301	38.40	39.90	1.565
Old soft boiled	36.82±0.680	34.90	38.00	3.693
Old hard boiled	40.97±0.520	39.60	42.00	2.539
Old fried	38.82±1.026	37.70	41.90	5.284
OMEGA-ENRICHED EGGS				
Fresh raw	35.90±1.025	33.80	38.20	5.709
Fresh soft boiled	34.80±2.278	28.30	38.50	13.093
Fresh hard boiled	36.77±1.388	32.80	39.00	7.550
Fresh fried	37.87±1.087	35.40	39.80	5.742
Old raw	38.00±0.481	36.90	39.20	2.533
Old soft boiled	37.52±0.822	35.60	39.50	4.381
Old hard boiled	36.52±1.577	31.80	38.30	8.636
Old fried	37.80±0.950	35.70	39.90	5.024

Table 2. Methyl esters of monounsaturated fatty acids (MUFA) in eggs [%]

EGGS (n = 4)	Mean ± s.e.	Minimum	Maximum	KV [%]
Fresh raw	40.35±1.568	36.50	43.50	7.770
Fresh soft boiled	44.55±0.679	43.00	46.30	3.048
Fresh hard boiled	44.22±0.661	42.60	45.40	2.991
Fresh fried	43.85±0.782	41.80	45.60	3.565
Old raw	40.80±0.918	38.90	43.30	4.502
Old soft boiled	44.87±0.421	43.90	45.90	1.876
Old hard boiled	37.00±0.754	35.40	38.90	4.075
Old fried	41.72±1.319	37.80	43.50	6.321
OMEGA-ENRICHED EGGS				
Fresh raw	42.55±1.937	38.40	45.90	9.103
Fresh soft boiled	43.92±1.853	39.80	48.00	8.440
Fresh hard boiled	41.37±2.141	37.90	47.30	10.348
Fresh fried	41.10±1.250	38.30	44.10	6.081
Old raw	39.87±0.758	37.80	41.10	3.900
Old soft boiled	38.97±1.344	35.30	41.60	6.895
Old hard boiled	38.75±2.621	33.90	46.20	13.526
Old fried	39.45±1.165	36.60	42.30	5.907

Table 3. Methyl esters of polyunsaturated fatty acids (PUFA) in eggs [%]

EGGS (n = 4)	Mean ± s.e.	Minimum	Maximum	KV [%]
Fresh raw	20.70±1.054	18.00	22.70	10.187
Fresh soft boiled	18.35±0.614	16.60	19.30	6.696
Fresh hard boiled	18.87±1.161	16.50	22.00	12.307
Fresh fried	17.72±0.131	17.50	18.10	1.484
Old raw	20.02±1.064	17.50	22.70	10.628
Old soft boiled	18.30±0.387	17.40	19.20	4.233
Old hard boiled	21.95±1.027	19.10	23.80	9.355
Old fried	19.45±0.330	18.70	20.30	3.397
OMEGA-ENRICHED EGGS				
Fresh raw	21.57±1.114	19.50	24.60	10.325
Fresh soft boiled	21.25±1.198	19.00	23.70	4.706
Fresh hard boiled	21.87±0.822	19.90	23.80	7.515
Fresh fried	20.90±0.141	20.50	21.10	1.352
Old raw	22.12±0.652	21.10	24.00	5.897
Old soft boiled	23.50±1.075	21.40	25.50	9.146
Old hard boiled	24.75±1.285	22.00	28.20	10.398
Old fried	22.75±0.463	21.90	23.60	4.069

Table 4. Methyl esters of omega-3 fatty acids (ω-3 FA) in eggs [%]

EGGS (n = 4)	Mean ± s.e.	Minimum	Maximum	KV [%]
Fresh raw	1.22±0.048	1.10	1.30	7.837
Fresh soft boiled	1.05±0.119	0.80	1.30	22.667
Fresh hard boiled	2.32±0.405	1.70	3.50	34.830
Fresh fried	0.12±0.125	0.00	0.50	200.000
Old raw	1.07±0.427	0.00	1.90	79.433
Old soft boiled	0.925±0.125	0.60	1.20	27.027
Old hard boiled	2.75±0.126	2.40	3.00	9.153
Old fried	0.30±0.173	0.00	0.60	115.467
OMEGA-ENRICHED EGGS				
Fresh raw	6.85±0.299	6.20	7.60	8.718
Fresh soft boiled	6.52±0.501	5.50	7.90	15.344
Fresh hard boiled	6.90±0.715	5.00	8.10	20.733
Fresh fried	5.47±0.533	4.10	6.70	19.467
Old raw	7.15±0.497	6.10	8.50	13.961
Old soft boiled	7.37±0.399	6.50	8.10	10.812
Old hard boiled	9.12±0.638	8.10	10.90	6.994
Old fried	7.55±0.441	6.60	8.60	11.673

Table 5. Methyl esters of omega-6 fatty acids (ω-6 FA) in eggs [%]

EGGS (n = 4)	Mean ± s.e.	Minimum	Maximum	KV [%]
Fresh raw	19.47±1.023	16.90	21.40	10.500
Fresh soft boiled	17.30±0.567	15.80	18.40	6.557
Fresh hard boiled	16.55±0.896	14.30	18.50	10.825
Fresh fried	17.60±0.227	17.00	18.10	2.583
Old raw	18.95±0.814	17.50	21.10	8.590
Old soft boiled	17.37±0.388	16.40	18.30	4.467
Old hard boiled	19.20±1.016	16.30	21.00	10.580
Old fried	19.15±0.386	18.70	20.30	4.033
OMEGA–ENRICHED EGGS				
Fresh raw	14.72±1.006	12.90	17.60	13.666
Fresh soft boiled	14.72±0.951	13.10	17.30	12.913
Fresh hard boiled	14.97±0.816	13.30	17.20	10.896
Fresh fried	15.42±0.413	14.40	16.40	5.356
Old raw	14.97±0.914	12.90	17.00	12.209
Old soft boiled	16.12±0.684	14,90	17.50	8.479
Old hard boiled	15,60±0.736	13.90	17.30	9.436
Old fried	15.20±0.212	14.80	15.70	2.791

Table 6. F-values (Analysis of variance)

Factor	SFA	MUFA	PUFA	ω-3 FA	ω-6 FA
P	–	–	23.291**	677.551***	37.541***
R	–	40.869***	16.022**	25.167**	6.822*
T	2.984*	3.365*	3.414*	15.932***	–
PR	–	–	–	23.386**	–

* = The differences between arithmetic means are significant on level of $\alpha \leq 0.05$
** = The differences between arithmetic means are significant on level of$\alpha \leq 0.01$
*** = The differences between arithmetic means are significant on level of $\alpha \leq 0.001$
Factor P = ω enriched eggs and eggs (2 values)
Factor R = fresh eggs and 4 weeks old eggs (2 values)
Factor T = kind of preparation (4 values)

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Prevention of Lipid Oxidation by Glucose Tolerance Factor

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Abstract

The antioxidant activity of GTF fractions (Crude – CR; and Partially Purified – PP), extracted from yeast, was examined. Dose dependent antioxidant activity of GTF was assessed, by testing its protective effect against oxidation of linoleic acid and beta-carotene. The oxidation of human low-density lipoprotein (LDL), incubated with or without GTF fractions, was investigated. In vivo antioxidant effect of GTF were also studied. Blood was taken from streptozotocin – diabetic Sprague Dawley male rats, divided into two groups: diabetic controls and diabetic treated with GTF. Lipoproteins were isolated and their sensitivity to oxidation was measured. The results clearly indicate that GTF functions as an antioxidant. We found that the effect of GTF in vitro was dose-dependent. We also observed that blood lipoproteins obtained from diabetic rats orally-supplemented with GTF, demonstrated a lower sensitivy to oxidation, compared with blood lipoproteins from control diabetic rats, or from healthy rats.

Keywords: Glucose Tolerance Factor (GTF); LDL; antioxidant; lipid peroxidation; β-carotene assay

INTRODUCTION

Glucose Tolerance Factor (GTF) is an anti-diabetic trivalent chromium (Cr^{3+}) compound, whose composition and chemical structure have not been defined yet (1, 2). There are several natural sources for GTF, the richest among them is brewer's yeast (2, 3). The role of chromium in glucose homeostasis and diabetes has been conclusively established in a series of studies conducted over the past few years (1–7). There is ample evidence in the literature that plasma lipid peroxides are elevated in diabetic humans, particularly in those with poorly controlled plasma glucose or angiopathy (8, 9). Increased levels of plasma lipid peroxidation products have also been reported in streptozotocin - induced diabetes in rats (10, 11). Low density lipoprotein has been reported to be more prone to oxidation in diabetics (12).

The purpose of the present study was to examine the activity of GTF, extracted and partially purified from yeast, as an antioxidant *in vitro* and *in vivo*.

EXPERIMENTAL

GTF was extracted and partially purified according to (13). Sprague Dawley mail rats weighing 130 g were injected sub cutaneously with a single dose of Streptozotocin (60 mg/kg b.w.) to induce diabetes. Five daily doses of GTF preparation were orally administered by a stomach tube to the GTF treated animals.

Evaluation of antioxidant activity of the examined materials is based on coupled oxidation of β-carotene and linoleic acid (14, 15). The method measures the consumption of ß carotene as a result of its oxidation process occurring in the reaction mixture. The natural anti oxidant examined can reduce the rate of ß carotene oxidation.

Oxidation of LDL was carried in a shaking bath with AAPH (A water soluble azo compound that produces radicals at a constant rate). Evaluation of LDL oxidation was determined by measuring the amount of TBARS (Thio Barbituric Acid Reactive Substances) (16, 17), and the amount of lipid peroxides, according to Niki et al (18).

RESULTS AND DISCUSSION

The relative antioxidant activity of the isolated crude GTF fraction (CR) (Fig. 1) and the partially purified GTF

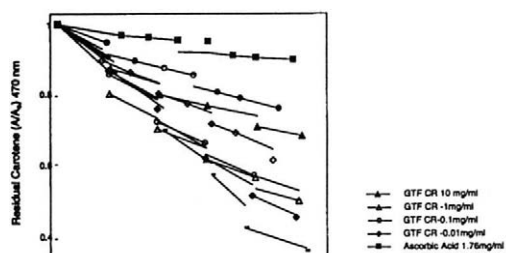

Figure 1. Anti oxidant activity of GTF CR (crude extract) compared with ascorbic acid activity in ß/carotene assay

(PP) (Fig. 2) were studied in the ß carotene linoleic acid system. The activities were compared with ascorbic acid activity. A dose dependent inhibition effect of both fractions of GTF is clearly evident from the figures. The highly purified preparation (Fig. 2) exhibits higher antioxidant activity when compared with the crude extract.

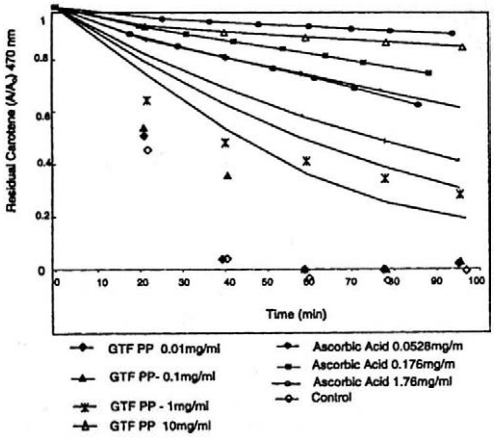

Figure 2. Anti oxidant activity of partially purified GTF (PP), compared with ascorbic acid activity in β-carotene assay

Protection of soybean oil against lipid peroxidation by GTF is presented in Fig. 3. Oil samples were incubated with or without GTF-CR (crude extract) in a bath shaker at 60°C for 53 hours. The results indicate that GTF is an efficient antioxidant.

Protection of human low density lipoprotein (LDL) in vitro against lipid peroxidation in the presence of GTF, is presented in Fig. 4. Thiobarbituric acid reactive substances (TBARS) were determined in human LDL after incubation with $CuSO_4$/2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). GTF samples inhibited the generation of TBARS and thus inhibited the oxidation process.

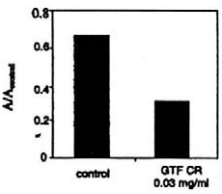

Figure 3. $A/A_{control}$ ratio of GTF CR after 53 hour in a bath shaker at 60°C. TBA extracted from oxidized soybean oil

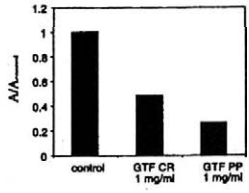

Figure 4. The effect of GTF extract on $CuSO_4$ induced LDL oxidation determined by TBARS assay

GTF exhibited also in vivo antioxidant activity in streptozotocin diabetic rats. $CuSO_4$ induced TBARS formation was markedly inhibited in the Very Low Density Lipoprotein (VLDL) and LDL from diabetic rats treated with GTF-CR for 5 days. In untreated diabetic controls the values of TBARS in VLDL and LDL fractions, were much higher (data not shown).

Our results clearly indicate that GTF functions as an antioxidant. We found that the effect of GTF in vitro was dose-dependent. We also observed that blood lipoproteins obtained from diabetic rats treated with GTF, demonstrated a lower sensitivity to oxidation, compared to blood lipoproteins from control diabetic rats.

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Antioxidant Activity of Dihydropyridine Derivatives

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Abstract

A series of 1,4-dihydropyridine derivatives was tested in lard, rapeseed and sunflower oils for their antioxidant activities. Under conditions of the Schaal Oven test, antioxidant activities were fair to good at the concentration levels of 0.02 or 0.05%. Diludin was the most active derivatives, followed by several structurally related derivatives. The activities were retained even in mixtures with protein, and in lesser degree, with starch or cellulose. Activities in lard and rapeseed oil were much lower at 100°C than under conditions of the Schaal Oven Test. Low activities were observed in lard in comparison with the effect of phenolic antioxidants. Activities of several 1,4-dihydropyridines to inhibit lipoxygenase-1 were of the same order as those of BHA and BHT, only Diludin was less active.

Keywords: antioxidants; dihydropyridines; lard; edible oils; lipoxygenase inhibition

INTRODUCTION

Most antioxidants are phenolic substances, possessing at least two hydroxyl groups in the 1,2- or 1,4-position, but nitrogen-containing heterocyclic compounds possess an antioxidant activity, too, and are used in the metabolism of living organisms. Several 1,4-dihydropyridine derivatives had pronounced free radical quenching activitiers (1). Diludin, the most active derivative of this group (Fig. 1) was found a good synergist of α-tocopherol (2). It was more efficient in rapeseed oil than butylated anisole (3). Efficiency of 1,4-dihydropyridines was also observed in a β-carotene-methyl linoleate system, in sunflower oil and in lipid emulsions (4). In this paper, we compare the antioxidant activity of Diludin with that of other 1,4-dihydropyridine derivatives.

EXPERIMENTAL

Material. The following 1,4-dihydropyridine derivatives were tested: OSI-119 (Diludin), its cyclohexyl and menthyl analogues, OSI-7283, OSI-7284. OSI-7375, OSI-7376, OSI-7377,

OSI-7378, OSI-5801, OSI-5802, OSI-2128, OSI-2780. OSI-2134, OSI-5151, OSI-123, OSI-397, IOS-541, PP-145, PP-275 (the structures are available from Prof. Duburs). All derivatives were prepared at the Institute of Organic Synthesis, Riga, Latvia, and the purity was tested by HPLC. Lipoxygenase-1 was isolated from soybeans (optimum activity at pH = 9.0). Pork lard, sunflower and rapeseed oils were commercial refined products.

R_1, R_6 = CH_3 (all compounds)

R_3, R_5 = $COOC_2H_5$ OSI 119 (Diludin)

R_3, R_5 = $COOC_6H_{11}$ cyclohexyldiludin

R_3, R_5 = $COOC_2H_4OC_3H_7$ OSI 7283

R_3, R_5 = $COOC_4H_9$ OSI 7284

R_3, R_5 = $COOC_2H_5$, R_4 = COOH PP-145

R_3, R_5 = CO-NH-C_6H_5 OSI 7375

Figure 1. Structures of the most active 1,4-dihydropyridine derivatives

Methods. The antioxidant activity was determined using the Schaal Oven Test at 40 and 60°C. The method using the ML-Oxipres apparatus (Mikrolab Aarhus A/S, Denmark), both applied in bulk lipids (5) and in mixtures of rapeseed oil with casein, egg albumin, microcrystalline cellulose and starch (6). The protection factor is expressed as the prolongation of induction period in comparison with the control. The lipoxygenase activity was determined with use of our modified procedure (7). Antioxidants were determined using the LCP 4000.1 micropump (Ekom, Prague), injector Rheodyne type 7725, a 250 mm × 4.6 mm column

packed with Hypersil ODS, 5 μm (Sigma, Aldrich), mobile phase: methanol and deionized water (9:1 v/v), with an addition of sodium acetate (0.05 mmol/L) and sodium-chloride (0.005 mol/L), and an electrochemical detector HP 1059 A (Hewlett-Packard) with a graphite measuring electrode, and the potential of +0.9 V.

RESULTS AND DISCUSSION

The antioxidant activities were tested using the Schaal Oven Test at 40 and 60°C at the concentration levels of 0.01, 0.02 and 0.05%. Good antioxidant activities were observed in sunflower oil containing 0.02% Diludin, 0.05% menthyl derivative, 0.05% OS-7376, 0.05% OSI-7383, 0.05% OSI-7284. Other derivatives had either only fair or weak activities or were entirely inactive. In rapeseed oil, 0.05% Diludin had an excellent activity, 0.01 or 0.02% Diludin, 0.02% OSI-4864, 0.02% OSI-5151, 0.02% OSI-123, 0.05% OSI-2134 and 0.05% OSI-397 had good activities. Diludin and OSI-7284 showed good activities in rapeseed oil emulsions (1:1) as well. The induction period was prolonged by 41% or 35% in rapeseed oil containing 0.02% Diludin and 0.02% OSI-7284, respectively. Generally, the efficiencies of all 1,4-dihydropyridines were lower than that of butylated hydroxytoluene (BHT) under the same conditions, and antioxidant activities were higher in rapeseed oil than in sunflower oil. Diludin or its analogues (Fig. 1) were the best antioxidants.

Antioxidant activities were lower at 100°C in the Oxipres apparatus as protection factors did not exceed 0.50 in lard and 0.25% in rapeseed oil (Table 1). Only the results of more active antioxidants have been included in the Table. It is interesting that 1,4-dihydropyridines were only weak antioxidants in lard. Activities in lard did not correlate with activities in rapeseed oil.

Table 1. Activities of 0.02% 1,4-dihydropyridines at 100°C in an Oxipres apparatus, expressed as protection factors (PF)

Antioxidant	PF in lard	PF in rapeseed oil
OSI-119 (Diludin)	0.48	0.23
OSI-7284	0.48	0.18
Menthyl Diludin	0.32	0.06
PP-275	0.32	0.09
OSI-2128	0.44	0.04
OSI-7283	0.44	0.19
OSI-7376	0.20	0.06
BHT	7.72	0.51

Similar activities were observed in mixtures of rapeseed oil with proteins, starch or cellulose (the maximum protection factor in Schaal Oven Test at 60°C was 0.53 in a mixture with casein (1:1) or 0.28 in a mixture with albumin (1:1). Similar activities were measured in mixtures in the ratio of 1:3 (0.33 in a mixture with egg albumin, 0.19 in a mixture with casein, 0.69 in a mixture with starch, and 0.14 in a mixture with cellulose). The activities of mixtures were very low at 100°C in an Oxipres apparatus.

Activities to inhibit lipoxygenase are expressed as the inhibition constant IC_{50}, i.e. the inhibitor concentration, which decreases the reaction rate by 50%. Results observed with some antioxidants are given in Table 2. Other derivatives were either not active or were insoluble under the test conditions. The lower is the IC_{50}, the more active is the antioxidant. The IC_{50} constant of butylated hydroxyanisole (BHA) was 0.155 mmol/L under the same conditions. It is evident that activities of 1,4-dihydropyridines were of the same order as that of BHA, and higher than BHT (IC_{50} = 0.599 mmol/L). Surprisingly, the activity of Diludin was lower, even when it was the most active antioxidant in rapeseed oil.

Table 2. Activities of different 1,4-dihydropyridines to inhibit lipoxygenase-1

Antioxidant	IC_{50} mmol/L
Diludin	1.35
Cyclohexyl derivative	0.243
Menthyl derivative	0.220
OSI-123	0.191
PP-145	0.182
OSI-2134	0.126
OSI-2780	0.196
OSI-7376	0.196
OSI-7377	0.124

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Oxidation Stability of Margarines with Monoacylglycerol Emulsifier Type

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Abstract

Lipid oxidation stability of several margarine emulsions with different monoglyceride emulsifiers was studied. Margarines contain 1-lauroylglycerol or the monoglycerides of coconut oil shows greater oxidation stability than margarines with the commercial monoglyceride emulsifiers (on the basis of palmitoyl-, stearoyl- and oleylglyceride). Accordingly, the interface structures created by molecules of monoglycerides of lower fatty acids (C12) have probably different properties than the interface structures created by monoglycerides of higher fatty acids (C16–C18). Thus, diffusion of oxygen across the interface of phases in w/o type emulsions influences velocity of the lipid oxidation.

Keywords: emulsion; lauroylglycerol; lipid; margarine; monoacylglycerol; oxidation

INTRODUCTION

Mechanisms of autooxidation reactions of lipids under various conditions are well known. The lipid oxidation of o/w emulsions (1–3) is more frequently investigated than w/o emulsion. In emulsion it is necessary to presume three "phases", the droplets of a dispersed phase, the continuous phase and the interface (1). These emulsions are thermodynamically unstable (particle size 0.1–10 μm). Molecules of the emulsifier (monoglycerides) are adsorbed at the interface. Diffusion of oxygen in the fat emulsions influences lipid oxidation because solubility of oxygen in water and in oil is different. The droplets of an aqueous phase represent dispersed environment in a margarine emulsion. Behaviour of continuous fat phase is more complicated. Liquid triglycerides represent a continuous phase, solid crystalized triglycerides of represent a further dispersed phase. It is not clear if solid triglycerides take part in autooxidation reactions. Important role in the initial state of the margarine autooxidation plays presence of fatty acids and triglycerides, presence of further oil soluble substances as tocopherols, carotenes, presence of water soluble substances as citric, lactic and ascorbic acid. Surface active substances (monoglycerides, phospolipids (4), ascorbylpalmitate) are concentrated at the interface.

It was found out during of testing of an antimicrobial activity (5) of lauroylglycerol that the fat emulsions w/o with content of lauroylglycerol displayed greater oxidation stability than the emulsions prepared with common monoglycerides. Therefore pilot types of margarines with lauric acid emulsifiers were prepared. Their oxidation stability was compared with the margarines prepared with commercial monoglyceride emulsifiers.

EXPERIMENTAL

The fat content of a model soft margarine was 73% w/w. Fatty acid composition of a fat blend was 13% palmitic acid, 5% stearic acid, 39% octadecenoic acids, 14% transisomers, 41% linoleic acid, 0.8% linolenic acid. Solid fat content of fat blend was 27% (10°C), 16% (20°C), 5% (30°C), 2% (40°C). Reconstituted whey was the aqueous phase which pH value was 4 and was set by lactic acid. Monolaurin (98% w/w of 1-lauroylglycerol), coconut oil fatty acid monoglycerides (86% w/w of MAG), coconut oil monoglycerides (60% w/w MAG, 30% w/w DAG), Emulsifier D (36% w/w of MAG of palmitic, stearic and oleic acid, control in series 1) and Polynol A (90 % w/w of MAG of palmitic and stearic acid, control in series 2) were used at the tested concentration (0.3 g per 100 g of emulsion). The storage of margarine were carried out at commercial package (250 g) at temperature 10°C and 20°C for 22 weeks. The peroxid value (PV), anisidine value and fatty acid content was determined during storage. Induction period (IP) and oxidation time ($\tau_{10°C}$) when the peroxid value reached 10 were calculated.

RESULTS AND DISCUSSION

The calculation of IP values was possible at all tested margarine at storage temperature 10°C (Table 1). At 20°C of storage, the course of lipid autooxidation takes place so

Table 1. Induction periode (IP – weeks) of margarines and the oxidation time ($\tau_{10°C}$, weeks) during the storage at 10°C and 20°C

	Series 1			Series 2			
	Emuls. D	Monolaurin	MAG of coconut oil (63%)	Monolaurin	MAG of coconut oil fatty acids (8 %)	MAG of coconut oil (63%)	Polynol A
IP 10°C	8–9	10	9	11	12	10	X
IP 20°C	8–9	10	8	11	X	X	10
$\tau_{10°C}$ (PV=10)	4	6	8.5	12.5	13.5	11.5	6.5
$\tau_{20°C}$ (PV = 10)	1.5	2	1.5	5	4	3	1.5

fast that the differences in the induction period (IP) values and the oxidation time ($\tau_{10°C}$) are minimal.

Figs. 1 and 2 present the dependence of peroxide value on the time of storage at 10°C for Series 1 and Series 2. Because was not possible to calculate IP at all tested margarine the time when the IP reached value 10 was determine to be oxidation time ($\tau_{10°C}$). Secondary oxidation products measured by the anisidine value displayed a mild increase which corresponds to a partial decomposition of hydroperoxides (date are not shown). Free fatty acid concentration in an oil blend was stabile in the range 0.1–0.3 w/w (date are not shown).

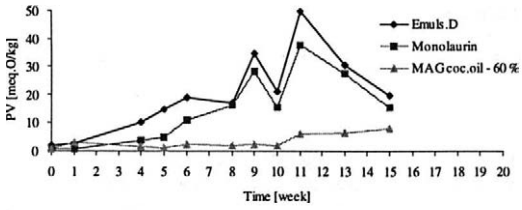

Figure 1. Dependence of PV during storage – Series 1

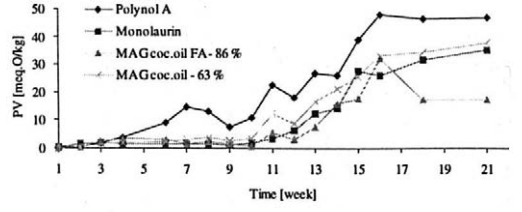

Figure 2. The dependence of PV during storage – Series 2

In comparison with the margarines in which monoglyceride emulsifier on the basis of palmitic, stearic and oleic acid (Emulsifier D and Polynol A), margarines produced with the use of lauric acid emulsifiers or emulsifier of coconut oil display greater oxidation stability. Differences in the IP values at the storage temperature 10°C are 2 weeks. If the oxidation time is compared, the differences are even more obvious (in the first experimental set min. 2–4 weeks, in the second experimental set min. 5 weeks – Table 1).

Oxidation stability of the margarine lipids with a monoglyceride emulsifier on the coconut oil basis is greater than the margarine prepared with monolaurin (1-lauroylglyceride).

The margarine produced with the emulsifier Polynol-A (mixture of palmitoyl- and stearoylglyceride) showed the lowest oxidation stability.

In the case of lipid oxidation of the w/o type of emulsion it is obvious that a transportation phenomena at the interface play an important role,especially, the diffusion of oxygen from water phase into oil phase. The interface structures created by molecules of lower faty acid (C8–C14) have probably different properties than the interface structures created by fatty acids with longer fatty acid (C16–C18).

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Application of Plant Extracts as Antioxidants in Fats, Oils and Emulsions

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Abstract

Antioxidant activities of acetone extracts of a 35 plants grown in Lithuania were tested in pork lard, rapeseed and sunflower oils. The following methods were used: Schaal Oven Test at 40°C, OXIPRES Method at 100°C and storage of emulsions at 40°C in presence of copper ions. Linear relations with statistically significant correlation coefficients existed between the results of different methods. An electrochemical detector could be used for the determination of components possessing oxidation potentials of active compounds, and thus, for the screening of extracts for their antioxidant activities. The highest antioxidant activity was observed in sage extracts, followed by sweetgrass, thyme, lemon balm and savoury.

Keywords: antioxidants; plant extracts; lard; edible oils; content of electrochemically active substances; methods for determination of antioxidant activity

INTRODUCTION

Natural antioxidants are now preferred to synthetic compounds for stabilization of edible fats and oils against oxidation under storage or deep frying conditions. Tocopherols are the most frequently used natural antioxidants (1). Extracts from rosemary leaves have also found commercial applications (2). Many plant extracts have been tested since the last 50 years, and most plants were found more or less active. The antioxidant activity is correlated with electrochemical behavior (3, 4) so that the use of voltammetric methods is justified as a screening method for the determination of antioxidant activity.

In this study, we examined a series of 35 extracts obtained from plants grown in Lithuania for their antioxidant activities.

EXPERIMENTAL

Material. Experimental plants were grown at the experimental botanical garden in Kaunas. Plant leaves were dried at 30°C, crushed, and extracted with acetone in a Soxhlet apparatus for 6 h. Pork lard, refined rapeseed and sunflower oils were commercial products. Their quality was in agreement with national quality standards.

Methods. The Schaal Oven Test was carried out at 40°C, oil layer 18 mm, and weight changes were monitored in 3–5 d intervals. The stability in the OXIPRES apparatus (ML Oxipres, Mikrolab Aarhus a/s, Denmark) was determined (5) at 100°C and at oxygen pressure of 0.5 MPa. Emulsions were prepared by mixing 0.5% lipid material with 0.5% soybean lecithin, 0.01% plant extract was added, and the oxidation was catalyzed with 10 mg cupric acetate (6); the volume was made up to 100 mL. The emulsion was stored at 40°C under shaking in the dark, and the progress of oxidation was monitored by measuring the ultraviolet absorption at 234 nm. The antioxidant activity was expressed as the protection factor (an indicator, how the induction period was prolonged after addition of an extract). The method for determining the electrochemical activity was developed on the basis of a HPLC method used for the analysis of antioxidants (7). Total active substances were measured using a reversed phase gradient elution, and an electrochemical detector HP 1049 A, provided with a graphite electrode set at the potential of +0.8 V (Hewlett-Packard). The electrochemical activity was expressed as the total area of peaks (in arbitrary units).

RESULTS AND DISCUSSION

A series of 35 plant extracts were tested in lard, rapeseed and sunflower oils at concentrations of 0.01, 0.02, 0.05 and 0.10%. Selected results at 0.05% concentrations (Table 1) show that sage extracts were more active than all other extracts. Relative values depended on the stabilized fat and on the method used. In addition to sage, thyme, sweetgrass, lemon balm, marjoram and savoury were particular-

Table 1. Antioxidant activities of plant extracts and contents of electrochemically active substances

Extracted plant	Schaal test		Oxipres test in lard	Emulsions of rapeseed oil	Area of active substances
	in sunflower oil	in rapeseed oil			
Sage	0.55–0.99	0.43–0.90	2.06–4.81	1.47–4.88	26.8–93.2
Sweetgrass	0.31–0.45	0.55–0.57	1.61–1.81	0.60–3.80	34.4–52.7
Thyme	0.27	0.23	2.06	1.94	40.1
Oregano	0.08	0.10	0.63	0.67	69.6
Marjoram	0.17	0.13	0.87	0.67	30.8
Savoury	0.15–0.28	0.19–0.21	0.67–0.74	0.73–1.33	16.2–25.7
Chamomile	0.12–0.15	0.26–0.35	0.06–0.31	0.60–1.53	9.9–32.1
Perilla	0.16–0.42	0.16–0.35	0.61–0.74	0.27–0.53	17.0–37.4
Dragonhead	0.15–0.28	0.12–0.27	0.30–0.70	0.40–1.27	4.5–27.8
Horehound	0.31–0.33	0.23–0.36	0.02–0.22	0.13–0.18	1.8–5.1
Quince	0.36	0.29–0.30	0.07–0.11	0.40–0.43	0.07–0.11
Costmary	0.20	0.18	0.35	0.20	19.3
Lovage	0.17	0.10	0.22	0.47	10.7
Borage	0.10–0.17	0.17–0.19	0.22–0.30	0.07–0.53	10.0–10.2
Artemisia	0.21	0.27	0.11	0.53	11.1
Lavender	0.19	0.13	0.04	0.33	2.2

ly active in lard at 100°C. Sage, sweetgrass, perilla, thyme and oregano were active in rapeseed oil, and sage, sweetgrass, perilla and lemon balm in sunflower oil. Sweetgrass is a perspective raw material. Other extracts showed moderate, weak or negligible activities. Protection factors were substantially higher in lard than in edible oils. They were higher in rapeseed oil than in sunflower oil. Activities were higher at 40°C than at 100°C, and no pronounced difference was observed between protection factors in bulk oil and in an emulsion. Concentration had moderate effect, too, but essentially, the dependence was nearly linear. Antioxidant activities of original extracts were higher than after removal of essential oils using deodorization.

Great differences were observed among total electrochemically active substances, reacting at the detection potential of +0.8 V (Table 1). Contents ranged from 0.00 in quince to 93.22 in sage, and sage, sweetgrass, oregano, thyme, marjoram, savoury and dragonhead extracts had high contents of substances reacting with the detector.

Linear correlations were observed between results of different methods. Examples are given (Table 2) for some substrates at 0.05% concentrations of extracts, however, the variability was rather high so that the results obtained with a method (or with a lipidic substrate) can be hardly used for prediction of antioxidant activities under different conditions.

Linear correlations exist between the antioxidant activites and the areas of electrochemically active substances in the extract (Table 2). Even in this case, great variability was found, but still, the method can be used for rapid screening. The extract showing only low response to the electrochemical detector would scarcely have a pronounced antioxidant activity, and an extract with high relative content of electrochemically active substances will probably show high antioxidant activity.

Table 2.Relations between results of various methods for determination of antioxidant activity and the response to the electrochemical detector

Regression equation	Correlation coefficient
B = –0.103 + 0.037 A	0.73
C = 0.175 + 0.004 A	0.79
D = 0.136 + 0.006 A	0.49
E = 0.144 + 0.031 A	0.55
D = –0.044 + 1.218 C	0.52
E = 0.215 + 2.549 D	0.88
D = 0.120 + 0.194 B	0.87

Notes: A = content of substances reacting in the electrochemical detector at +0.8 V (arbitrary units); B = protection factor in lard (Oxipres, 0.05% extract); C = protection factor in sunflower oil (Schaal, 0.05%); D = protection factor in rapeseed oil (Schaal, 0.05% extract); E = protection factor in rapeseed oil emulsions; N = 35

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Antioxidative Effect and Composition of Allspice in Rape-Seed Oil

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Abstract

The composition and antioxidative effect of the ethanol extract from allspice was studied in rape-seed oil. Two major allspice extract components are eugenol (52.6%) and methyleugenol (25.1%), the content of the other compounds was lower than 3%. The allspice extract was added to oil and samples were stored in the dark at 40°C. The results of the peroxide and thiobarbituric acid values have shown that the allspice extract has an antioxidative effect. Its action is associated with the high content of eugenol.

Keywords: allspice; antioxidant effect; rapeseed oil

INTRODUCTION

Lipids are one of the most important food components. As lipids oxidize they form hydroperoxides, which are susceptible to further oxidation and decomposition to secondary products such as aldehydes, ketones, alcohols etc. The oxidation products of lipids decrease the nutritional, sensory and hygienic value of foods. One of the most significant methods of prevention from lipid oxidation is the addition of natural and synthetic antioxidants. Some of the synthetic antioxidants such as BHA, BHT, etc. are permitted but the present-day trend is to use natural antioxidants. An interest in using extracts from natural plants for stabilization of fat-containing foods has been increasing. The rosemary and sage extracts play an important role, particularly rosemary is widely used in the food-processing industry. The antioxidative effect of rosemary extract in oil, oil-in-water emulsion and meat was investigated and confirmed (1, 2). Recently we have tested allspice extracts for an antioxidant effect in rapeseed oil and the results were reported (3). The aim of this study was to analyse the composition and antioxidant effect of allspice in rapeseed oil.

EXPERIMENTAL

Extraction of allspice: Ground allspice was obtained from the firm Kotanyi GmbH (Wolkersdorf, Austria). 10% allspice extract was prepared by extracting with 96% ethanol for 48 h at room temperature and at the occasional stirring. The extract attained was used after filtration.

Identification of allspice components: The composition of the allspice extract was detected by gas chromatography combined with mass spectrometry after filtration through anhydrous sodium sulphate.

The MS 25 RFA instrument from Kratos, Manchester, equipped with a Chrompack chromatographic column CP Sil & CB (25 m × 0.32 mm) was used for the GC-MS analysis; film thickness was 0.12 μm. Conditions for the GC-MS analysis were following: temperature program: 2 min isothermically at 50°C, then temperature increase of 2°C /min up to 260°C ; carrier gas: helium at the rate of flow of 1 ml/min; ionizing electron energy: 70 eV; ionization current: 100 μA; ion source temperature: 250°C ; scan rate of mass spectra equal to 0.6 s/decade.

Identification of mass spectra was performed by their interpretation and by comparison with the mass spectra library (NIST193).

Antioxidative effect of allspice: Allspice was added to oil samples (which is the product of the firm Palma-Tumys, Inc., Bratislava) as ethanol extract in the concentration of 05 % (wt). The samples were stored in sealed wide-mouth bottles for 48 days at 40°C in the thermostat. The antioxidant activity of the allspice extract was expressed as the decrease in the rate of peroxide and thiobarbituric acid reactive products formation in compare to the check sample during the storage period.

During the analysis of the change in lipides the following methods were used:

- determination of the peroxide value during the titration method (4);
- determination of the thiobarbituric acid reactive products (TBARP) by means of spectrophotometry using the direct method (4).

RESULTS AND DISCUSSION

The composition of the allspice extract components which was obtained by quantification of the GC-MS chro-

matogram is indicated in Table 1. The presence of 37 compounds was detected in allspice extract; all of them were identified.

The major allspice extract components are eugenol (52.6%) and *o*-methyleugenol (25.1%), the content of other compounds was less than 4%. Methylchavicol and *o*-methyleugenol have very similar structure to eugenol. Eugenol has an expressive odour, which can limit its use in food as an antioxidant. Other alcohols, which were identified in allspice extract, were 4-terpineol (0.5%), α-terpineol (1.36%) a globulol (0.6%), but these compounds do not contain phenolic ring, and their contents are much lower than that of eugenol. Therefore the antioxidant effect of allpice extract is dependent on its eugenol content.

Table 1. The composition of the allspice extract

Pike No.	Compounds	%
216	β-myrcene	2.2
282	eucalyptol	1.6
312	ocimene	0.2
409	β-linalool	0.6
559	4-terpineol	0.5
587	α-terpineol	1.4
601	methylchavicol	0.2
691	carvone	0.4
723	*p*-allylphenol	0.3
774	*p*-propenylanisole	0.1
792	2,4-decadienal	0.1
811	*p*-cymene-2-ol	0.1
939	eugenol	52.6
984	β-elemene	0.8
1020	methyleugenol	25.1
1037	β-caryophylene	3.5
1097	α-caryophylene	0.8
1136	τ-selinene	0.1
1146	β-cubenene	0.1
1156	cudesmia-4(14), 11-diene	0.3
1172	1,2,3,4,4a,5,6,8a-octahydro-4a, 8-di-methyl-2-(1-methylethenyl)-, [2R-(2a,4aa,8ab)]-naphtalene	0.4
1196	α-farnesene	0.1
1223	δ-cadinene	0.1
1328	caryophylene oxide	1.1
1374	(+)-(R)-a-ionol	0.3
1451	globulol	0.5
1878	methylester palmitic acid	0.7
1917	2,6,11,15-tetramethyl-hexadeca-2,6,10,14-pentaene	0.4
1944	palmitic acid	0.9
2117	linoleic acid	0.7
2126	methylester linolenic acid	0.4
2143	fytol	0.1
2162	methylester stearic acid	0.2
2185	kyselina linoleic acid	1.5
2209	ethyllinoleate	0.1
2219	stearic acid	0.3
2392	n-nonacosane	2.0

The allspice extract was added to oil in the 0.5% concentration and samples were stored for 48 days at the temperature of 40°C. Changes in the peroxide and thiobarbituric values are demonstrated in Figs. 1, 2.

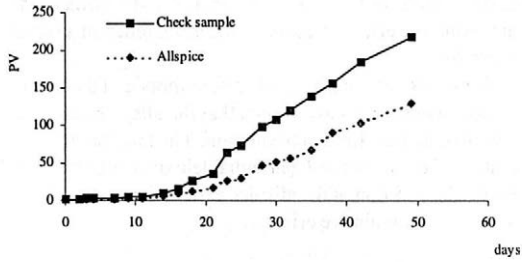

Figure 1. The changes of peroxide value (PV, meq/ kg oil) during the storage

The peroxide values of the check sample (pure oil) started to increase more intensively from the 11th day and after the 17th day it exceeded the value of 20 meq/kg, which is often denoted as the end of the induction period (1). The peroxide values of the samples with the addition of allspice increased later and the induction period was finished as late as after 21 days. It is assumed that the allspice extract had the inhibition effect on the production of hydroperoxides in oil and thereby on the extension of the induction period of stored oil. Malondialdehyde reacts with thiobarbituric acid and produce a red product, and the absorbance is measured at 540 nm. It is remarkable that even the amount of malondialdehyde contained in samples with the addition of allspice extract was during the storage lower than in the check sample. Malondialdehyde is a degradation product of hydoperoxides, and therefore accumulates later. The thiobarbituric acid reactive products increase more explicitly only after 21 days. The production of malonaldehyde was retarded by the allspice extract.

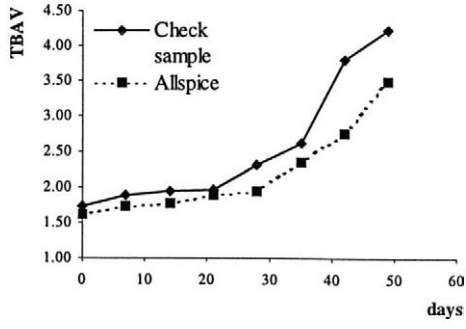

Figure 2. The changes of thiobarbituric acid value (TBAV, A1cm1%) during the storage

The antioxidative effect is frequently connected with phenolic compounds because they are good donors of the hydrogen atom and their radicals are relatively stable (5). Radicals of the phenolic compounds are stabilized by delocalization of an unpaired electron to the aromatic cycle. This is associated with the antioxidative effect of phenolic compounds since the more stable is the radical the better is the antioxidant. Such a structure is characteristic of eugenol, which has the 52.6 % proportion in the extract. The antioxidative effect of eugenol was researched in another work (6).

The results of a large number of authors and those presented in our work have shown that the allspice extract is effective against lipid autoxidation. The fact that the formation of peroxides and malondialdehyde in oil was decelerated by addition of the allspice extract, it gives evidence about its antioxidative effect.

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The Influence of Extracts and Essential Oils from Various Spices on the Oxidation Stability of Lard

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Abstract

The antioxidant properties of hexane extracts and essential oils from cinnamon, black pepper, fennel as well as hexane extracts from ginger and turmeric was studied in the model system of lard. The Shaal Oven Test was used to evaluate the antioxidant effect of spice treatments. The results showed that all spice extracts and essential oils had antioxidant effect on lard. Ginger extract was the most effective treatment, followed by turmeric extract. The group of the other extracts and oils possessed a weaker antioxidant effect. We didn't notice significant difference of antioxidant effect between extract and essential oil cinnamon, fennel and black pepper.

Keywords: spices; antioxidant; lipid

INTRODUCTION

Spices are important ingredients in food industry. They help to improve the flavour, taste as well as colour of foods. Because there's suspicion about possibility of carcinogenic properties of common used synthetic antioxidants and a growing trend to replace synthetic compounds with natural ones recently, they have become the subject of numerous studies on their antioxidant utilization. Antioxidant effect of various spices in different model systems was reported. Rosemary and sage have been shown to be very good antioxidants and been used commercially, but the other spices are also potential antioxidants (1–5). In most studies spices were studied in form of organic extract, less information is about essential oils obtained by steam distillation. Thus, the aim of our study was to examine and compare the antioxidant effect of essential oils and hexane extracts from cinnamon, black pepper, fennel as well as hexane extracts from ginger and turmeric.

EXPERIMENTAL

All spices and lard were obtained from commercial sources.

Preparation of essential oil by steam distillation. Spices (20 g potion) were ground using a vibration mill and subjected to steam distillation in a Clevenger apparatus (6). Essential oil was diluted with hexane and mixed with lard to a concentration of 0.1% with regard to the dry matter. The yields of oils obtained by steam distillation were as follows: cinnamon: 1.0% (v/w), black pepper: 2.5% (v/w), fennel: 2.0% (v/w).

Hexane extraction of essential oils. Spices were ground on a vibration mill and 10% (w/v) slurries made in hexane. These were left in the dark for 3 days at room temperature with occasional stirring. After sedimentation, the supernatant solution was mixed with lard to a concentration of 0.1% of spices with respect to the dry matter.

Evaluation of antioxidant effect. The *Schaal* Oven Test was used for measuring antioxidant effect of spice treatments (7). Samples of lard (25 g) were placed in 150 ml beakers and stored aerobically in a thermostat at 60°C. The peroxide value of samples with and without the addition of spice essential oil was measured iodometrically during storage (7). Samples were measured triplicated and results presented as mean value ± standard deviations. The induction period was determined graphically from the course of oxidation (7) and the antioxidant index was calculated as the ratio of the induction period of samples with addition of spices to the induction period of samples without addition of spices (2).

RESULTS AND DISCUSSION

The results in Table 1 show that the peroxide value increases during storage; furthermore, the most rapid increases in peroxide value were observed in control samples. These samples had the shortest induction period (Table 2). Among the studied spice treatments, the extract from ginger was showed to be the most effective antioxidant; this one inhibited the oxidation of lard by a factor of 5 in comparison with the control. Turmeric also had a very good antioxidant effect on lard, but these other spice applications showed only a mild influence on oxidation stability of lard. The two preparations (extract and essential oil) from cinnamon, black pepper, fennel showed no significant difference in antioxidant properties. It is probable that princi-

Table 1. Changes of peroxide value [μg O_2/ g] in spice-treated lard during storage

Day	Control	Ginger	Turmeric	Cinnamon		Black pepper		Fennel	
		E	E	E	O	E	O	E	O
0	29.8±0.2	29.8±0.2	29.8±0.2	29.8±0.2	29.8±0.2	29.8±0.2	29.8±0.2	29.8±0.2	29,8±0.2
1	30.0±0.4	29.3±0.4	28.4±0.2	29.4±0.8	29.7±0.4	29.4±0.5	29.5±0.2	29.4±1.6	29,3±0.7
2	73.8±1.3	37.5±0.4	36.8±1.0	35.4±0.3	37.3±0.3	36.2±1.1	43.9±0.9	36.7±0.6	44,7±0.3
3	101 ± 3	36.7±1.0	29.8±0.2	44.0±2	43.5±1.4	58.8±0.8	58.7±0.8	63.7±1.7	59.0±1.4
4	154±3	43.1±0.2	36.9±1.1	65.3±1	64.3±1.7	94±1	92±2	95±2	96±1
5	251±6	43.8±0.9	51±2	115±4	115±1	166±1	165±8	184±4	185±1
6	355±16	43.9±0.3	59±3	185±9	183±4	250±3	247±0	257±7	257±3
7	478±20	35.9±0.2	96±2	275±2	271±6	351±3	351±4	369±12	374±10
8	635±28	44.1±0.6	169±5	372±2	371±4	496±1	496±6	484±5	485±3
10	854±18	43.8±0.5	324±5	552±3	554±11	690±18	690±1	687±14	687±6
12		57.3±0.6	512±1						
14		74±0.3	712±3						
16		117±3	895±13						
18		166±1							
20		279±6							
22		473±1							
24		652±1							

E: extract, O: essential oil

Table 2. Induction period and antioxidant index of spice treatments

	Control	Ginger	Turmeric	Cinnamon		Black pepper		Fennel	
		E	E	E	S	E	S	E	S
Induction period [day]	3.48	17.2	7.0	4.4	4.5	4.1	4.1	4.0	4.1
Antioxidant index	–	4.95	2.02	1.27	1.30	1.17	1.17	1.14	1.17

ple antioxidant components of these spices are volatile and are present in both the extract and essential oils in the same amount.

Essential oils obtained by steam distillation contain volatile components, which usually have the characteristic aroma of individual spices. Their application in food industry as an application of essential oil is given priority because it is not necessary to use and remove the solvent. The antimicrobial properties of essential oils have also been widely investigated, but there have been few studies on their antioxidative properties. The antioxidant effect of essential oil from caraway, sage, cumin, rosemary, thyme, clove and their components on the oxidation of linolenic acid has been examined (8, 9). The fact that the antioxidant effect of solvent extract is better than those of essential oils was confirmed in a study on sunflower oil stability in the presence of Turkish spices (10).

From our results, it's possible to confirm the antioxidant properties of ginger, turmeric, cinnamon, black pepper and fennel. Ginger showed to be the best antioxidant among studied spices. Cinnamon, black pepper and fennel have about the same antioxidant effect on lard in both forms: hexane extract and essential oils.

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Antioxidant Activity of Ginger Extract in Ground Pork Patties

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Abstract

Lipid oxidation is a significant problem relative to off-flavour and off-odour, and warmed-over flavour appears to be related to lipid oxidation in meat. The objective of this study was to examine the effect of ginger extract on the lipid stability in ground pork patties. Antioxidant activity was observed at 0.5% w/w addition of ethanolic ginger extract. The extract decreased triacylglycerol hydrolysis, hydroperoxide formation and reduced the maximum of peroxide value. During the period of storage, the formation of primary and secondary oxidation products were less intensive than that of the control sample.

Keywords: antioxidant; ginger; ground pork

INTRODUCTION

Oxidation of lipids has detrimental effects on color, flavor, texture, and nutritional value of foods. Although synthetic antioxidants are effective, their use has been related to health risks. Accordingly, there is a strong need for effective antioxidants from natural sources. Sources of natural antioxidants are spices and herbs, and such materials have been used throughout the history not only for flavoring but also for preservative properties.

Oxidation of lipids is an autocatalytic process where the products of initial reactions propagate the oxidative process resulting in rancidity. Moisture, prooxidant pigments, storage, contribute to lipid oxidation of meat products.

Herbs in the Family *Zingiberaceae* have been reported to show active antioxidant components [1]. Our objective was to determine the antioxidant activity of ginger ethanol extract on pork.

EXPERIMENTAL

Material. Commercially available dried ground roots of ginger (MASPOMA Zvolen) were extracted by 96% ethanol at room temperature for 48 hrs. Extracts were added to fresh ground pork patties (0.5% w/w) prior to roasting for 35 min at 180°C. Three types of samples were prepared: i) raw meat patties – without any heat or extract treatment, analysed on day 0 only, ii) roasted meat patties extract treated, iii) roasted meat patties without extract treatment as control. Samples were prepared in triplicates. The latter two types were held at 4°C for 21 days.

Methods. Meat patties were analysed for fat content on dry basis and dry matter on days 0 and 21 according to Pribela (2). Fat was extracted according to Folch *et al.* (3). Acid, peroxide, and thiobarbituric acid values were analysed on days 0, 7, 14, and 21 according to Davídek (4). The amount of conjugated dienes and trienes were assessed spectrophotometrically (5) on days 0 and 21 at 232 nm and 268 nm, respectively.

RESULTS AND DISCUSSION

On day 0, raw meat patties contained 27.68% of dry matter, roasted meat patties 42.69%, and roasted meat patties treated with extract 34.54%. The amounts of dry matter did not change significantly during the period of storage. There was an indirect relation between fat and dry matter amounts. The greatest amount of fat was in roasted meat sample without extract addition, 13.70%, in raw meat sample it was 9.57%, in extract treated roasted meat sample 11.09%.

Acid values. Changes in acid values (AV) are reported as mg KOH/g of fat (Fig. 1).

In the beginning of storage, there was the greatest acid value in raw meat samples (AV = 13.610 mg KOH/g of fat). The degree of hydrolysis was 1.9 times lower in extract treated roasted patties (AV = 3.884 mg KOH/g of fat) than

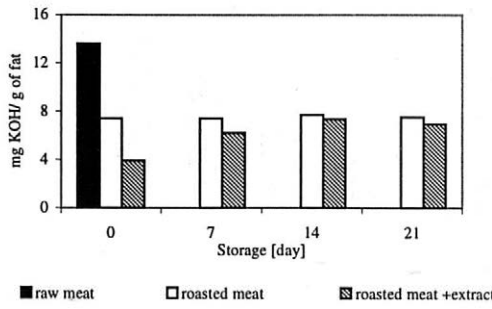

Figure 1. Changes in mean acid values of ground pork patties

that in controls – roasted samples without additives (AV = 7.408 mg KOH/g of fat). During the storage, acid values of extract treated samples increased; on day 21, there was a slight decrease. Storage did not affect acid values of control samples significantly. Acid values of samples with ginger extracts were lower than of those without additives during the whole period of storage.

Peroxide values. On day 0, the lowest amount of hydroperoxides was found in raw meat, the greatest in roasted meat without extract added. Peroxide values (PV) of extract treated samples are higher than those of raw meat due to heat treatment (Fig. 2). Ginger extract decreased the PV of samples by 37.3% when compared to controls – roasted meat samples without extract addition. PV showed there was the time of maximum peroxide value on day 14 for both

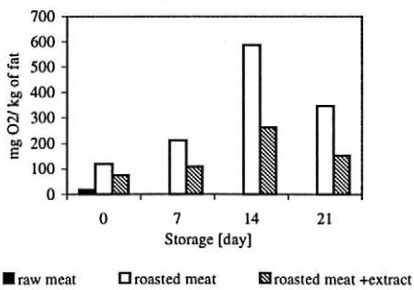

Figure 2. Changes in mean peroxide values of ground pork patties

types of samples: 586.25 mg O_2/kg in the sample without extract, 262.46 mg O_2/kg in the extract treated sample. On day 14, there was a rapid increase in amounts of hydroperoxides in control. Ginger extract effectively decreased the PV, so the maximum of PV in extract treated samples is 2.2-times lower than that of untreated ones. On day 21 the amounts of hydroperoxides decreased. The amounts of primary products of autooxidation in samples with ginger extract were lower that in controls during the storage.

Thiobarbituric acid values. Changes in thiobarbituric acid (TBA) values of samples with and without extracts added showed an increase: the amount of substances able to react with thiobarbituric acid increased during storage (Fig. 3).

TBA values of samples with extract treatment were lower by 17.8% when compared to control samples on day of

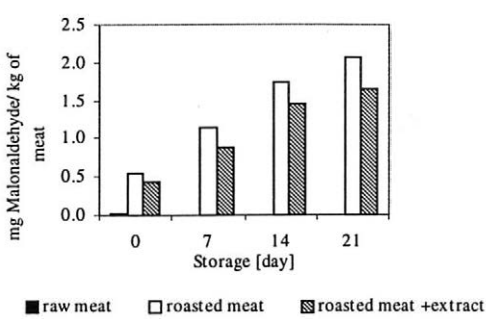

Figure 3. Changes in mean TBA values of ground pork patties

sample preparing. On the last day of analyses, TBA values of extract treated samples were lower by 20.6% than those of controls. However, malonaldehyde can react with other components such as peptides, amino acids and amines forming complexes that would not be detected by TBA tests. Within the whole period of storage, TBA values of extract treated samples were lower than TBA values of untreated samples.

Conjugated dienes and trienes. As for the content of conjugated diene and triene hydroperoxides in the samples with and without ginger extract (Figs. 4 and 5), antioxidant activity of the extract was confirmed: when comparing the data obtained on days 0 and 21, the absorbance of diene hydroperoxides in samples without extract increased by 11.6%, while that of extract treated samples increased by 6.4% only. The amount of conjugated triene hydroperoxides was higher by 51% in the samples without extract, there was an increase by 21.5% in the samples with ginger extract.

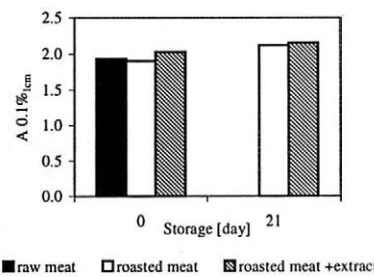

Figure 4. Changes in mean amounts of conjugated dienes in ground pork patties

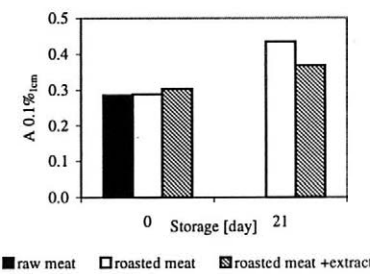

Figure 5. Changes in mean amounts of conjugated trienes in ground pork patties

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Antioxidative Activities of Evening Primrose and Rapeseed Extracts in Rapeseed Oil

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Abstract

The present study was carried out on the antioxidative activities of ethanol, 70% ethanol, acetone, 70% acetone, water, ethyl acetate, and hexane extracts of evening primrose (EP) meals and rapeseed (RS) meals. The extracts were characterised by a number of phenolic compounds. The antioxidative activities of extracts were studied on Oxidograph apparatus at 110°C. Rapeseed oil was used as a substrate. As for the observed experimental results we can state that the ethanol and 70% acetone extracts contained the largest amount of phenolic compounds, but the strongest antioxidative activity was found out in the case of ethyl acetate extract of EP meals and ethanol extract of RS meals.

Keywords: natural antioxidant; evening primrose; rapeseed

INTRODUCTION

The discovery of the use of antioxidants to increase the storage life of foods has made possible the marketing of many new products. During the last few decades, natural alternatives for synthetic antioxidants have been studied. The antioxidative activity of many extracts has always been attributed to their phenolic constituents (1).

Similar to other plant materials, evening primrose (*Oenothera biennis* L.) and rapeseed (*Brassica napus* L.) meals might be used as a potential source of antioxidants for foods. Phenolic compounds present in meals are chemically diverse, but generally belong to the phenolic acids, flavonoids and related compounds. These phenolic compounds can retard lipid oxidation by donating a hydrogen atom or an electron to chain-initiating free radicals. They can neutralise the substrate-derived free radicals such as fatty acid free radicals and alkoxy radicals and they also are capable of scavenging reactive oxygen species (ROS), namely, hydrogen peroxide, superoxide and hydroxyl radicals (2–4).

The objectives of this study were to prepare the extracts of evening primrose and rapeseed meals with various solvents, to determine the content of phenolic compounds in the extracts, and to evaluate their antioxidant properties in rapeseed oil.

EXPERIMENTAL

All materials and chemicals used were commercially available. Evening primrose (EP) meals and rapeseed (RS) meals were industrial materials obtained from Flaveco company, Czech Republic, after extraction of oil by supercritical CO_2 or Palma-Tumys, Slovak Republic, after extraction of oil by hexane. Edible rapeseed oil was obtained from the local fat factory Palma-Tumys, Bratislava (acid value = 0.20 mg KOH/g, peroxide value = 0.29 mmol $^1/_2$ O_2/kg and iodine value = 111.6 g I_2/100 g). Rapeseed oil fatty acids were separated as methylesters (14:0 = 0.06%, 16:0 = 4.76%, 16:1 = 0.30%, 18:0 = 1.80%, 18:1 = 62.12%, 18:2 = 18.78%, 18:3 = 6.63%, 20:0 = 0.60, 20:1=1,46%, 22:0 = 0.37% and 22:1 = 0.28%). Tocopherols (52.3 mg/100 g) and carotenoids (0.92 mg/100 g) were determined in rapeseed oil by high-pressure liquid chromatography (HPLC).

Preparation of the crude extracts. Extracts were prepared by mixing 20 g of dried material with 180-ml solvent (ethanol, 70% ethanol, acetone, 70% acetone, water, ethyl acetate and hexane), under refluxing conditions for 30 minutes and macerating overnight at room temperature. Each suspension was filtered, the residues mixed with another portion (20 ml) of the extraction solvent, and procedure was repeated two times. The filtrates were combined, and solvents evaporated. Extract yields, total phenols in the crude extracts, and their antioxidant activities in rapeseed oil were determined.

Determination of total phenols. The content of total phenols, as equivalents of caffeic acid (for evening primrose extracts) and sinapic acid (for rapeseed extracts), in each extract was determined using the Folin – Denis reagent (2). Total extracted phenols were expressed as milligrams of acid equivalents per g of crude extract.

Evaluation of antioxidant activities. The Oxidograph apparatus at 110°C was employed to monitor of the oxidation of rapeseed oil with or without addition antioxidants. The antioxidant activities of added crude EP extracts, RS extracts, and synthetic antioxidants were expressed as induction periods (IP). The IP was determined by the method of tangents to the two linear parts of the kinetic curve. The antioxidant activities of extracts were compared with selected synthetic or commercial antioxidants such as butulated hydroxytoluene (BHT), ascorbylpalmitate (AP) or tocopherolacetate.

RESULTS AND DISCUSSION

Yield and total phenols of crude extracts. As it is known, the use of various solvents is a common method for the extraction of several biologically active substances, particularly phenols. Many solvents can be used successfully for the extraction of oilseeds. In our studies we decided to test seven various solvents – ethanol, 70% ethanol, acetone, 70% acetone, water, ethyl acetate and hexane to choose the appropriate one (Table 1). The exact compositions of evening primrose and rapeseed meals are not known yet. Antioxidant compounds present in extracts may have various structures - from small phenolic acids to flavonoids and polymerized tannins. Our data demonstrate that the application of the acetone-water mixture for the extraction of phenolic compounds from EP and RS meals yielded the most phenols of all. The same results in the case of EP were found out by Wettasinghe et al. They proposed optimum extraction conditions – 56% acetone, 71°C, and 47 minutes (2).

Table 1. Crude extract yields and total phenols (for evening primrose as caffeic acid equivalents, and for rapeseed as sinapic acid equivalents) in extracts of evening primrose and rapeseed meals

Extract	Yield (% w/w)		Total phenols (mg/g)	
	Evening primrose	Rapeseed	Evening primrose	Rapeseed
Ethanol	6.5	9.9	333	113
70% ethanol	4.6	18.9	181	90
Acetone	3.5	1.9	335	89
70% acetone	8.6	14.2	404	117
Water	4.0	17.9	52	77
Ethyl acetate	1.4	2.4	69	100
Hexane	0.3	2.9	14	44

Antioxidative activity of crude extracts. All extracts showed varying degrees of antioxidant activity, except hexane extract of EP meals, 70% acetone and water extract of rapeseed meals (Table 2). As we expected, the hexane and water extracts gave the least amount of components with the lowest antioxidant activity. The strongest antioxidative activity was observed using ethyl acetate extract of EP meals. The mechanism by which polyphenols inhibit oxidation of oil seems to be rather complex. Antioxidant mechanisms for flavonoids in food products may involve free radical scavenging, metal chelation, and oxygen free radical scavenging. Polyphenols may interact with propagation reactions and with reactive oxygen species capable of initiating lipid oxidation. It seems that not only concentrations of polyphenols, but also the synergistic action between polyphenols is very important. Some comparative experiments were carried out with several commercial antioxidants. As it is obvious from Table 2, the ethyl acetate extract of EP meals exhibited as strong antioxidative activity as synthetic antioxidant, butylated hydroxytoluene, and ascorbylpalmitate, or tocopherolacetate did. Both synthetic and natural antioxidants play a crucial role in preventing or delaying oxidation in food systems. However, safety of synthetic phenolic antioxidants has been questioned. Therefore, it exists strong demand for adding acceptable natural antioxidants. According to our preliminary results the evening primrose meals can be taken into consideration as one good source of natural phenolic antioxidants.

Table 2 Antioxidative activities of crude extracts of evening primrose and rapeseed meals in rapeseed oil determined by Oxidograph apparatus at 110°C

	Induction period (h)	
Control	5.00	
Addition of extracts (0.1%)	Evening primrose	Rapeseed
Ethanol	5.35	5.25
70% ethanol	5.81	5.05
Acetone	5.75	5.00
70% acetone	5.36	4.87
Water	5.38	4.87
Ethyl acetate	6.86	5.14
Hexane	4.74	5.00
Addition of commercial antioxidants (0.01%)	Induction period (h)	
Butylated hydroxytoluene	6.89	
Ascorbylpalmitate	6.17	
Tocopherolacetate	5.31	

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Antioxidants Extracted from the Saltbush Plant

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Abstract

Several antioxidant fractions were extracted and partially purified from saltbush leaves. The activity of the different fractions was examined in the FRAP (Ferric Reducing Antioxidant Power) assay. When compared with known commercial antioxidants, the compounds extracted from the saltbush exhibited a high antioxidant activity, which may indicate a potential use of the natural compounds for food preservation and medical applications.

Keywords: antioxidants; saltbush; natural extracts; FRAP (Ferric Reducing Antioxidant Power) Assay

INTRODUCTION

Oxidative damage by free radicals is the cause of diverse diseases. Oxygen free radicals are generated during ischemia, tissue damage, necrosis, inflammation and aging (1–3). Oxidized LDL is the major cause of atherosclerosis and cardiovascular diseases (4–6). Oxygen free radicals are massively produced in cases of diabetes mellitus, where the level of lipid peroxides are highly elevated, causing severe complications like renal failure, atherosclerosis and CVA (2, 3, 7). Other diseases like cancer are also associated with free radical formation (8). Protection of cellular constituents is one of the most important targets of modern medicine.

Antioxidants are also principle ingredients to protect the quality of food by inhibiting oxidative break down of their lipid components. Many of the materials utilized to inhibit oxidative degradation are toxic for mammals (9). Natural antioxidants are widely distributed in plant tissues, among them β-carotene, vitamin E and C, flavonoids and glutathione, have been reported to have a high antioxidant activity (10–12). The Saltbush, (*Atriplex halimus*), is a traditionally anti diabetic plant grown in arid and semi-arid areas in the Mediterranean and Saharo-Arabian deserts (13). We found that the crude Saltbush extract and fractions purified from this extract possess high antioxidant activity.

The purpose of the present study was to extract and isolate active antioxidants derived from a natural source – the saltbush plant.

EXPERIMENTAL

Extraction and isolation of constituents. Powdered dry leaves of saltbush (200 g), were refluxed twice in a mixture of water (700 ml) and methanol (700 ml) for 4 hours, to obtain (after filtration and solvent evaporation), an aqueous crude extract. (36 g). The crude organic extract (16 g), was obtained by reflux of the dry leaves (185 g) twice in acetone for 2 hours, filtration and evaporation of the solvents. The crude extracts were purified using size exclusion membranes and different chromatography columns: silica gel, G-15, LH-20 and C-18. Fractions eluted from the different columns were combined according to their TLC profile, and examined for antioxidant activity using the FRAP assay. Higher degree of purification was achieved using HPLC (gel filtration and C-18 columns). Further purification and identification of the active compounds are currently in progress.

Evaluation of antioxidant activity by the FRAP assay. The evaluation of antioxidant activity was based on the FRAP (Ferric Reducing Antioxidant Power) assay (14). Antioxidants are used as reductants in a redox – linked colorimetric method. The change in absorption at 593 nm monitors the reduction of ferric complex (Fe III) to the ferrous form by the examined antioxidant.

RESULTS AND DISCUSSION

Extraction of saltbush leaves with an organic solvent (acetone) or a mixture of water and methanol provides several active antioxidant fractions. Partial purification of the extracts using size exclusion membranes and different chromatography procedures, resulted in 3 active fractions, as demonstrated in Figs. 1 and 2. F1, the organic fraction, was found to be the most active fraction. Its antioxidant activity was 12 fold higher then the crude organic extract (Fig. 2). Two aqueous fractions were isolated: F2, having a relatively low M.W., and F3, having a higher M.W. Both aqueous fractions had higher rate of antioxidant activity than

the crude aqueous extract, as presented in Figs. 1 and 2. The rate of their antioxidant activity was 2.13 and 2.7 fold higher respectively than the rate measured for the crude aqueous extract. Comparison of the antioxidant activity of the partially purified fractions (F1, F2, F3) from the saltbush plant, with commercial antioxidants – gluthatione, β-carotene, cystein and ascorbic acid, is presented in Fig. 3. All three fractions isolated from the saltbush leaves showed higher antioxidant activity than gluthatione and b-Carotene, while the organic fraction, F1, was found to be most active among them.

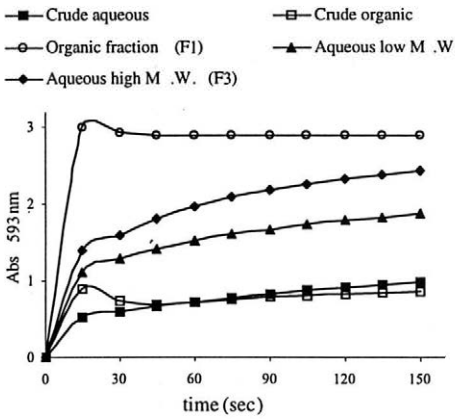

Figure 1. Antioxidant activity of compounds isolated from the saltbush leaves measured in the FRAP assay (2.5 mg/ml) of crude aqueous extract (■), crude organic extract (□), purified organic fraction F1 (○), low M.W. aqueous fraction F2 (▲), and high M.W. aqueous fraction F3 (♦)

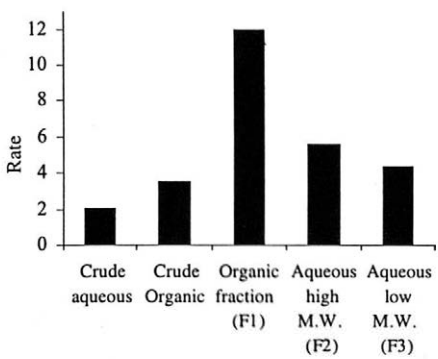

Figure 2. Comparison of antioxidant activity of the crude extracts and the purified Fractions derived from saltbush leaves, measured in the FRAP assay

Commercial antioxidants are generally synthetic compounds, and there has been a growing interest in replacing them with natural ingredients (15). Furthermore, possible toxicity of synthetic antioxidants has been a subject of many clinical studies (9). Therefore, evaluation of

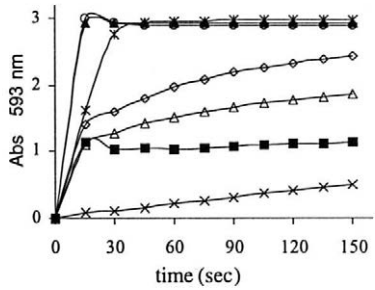

Figure 3. Antioxidant activity in purified fractions from the saltbush in FRAP test, compared with Commercial anti oxidants; increase in absorbance at 593 nm. For 100 µl solution (2.5 mg/ml). Organic fraction, F1 (○); low M.W. aqueous fraction, F2 (Δ); high M.W. aqueous fraction, F3 (○); Glutathione (x); L-Cysteine (•); ß-Carotene (■); Ascorbic acid (▲)

antioxidative activity of naturally occurring substances has been of high interest in recent years. The present study showed that saltbush leaves contain several potent antioxidant compounds, as determined in the FRAP assay. When compared with commercial antioxidants on a weigh basis, the natural compounds exhibited high antioxidant activity. Taking into account that the natural extracted compounds are partially purified, their relative antioxidant activity is even higher.

Our results may suggest a possible future use of saltbush ingredients for protection of certain foods against the development of oxidative rancidity. Moreover, the supplementation of the antioxidants isolated from the saltbush plant to human diet, may decrease the level of oxidative products in their tissues, and thus create a certain protection against diseases related to accumulation of oxidative products. The precise chemical identity of the antioxidant compounds derived from the saltbush have not been elucidated yet. Further work is required to purify and identify these compounds, but still, the high potential of the naturally occurring antioxidants extracted from the saltbush is clearly evident.

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Autoxidation of Triacylglycerol Species in Groundnut Oil

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Abstract

The oxidation of triacylglycerols was studied in their natural mixture as present in groundnut oil. The oil was oxidized under Schaal test conditions at 40, 60 and 80°C. Triacylglycerols were separated by reversed-phase HPLC. Fatty acid composition of triacylglycerol fractions was evaluated by capillary GLC with flame ionization detection. The rate of oxidation decreased with the increasing saturation degree of triacylglycerols. The content of polyunsaturated fatty acids (in triacylglycerols) has major influence on the oxidation rate.

Keywords: autoxidation; fatty acids; groundnut oil; HPLC; peanut oil; triacylglycerols

INTRODUCTION

The oxidation of lipids is a very large problem in fats and fat containing foods. Non-enzymatic (autoxidation) and also enzymatic oxidation of lipids have a very negative influence on the nutritional and sensory quality of foods.

Many reports were published on the of oxidation of simple triacylglycerols, but oxidation of natural triacylglycerol mixtures as they occur in the edible oils is still a very complicated problem. Different triacylglycerols may interact, and other compounds in the natural material, e.g. tocopherols, other antioxidants etc., contribute to the oxidation rate (1–3).

EXPERIMENTAL

Material: Refined groundnut oil, Setuza a.s. (Ústí n.L., Czech Republic), peroxide value 3.3 meqn O_2 /kg.

Methods of oxidation:

1. 20 mm layer of oil, free access of oxygen (without stirring), 80°C;
2. 1 mm layer of oil, 40°C, free access of oxygen, in the dark;
3. 1 mm layer of oil, 60°C, free access of oxygen, in the dark.

Analytical methods:

1. Oxygen absorption – weight changes (symbol d m in Table 2);
2. Content of hydroperoxides – peroxide value (4);
3. Separation of triacylglycerols and oxidation products: By the HPLC in the reversed phase Purospher RP-18e with refractometric detection.
4. Identification of main triacylglycerol species: Fatty acid composition of HPLC triacylglycerol fractions was evaluated, after conversion into methyl esters, by capillary GLC (column SP 2560; 100 m × 0.25 mm) with flame ionization detection.

RESULTS AND DISCUSSION

Chromatograms of fresh and more oxidized oils are shown in Fig. 1. Triacylglycerols were identified using the published data (5) (Table 1).

Figs. 2 and 3 show changes of evaluated factors during the oxidation. Kinetic characteristics of oxidation are given in Table 2.

During the induction period (Schaal test conditions) oxygen is absorbed, and the most unsaturated minor triacylglycerols A and B are rapidly decomposed. The oxidation of major triacylglycerols (C, D, E) and the formation of oxidized triacylglycerols proceed at substantial rates only in the stage of rapid oxidation after the induction period. Less pronounced differences were observed in a thin layer.

Slopes of time dependence during the induction period decrease with the decreasing unsaturation of triacylglycerols. The second stage of rapid oxidation is best expressed using an exponential equation. The effect of unsaturation is less evident in thin layers than under Schaal test conditions, where the oxygen diffusion becomes the rate governing factor.

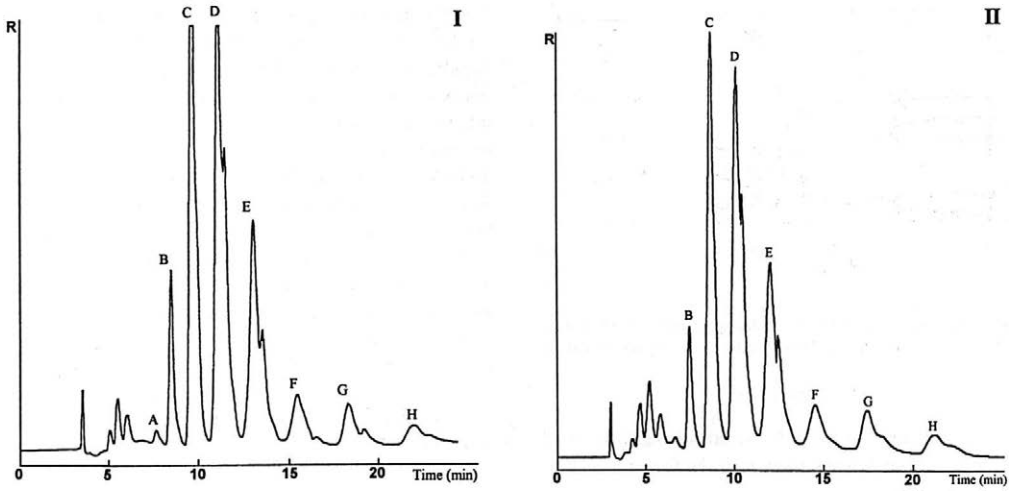

Figure 1. Triacylglycerol composition of groundnut oil (I = fresh oil; II = oil after 41 days of heating at 80°C)

Table 1. Triacylglycerols identification

Peak	RT	ECN	Triacylglycerol	Fatty acids
A	7.33	40	LLLn	P = palmitic
B	8.33	42	LLL	S = stearic
C	9.73	44	OLL(80%), PLL	Ar = arachic
D	11.29	46	OOL(86%), POL	Be = behenic
E	13.05	48	POO(47%), SOL(23%), PO(10%), POX(10%), OGdL(10%)	O = oleic X = C18:1/11c
F	15.25	50,52	SOO(35%), PSO(35%), OGdGd(30%)	Gd = gadoleic
G		52	ArOO(60%), PSGd(26%), SSO(14%)	L = linoleic
H		54	BeOO(9O%), SArO(10%)	Ln = linolenic

Arachic, behenic, lignoceric, gadoleic and higher acids are present also in other fractions, which are not eluted or imperfectly resolved

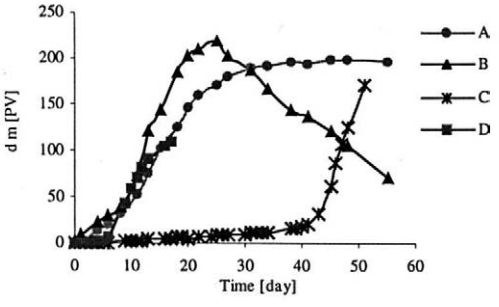

Figure 2. Course of weight changes (d m) and peroxide value (PV) during the oxidation; A = d m, 80°; B = PV, 80°C; C = d m, 40°C, thin layer; D = d m, 60°C, thin layer

Table 2. Changes during peanut oil oxidation (weight changes [dm/dt], peroxide value [PV], oxidation products formation [OX] and triacylglycerol content decrease)

	IP	LS	R^2	EXP (ln y)	R^2
80°C, Schaal test					
d m	8.2	3.98	0.99	3.40 + 0.0726x	0.89
PV	7.9	4.41	0.99	3.02 + 0.1162x	0.89
OX	22.8	0.25	0.92	1.24 + 0.0579x	0.98
B	7.4	−0.09	0.86	2.46 − 0.0748x	0.94
C	24.7	−0.11	0.93	3.78 − 0.0419x	0.89
D	30.1	−0.04	0.80	3.76 − 0.0156x	0.98
E	31.1	−0.03	0.71	3.11 − 0.0083x	0.90
F	32.8	−0.04	0.76	1.78 − 0.0089x	0.86
40°C, thin layer					
d m	42.8	0.37	0.98	−8.52 +0.2824x	0.98
OX	41.1	0.08	0.88	−6.81+ 0.2183x	0.91
B	38.3	−0.04	0.96	7.78 − 0.1532x	0.93
C	41.4	−0.05	0.81	9.05 − 0.1417x	0.96
D	41.7	−0.04	0.88	9.83 − 0.1537x	0.88
E	43.5	−0.01	0.65	4.59 − 0.0393x	0.93
F	40.8	−0.01	0.81	3.50 − 0.0429x	0.68
60°C, thin layer					
d m	5.7	0.41	0.96	−0.67 +0.4499x	0.91
OX	11.7	0.38	0.98	−0.821 + 0.2898x	0.81
B	13.6	−0.11	0.92	4.42 − 0.2277x	0.80
C	11.6	−0.19	0.99	7.21 − 0.3514x	0.99
D	11.7	−0.19	0.95	6.71 − 0.2875x	0.99
E	11.7	−0.03	0.94	4.17 − 0.0973x	0.90
F	12.4	−0.01	0.94	3.52 − 0.1329x	0.99

IP = induction period; LS = line slope during the induction period; R^2 = regression coefficient; EXP = logarithmic expression of exponential equation at 2nd stage of oxidation

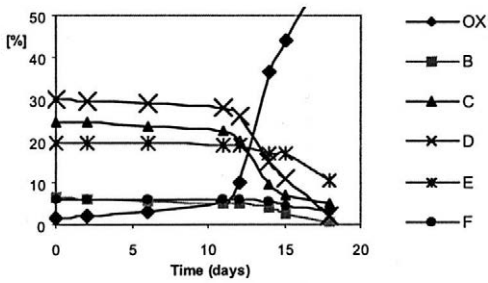

Figure 3. Course of oxidation products formation (OX) and triacylglycerols (as in Fig. 1) decomposition during the oxidation

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The Aspects of Cocoa Butter Equivalents in Chocolate

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Abstract

The EU is on the verge of accepting new Standard of Identify for chocolate. According to the new standard the incorporation of cocoa butter equivalents up to 5% of the weight of the product should be allowed and it still be called chocolate. The six specifics fat could be used: illipe, palm oil, sal, shea, kokum gurgi and mango kernel. Products that contain these fats will have to add the statement to the label, also contains vegetable fat other then cocoa butter. The reasons are not only due to economic effects but also these fats can improve properties of product. Therefore it is desirable that some analytical method be made available which would quantitatively determine the amount of added fat to chocolate. The objective of this work is to develop method or methods, which can detect and quantify symmetrical triacylglycerol cocoa butter equivalents (CBE) when used at the 5% level in chocolate. The results obtained by GLC on two silica-fused capillary columns (30 m × 0,32 mm, 50mm, polyethylene glycol, Innowax Hewlett Packard) and (100 m × 0,25 mm, 0,2 μm, SP-2560, Supelco, USA) will be statistically compared with NMR and IR data.

Keywords: cocoa butter equivalents; NIR; FTIR; NMR; capillary gas chromatography

INTRODUCTION

Throughout the world there is an increasing interest in the use of fats to partially replace cocoa butter in chocolate. The reason is that cocoa butter replacers can also provide product improvement. In the United Kingdom, Denmark and Ireland the addition of 5% cocoa butter replacers, except milk fat, are permitted. The determination of added fats has been the subject of much research. Several methods are available for determining total *trans* content of fats by infrared (IR) spectroscopy. Usually a strongly sloping background reduces the accuracy of the quantitation, especially at *trans* levels near and below 2–5% (1). Useful is comparison of capillary-column gas chromatography and single-bounce horizontal attenuated total reflection infrared spectroscopy. GC may be more accurate than SB-HATR or ATR for quantitation of total *trans* fatty acids. The lower limit of quantitation by SB-HATR is about 1% (2). CBEs were studied by thin layer chromatography (TLC), gas liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS) to determine the fatty acids at the sn-2 position and the sterol composition. Differences found in sterol compositions did not present any practical interest because of relatively small amounts present in CBE (3). The triacylglycerol gas liquid chromatography provides information on the composition of a fat according to the carbon number of the triacylglycerols. Examination of the data for a wide range of cocoa butters shows that a straight-line relationship between the C_{50} and C_{54} contents exists (4). The determination of cocoa butter replacements through the analysis of triacylglycerols with 50, 52 and 54 carbon atoms was judged satisfactory (5).

Differential scanning calorimetry measured crystallization and melting characteristic of commercial samples of anhydrous milk fat, cocoa butter and hydrogenated palm kernel stearin in ternary blends (6). Near infrared spectral patterns of oils reflect their fatty acid composition (7). Oil samples were analysed by both NIRS and gas-liquid chromatography. An accurate resolution of four of the five sunflower oil types was achieved by using the spectral information at two wavelengths (8).

EXPERIMENTAL

Material. Natural variation of cocoa butter was studied on four samples of cocoa butter (origin from Ghana, Equador, Ivory Coast, Indonesia). A matrix of samples was produced by blending cocoa butter equivalents (CBEs) in various ratio (5, 10, 15, 20, 25, 50, 75 and 100% of CBE – five types A, B, C, D, E). Fat was extracted by Soxhlet apparatus from three types of commercial samples (1, 2, 3), extraction written in Standard Methods of the Oils and Fats Division of the I.U.P.A.C.

Capillary gas chromatography. Fatty acid methyl esters (FAME) were prepared as described in CSN ISO 5509. GC was performed in a gas chromatograph Labio GC 07, equipped with a flame ionisation detector (FID). Used capillary column was Fused Silica Capillary Column INNO-Wax 19091a-213, 30 m × 0.32 mm × 0.50 μm, Hewlett Packard and the following temperature program was used: initial temperature 120°C; rate 10°C/min; final temperature 240°C. The injector and detector temperature were 220°C, 280°C, respectively. Nitrogen was used as the carrier gas; flow 0.7 ml/min. 1 μl was injected, splitter 1:30. The fatty acid methyl esters were identified by comparison of their retention times with those of known standards.

The results from capillary column SP-2560 (100 m × 0.25 mm, 0.2 μm, Supelco, USA) will be added.

FTIR. Fourier transform infrared spectrometer NICOLET 740 (Instrument Corporation, USA), consisting of a NICOLET 680 DSP workstation was used. The optical bench included a quality air bearing, a potassium bromide (KBr) substrate beam splitter, and a TGS detector, appodisation: Happ-Genzel. The ATR accessory contains a ZnSe 45° crystal. Samples were heated at 60°C and then they were placed on the horizontal ZnSe sampling surface of the ATR cell. 256 scans were collected at 4 cm^{-1} resolution.

NIR. NIR spectrometer NICOLET AVAR 360N (Instrument Corporation, USA), a TGS detector, a KBr substrate beam splitter, appodisation: Happ-Genzel were used. The spectra from 10 000 cm^{-1} to 4 000 cm^{-1} were recorded and 111 scans were collected at 4 cm^{-1} resolution.

NMR. Bruker Minispec, IBM PC/20 (IBM Instrument Inc.) was used to study solid fat content. Filtrated and well-mixed samples were poured to the test tubes. Samples were melted at 80°C before holding at 60°C for 5 min. Then samples were cooled to 0°C and held for 90 min. The test tubes were transferred to a 26°C incubator and held for 40 hours for stabilization. The stabilized samples were again cooled to 0°C and held for 90 min before being held at 10°C for 60 min prior to measurement. SFI melting curve were recorded at 10, 15, 20, 25, 30, 35, 40 °C always held for 60 min. Olive oil was used as a standard. Value of SFC was calculated from equation: $(1 - x/y^*s)^*100$; x – signal of sample at temperature t (°C), y – signal of sample at temperature 60°C, s – signal of olive oil at temperature t (°C).

RESULTS AND DISCUSSION

The content of fatty acids was determined in the samples of cocoa butter origin from different production areas, in the samples of CBEs, in the commercial samples and in the samples of CBE mixtures with cocoa butter by capillary gas chromatography. The content of fatty acids was determined also. The content of palmitic acid (16:0) increased with increasing content of CBE in mixtures. Contrary wise, the content of stearic acid (18:0) decreased with increasing content of CBE.

Differences of fatty acid content were significant in the blends of CBEs B and D. Less significant differences occurred in results of samples C and E. Fatty acid composition of CBE A was nearly similar to that of cocoa butter. Calibration plot of C16:0/C18:0 weight ratio were evaluated by the linear regression method to determine the content of CBE in the mixtures. The content of CBE in the commercial samples was calculated from the regression equations (Table 1). Calculated content of CBE B and D approached to expecting value of 15% CBE in the commercial sample fats unlike CBE A, C and E (Table 2). Just GLC is not available determine current type of the CBE in the commercial samples by used capillary column. Therefore FTIR, NIR and NMR were used in addition to GLC.

Table 1. Regression equations and correlation coefficient of callibration plots

CBE	Regression equation	Correlation coefficient
A	y = 0.0021x + 0.703	0.9991
B	y = 0.0173x + 0.6585	0.9855
C	y = 0.0055x + 0.6905	0.9981
D	y = 0.0206x + 0.5903	0.9701
E	y = 0.0056x + 0.6906	0.9974

Table 2. Calculated content of CBE (wt%) in the fat of the commercial samples

Samples	A	B	C	D	E
1	59.2	9.8	24.9	11.5	24.4
2	49.2	8.5	21.0	10.5	20.6
3	66.8	10.7	27.8	12.3	27.3

The FTIR determination measures the unique C-H out-of-plane deformation absorption at 966,4 cm^{-1} for analytes that contain isolated *trans* double bonds. 2nd derivative spectra were estimated. Fig. 1 presents 990–940 cm^{-1} region of 2nd derivative spectra of cocoa butter and CBE A. The highest content of *trans*-fatty acids was determined in CBE B. CBE A, D, C had less *trans*-fatty acids and the

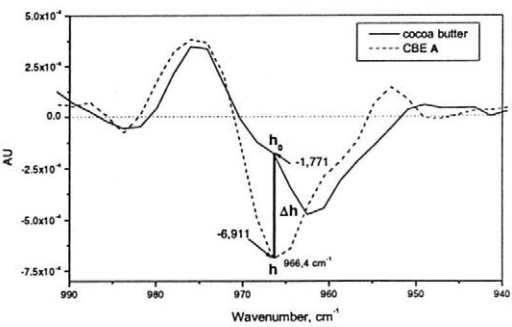

Figure 1. Second derivative FTIR spectras of cocoa butter and CBE A

lowest content of *trans*-fatty acids was checked in the CBE E. A calibration plot of "difference between Arbitrary Units of cocoa butter and those of CBE mixtures" vs. "percentage" CBE was generated for reference mixtures. The best statistic results were achieved in calibration plot of CBE B and A (correlation coefficients 0.9991 and 0.9963, consequently). Correlation coefficients of CBE C, D and E are 0.9671, 0.9553 and 0.9350, consequently. Small differences between spectres caused worse correlation coefficients.

For commercial samples, the percentage CBE was calculated from the difference between Arbitrary Units of cocoa butter and those of the observed absorption band at 966.4 cm^{-1} by the regression equation that describes the calibration plot. Commercial samples 1 and 3 were out of range of all CBE calibration plots. Explanation of it could be that extracted fat of commercial samples could contain the other type of CBE. Further reason could be fat extraction when non-fatty substances could be extracted and could absorb infrared light. Calculated values for sample 2 are from regression equations: calculated content of CBE A 26.17%, CBE B 19.78%, CBE C 67.36%, CBE D 48.40%, CBE E 58.96%.

NIR spectral patterns reflect fatty acid composition, so they can be used for the detection of adulteration with foreign fats. NIR spectral patterns, first-derivative and second-derivative NIR spectral patterns of mixtures were evaluated by program TQ-Analyst. TQ-Analyst allowed creating of calibration plots of "assumed percentage CBE" vs. "calculated percentage CBE". The best correlation coefficient was achieved for CBE A ($R = 0.9999$) in the NIR spectral pattern on the 5757–4516 cm^{-1} region. The correlation coefficient $R = 0.9881$ was achieved for CBE C in the first-derivative NIR spectral pattern on the 8875–7785 cm^{-1} region. The other CBE calibration plots were not useful because correlation coefficients were lower than 0.7. The commercial samples were not measured.

Pulsed nuclear magnetic resonance (NMR) is one of the standard methods that determine the solid fat content of fats. Analyses showed that the CBE D could be distinguished in mixture with cocoa butter with respect to their solid fat content at different temperatures. The CBEs A, B, C and E can be distinguished in mixture with cocoa butter in the case of content of CBE higher than 20%. SFI curve of these CBE mixtures with CBE content 5–20% showed small differences. The commercial samples were not measured.

Work is in progress with other inadmissible fats used as cocoa butter replacers and other permitted fats for the final formulation of the methods.

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Preventive Effects of Components of High-Oleic (Sunoleic) Peanuts against Life-Style Related Diseases

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Abstract

Compared to the conventional Virginia peanut oil, SunOleic peanut oil has substantially lower content of polyunsaturated triacylglycerols, lower linoleic acid and higher oleic acid contents. No great differences were observed in tocopherols, but the phospholipid composition is different. SunOleic peanut oil is substantially more resistant against oxidation than Virginia peanut oil. As SunOleic oil expresses anticancer and lysolecithin-increasing activities as well as a preventive effect against hyperlipidemia, it is a new food material suitable for the production of healthy and functional foods.

Key words: peanut oil; high oleic oil; anticancer activity; hyperlipidemia; erythrocytes

INTRODUCTION

In recent years, in addition to the excessive dietary intake of lipids, unbalanced fatty acid composition has become a problem of life style not only in Japan, but also in Europe and Northern America. In particular, an overintake of linoleic acid increases the occcurrence of civilization diseases, accompanying lipid peroxidation and eicosanoid imbalances (1-3). On the other hand, it is known that rates of mammary and colon cancer incidences are low among nations living along the Mediterrean coast, who have high intake of olive oil, which has high oleic acid and low linoleic acid contents. It is believed that the reason of this phenomenon is lower level of prostaglandin E_2 (PGE_2). This compound plays an important role in the growth of cancer cells. It is biosynthetised from linoleic cid, while it is not formed from oleic acid. From this standpoint, it is desirable to consume edible oils similar in their fatty acid composition to olive oil.

EXPERIMENTAL

Material. Peanut oil was extracted from kernels of SunOleic cultivar, developed in 1995 at the Institute of Food and Agricultural Sciences, Universty of Florida, USA (4), by crossbreeding the Sunrunner and high-oleic cultivars. The conventional Virginia peanut oil was prepared in the same way from Chinese kernels. The triacylglycerol composition of SunOleic oil was close to that of olive oil (Table 1). The stability against oxidationn was very high (5). Safflower oil was a high-linoleic commercial product.

Experiment 1. Determination of modifying actions against lung carcinogenesis: SunOleic peanut oil or high-linoleic safflower oil were fed to 6-week old female A/J mice at concentration of 10%, and methyl nitroso urea (MNU, 150 mg/kg, i. p.) was administered as a carcinogen. A mon-

Table 1. Composition of conventional and high oleic peanut oils

Components	Virginia oil	SunOleic oil
Linoleic acid (%)	30.2	2.6
Oleic acid (%)	51.8	81.7
Polyunsaturated triacylglycerols ECN 40–46 (%)	50.7	8.6
Monounsaturated triacylglycerols ECN 48 and more (%)	44.3	86.6
Tocopherols (mg/kg)	303	353
Gamma-Tocopherol (mg/kg)	134	201
Phosphatidylcholine (% P)	65.2	46.2
Lysophosphatidylcholine (% P)	7.2	6.6
Phosphatidylethanolamine (% P)	4.0	5.0
Phosphatidic acids (% P)	8.7	15.5
Phosphatidylinositol (% P)	12.9	23.3
Phosphatidylserine (% P)	2.0	1.3
Oxidative stability (Oxipres, h)	12.0	79.8

th after beginning of the inoculation of MNU, lungs were removed from the mice, and cycloxygenase (COX-1,2) and Ras signal transduction for carcinogenic cell proliferation were determined using immunosedimentation and immunoblotting techniques.

Experiment 2. Determination of preventive effects against hyperlipidemia: Crude SunOleic or Virginia peanut oils were administered to 6-week old male ICR mice in doses of either 0.2 mL/d or 0.4 mL/d, and blood serum biochemical test values and intrahepatic lipid concentrations were measured. The same procedure were undertaken on hyperlipidemic mice.

Experiment 3. Determination of erythrocyte membrane lysolecithin increasing effect: Crude SunOleic or Virginia peanut oils were administered to 6-week old male ICR mice in doses of 0.4 mL/d, and the phospholipid composition in the erythrocyte membrane was quantified using HPLC.

Experiment 4. Determination of anticancer effect against sarcoma 180: Sarcoma 180 cells were inoculated into the right axillary hypoderm. Crude SunOleic or Virginia peanut oil were administered to 6-week old male ICR mice in doses of either 0.2 mL/d or 0.4 mL/d, and the rates of tumor proliferation suppression were measured.

RESULTS AND DISCUSSION

In the first experiment, the phosphorylation of MEK was inhibited both in the MNU group and in the control group. No evident difference was found concerning COX 1,2. From these results, it may be concluded that SunOleic peanut oil inhibits cigarette-related tobacco-induced lung tumorigenesis.

In the second experiment, after 3 weeks of feeding high-oleic and control oil, biochemical serum analysis (T-Chol, T-Bil, GOT, GPT, TG, LDH, AP, HDL-C, Ca, UA, BUN, Alb, Gu, T-Pro) was carried out, and hepatic-lipid concentration (T-Chol, TG) was determined. No significant differences in blood biochemical test values were observed in the SunOleic peanut oil-administered group, when compared with the control group, but there was a reduction in intrahepatic triacylglycerols and lipid droplets. No effect indicating improvements were observed, however, in the hyperlipidemic rats (Tab. 2). It is probable that the large quantities of oleic leacid, in addition to trace elements, contributed to these results.

Table 2. Hepatic triacylglycerol concentration in mice

Group of mice		Virginia oil		SunOleic oil
		Mean value	Range	Mean value Range
Control	165	128–213	165	128–213
0.2 mL/d	145	100–190	175	125–223
0.4 mL/d	205	143–295	93*	50–135

*Significantly different ($P \geq 0.005$)

In the third experiment, 3 weeks after the start of feeding 0.4 mL/d of crude peanut oil, erythrocyte membrane phospholipids were determined using HPLC. The results show (Table 3) that the SunOleic oil administered group had a significantly increased amount of lysolecithin in comparison to the control group and the Virginia peanut oil group. Sunoleic peanut oil thus increases the fluidity and ductulity of erythrocytes.

Table 3. Phospholipid composition of erythrocyte membranes of mice

Phospholipid Class	Group		
	Control	SunOleic	Virginia oil
Phosphatidylcholine	73	64	69
Phosphatidylethanolamine	11	10	7
Sphingomyelin	5	5	6
Phosphatidylserine	8	6	8
Lysophosphatidylcholine	4	13*	7
Phosphatidylinositol	6	7	9

*Statistically significant ($P \geq 0.005$)

In the fourth experiment, rates of tumor proliferation suppression were measured after 3 weeks of the feeding experiment. Rates of tumor proliferation suppression (%) were 7 and 0 in groups fed 0.2 and 0.4 mL/d of Virgnia oil, respectively, and 4 and 68 in groups fed 0.2 and 0.4 mL/d in the SunOleic group. In the last group, the result was significantly higher (P 3 0.005). SunOleic peanut oil decreased the tumor proliferation at the promotion stage of cancer when compared with the Virginia peanut oil group. SunOleic peanut oil thus possesses an anticancer effect.

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th after beginning of the inoculation of MNU, lungs were removed from the mice, and cycloxygenase (COX-1,2) and Ras signal transduction for carcinogenic cell proliferation were determined using immunoblotting techniques.

Experiment 2. Determination of preventive effects against hyperlipidemia: Crude SunOleic or Virginia peanut oils were administered to 6-week old male ICR mice in doses of either 0.2 ml/d or 0.4 ml/d, and blood serum biochemical test values and intrahepatic lipid concentrations were measured. The same procedure were undertaken on hyperlipidemic mice.

Experiment 3. Determination of erythrocyte membrane lysolecithin increasing effect: Crude SunOleic or Virginia peanut oils were administered to 6-week old male ICR mice in doses of 0.4 ml/d, and the phospholipid composition in the erythrocyte membrane was quantified using HPLC.

Experiment 4. Determination of anticancer effect against sarcoma 180: Sarcoma 180 cells were inoculated into the right axillary hypoderm. Crude SunOleic or Virginia peanut oil were administered to 6-week old male ICR mice in doses of either 0.2 ml/d or 0.4 ml/d, and the rates of tumor proliferation suppression were measured.

RESULTS AND DISCUSSION

In the first experiment, the phosphorylation of MEK was inhibited both in the MNU group and in the control group. No evident difference was found concerning COX-1,2. From these results, it may be concluded that SunOleic peanut oil inhibits cigarette related tobacco-induced lung tumorigenesis.

In the second experiment, after 3 weeks of feeding high-oleic and control oil, biochemical serum analysis (T-Chol, T-Bil, GOT, GPT, TG, LDH, AP, HDL-C, CS, UA, BUN, Alb, Glu, T-Pro) was carried out, and hepatic-lipid concentration (T-Chol, TG) was determined. No significant differences in blood biochemical test values were observed in the SunOleic peanut oil-administered groups when compared with the control group, but there was a reduction in intrahepatic triacylglycerols and lipid droplets. No effect indicating improvement were observed, however, in the hyperlipidemic rats (Tab. 2). It is probable that the large quantities of oleic acid, in addition to trace elements, contributed to these results.

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	Mean value	Range	Mean value	Range
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*Significantly differing (P ≤ 0.005)

In the third experiment, 3 weeks after the start of feeding 0.4 ml/d of crude peanut oil, erythrocyte membrane phospholipids were determined using HPLC. The results show (Table 3) that the SunOleic oil administered group had a significantly increased amount of lysolecithin in comparison to the control group and the Virginia peanut oil group. Sunoleic peanut oil thus increases the flexibility and ductility of erythrocytes.

Table 3. Phospholipid composition of erythrocyte membranes of mice

Phospholipid Class	Control	SunOleic	Virginia oil
Phosphatidylcholine	73	69	70
Phosphatidylethanolamine	11	10	7
Sphingomyelin	5	5	6
Phosphatidylserine	5	6	5
Lysophosphatidylcholine	4	13*	[illegible]
Phosphatidylinositol	6	7	9

*Statistically significant (P ≤ 0.005)

In the fourth experiment, rates of tumor proliferation suppression were measured after 3 weeks of the feeding experiment. Rates of tumor proliferation suppression (%) were 7 and 0 in groups fed 0.2 and 0.4 ml/d of Virginia oil, respectively, and 4 and 65 in groups fed 0.2 and 0.4 ml/d in the SunOleic group. In the last group, the result was significantly higher (P ≤ 0.005). SunOleic peanut oil decreased the tumor proliferation at the promotion stage of cancer when compared with the Virginia peanut oil group. SunOleic peanut oil thus possesses an anticancer effect.

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D VITAMINS, PHYTOCHEMICALS AND MINERALS

Stability of Selected B-Vitamins during High Pressure Processing of Meat

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Abstract

Retention of thiamin, riboflavin, vitamin B_6, niacin and pantothenic acid during high pressure processing of pork, beef and chicken meat was examined. Fresh meat samples were vacuum packed and subjected to the effect of the high pressure of 200, 400 and 600 MPa for 10 min at room temperature. High pressure meat treatment had merely a minimal influence on the content of the examined B-vitamins. Small losses were only observed in thiamin, their extent was not influenced by meat treatment conditions. Changes in retention values of other factors examined approximately corresponded to the experimental error of the respective determination methods.

Keywords: high pressure processing; meat; B-vitamins; retention

INTRODUCTION

In recent decade high isostatic pressure technology (pascalization) is considered as one of the prospective methods of nonthermal food preservation. High pressure treatment inactivates a number of enzymes and vegetative forms of microorganisms contained in foods, whereas sensory and nutritional properties remain practically unaltered (1).

A number of authors studied the influence of high pressure on some food components, enzymatic reactions or microorganism behaviour. However, very little is known about the influence of pascalization on vitamins. Kłoczko and Radomski (2), examining vitamin C retention in carrot and apple juice after high pressure treatment, have only found 5% losses. Farr (3) has found no decrease at all of vitamin C content in pressure treated citrus juices. De Ancos and Cano (4) subjected carotenoids isolated from Spanish persimmon to high pressure without observing their loss. High pressure processing of eggs also resulted in no loss of riboflavin, folacin and thiamin (1).

Meat processing is a very important sector for high pressure application. High pressure treatment changes meat texture. This is already currently utilised in Japan for purposeful processing of fish meat. Meat is a good source of B-vitamins. Culinary processing (5, 6) and preservation (7) result in losing some of them. It is therefore desirable to know whether these factors remain retained after meat treatment with high pressure. Up to this time, only Bognár *et al.* (8) studied the influence of high pressure on thiamin retention in pork.

The purpose of the present paper is to examine retention of thiamin, riboflavin, vitamin B_6, niacin and pantothenic acid during high pressure treatment of pork, beef and chicken meat.

EXPERIMENTAL

Meat samples and the conditions of their high pressure treatment are listed in Table 1. The respective samples were purchased from local retail stores. One part of each sample was kept separately as an untreated sample. The rest was vacuum packed and treated by high pressure using the CYX 6/0103 equipment (ŽĎAS a.s. Žďár nad Sázavou), following the parameters shown in Table 1. Tap water was used as a pascalization medium. The respective high pressure treatments were replicated twice under the same conditions, except for pork leg with three replications.

The samples of untreated and treated meat were homogenised and used for the following determinations:

Dry matter

Thiamin – HPLC method with fluorescence detection

Riboflavin – lumiflavin method (Czechoslovak Standard 560054)

Vitamin B_6 – microbiological assay using *Saccharomyces uvarum* ATCC 9080 (Czechoslovak Standard 56 0056)

Table 1. Summary of material used and of high pressure treatment conditions

Treatment No.	Sample	Processing pressure (MPa)	Processing time (min)	Sample temperature (°C)	Medium temperature (°C)	Room temperature (°C)
1	Chicken (breast)	400	10	13.5	15.0	17.0
2	Chicken (breast)	600	10	13.5	15.0	17.0
3	Pork (ham)	200	10	17.7	18.9	20.8
4	Pork (ham)	400	10	18.2	18.9	20.8
5	Beef (rump)	200	10	12.2	16.2	17.6
6	Beef (rump)	400	10	12.3	16.2	17.6
7	Beef (rump)	600	10	13.6	13.5	18.0

Niacin – microbiological assay using *Lactobacillus plantarum* ATCC 8014 (Czechoslovak Standard 56 0051)

Pantothenic acid – microbiological assay using *Lactobacillus plantarum* ATCC 8014 (Czechoslovak Standard 56 0060)

Each vitamin determination in a sample, treated or untreated, was replicated at least twice.

The data on vitamin content in a sample were related to dry matter content and retention values in dry matter were evaluated as "apparent retention" (AR) (9).

RESULTS AND DISCUSSION

Table 2 lists retention values for respective vitamins in meat after high pressure treatment under the conditions specified in Table 1. Retention values were calculated as means of two or three independent high pressure experiments and from at least two replicate determinations for each vitamin value.

Table 2. Apparent retention values of vitamins in meat after high pressure processing (%)

Sample No.	Treatment				
	Thiamin	Riboflavin	Vitamin B_6	Niacin	Pantothenic acid
	Chicken (breast)				
1.	103	109	99	100	95
2.	97	109	104	100	90
	Pork (ham)				
3.	84	106	90	92	89
4.	87	111	92	99	95
	Beef (rump)				
5.	87	99	95	95	96
6.	87	99	92	101	103
7.	85	110	99	97	110

Retention values of all factors observed fluctuated in the range of 84–111%. Variation coefficients obtained in the validation of all the analytical procedures applied were found to be close to 10%. It can therefore be concluded that meat treatment with high pressure exhibited only a minimal influence on the content of the observed B-vitamins. Small losses were only recorded in thiamin. Its retention in pork and beef meat ranged between 84–87%. Bognár *et al.* (8) measured thiamin losses in pork meat treated with high pressure of 100–250 MPa for 10 min at room temperature and found retention values ranging between 99–101%.

The experiments performed did not prove any correlation between the conditions of meat processing by high pressure and retention of the observed B-vitamins. From the viewpoint of the stability of these vitamins, high pressure treatment appears more suitable than thermal ways of preservation and culinary processing.

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Comparison of the Methods of Determining the Tocopherol Dimers Generated during Oxidation of Fats

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Abstract

In the paper possibilities of separation of tocopherols and products of their mild oxidation occurring in the plant as well as animal material are compared – the methods applied are the adsorption column chromatography and high performance liquid chromatography. The test were carried out using HPLC with normal and reversed phases. Best results and the shortest time were obtained in the case of HPLC with reversed phases. Precise isolation of homologous tocopherols and their dimers from the mixture but the longest separation time were characteristic of the adsorption chromatography.

Keywords: tocopherols; dimers; separation; chromatography methods; HPLC

INTRODUCTION

Tocopherols are being determined in biologic material, foodstuffs and animal fodder in order to enable later precise determination of their decomposition, e.g. during technologic processes, elongation of the food shelf-life or food storage (4). The most important thing is to determine their exact content while deciding about addition of alpha-tocopherol (alpha-T) as an antioxidant applied e.g. in margarine or baby food. During autoxidation of fats tocopherols act as inhibitors, and their metabolites which are being generated – dimers – maintain antioxidant properties (9). These compounds can be traced in plant as well as in animal material and also in human organisms (5). So far their role in antioxidant processes has not been established – one of the reasons for this may be difficulty in their determination.

Development of modern analytical methods of determining chemical compounds occurring e.g. in plant oil brought about numerous papers on tocopherol isolation, some of them concern separation of alpha-T dimers, but studies referring to the simultaneous separation of remaining homologues together with their dimers are still lacking.

This paper presents attempts of separation of tocopherols and their dimers using various chromatographic methods aiming at finding the one that would be most effective and allow simultaneous determination of all tocopherols together with their dimers.

EXPERIMENTAL

Standards of apha-T, gamma-T and delta-T supplied by Eisai/Japan were used for synthesis of homologous dimers by the Nilsson procedure (5).

Separation of the tocopherols and their dimers was carried out using:

- *adsorption column chromatography* filled with $CaHPO_4$ with the solvent being extraction naphta with diethylether with changing gradient (1–10%)
- *HPLC with normal mobile phases* using the column Radial-Pak Liquid Chromatography Cartridge 8 NuSi, 4μ and 100 mm, with the solvent being isooctane and 2-propanol, with fluorymetric detection, excitation wavelength: 206 nm and absorption 304 nm (8)
- *HPLC with reversed mobile phases* using the LiChroCart Lichrospher 100RP-18 (4 × 4 mm) and LiChroCart Lichrospher 100RP-18 (25 × 4 mm) columns with separating mixture being methanol and dichloromethane, UV/VIS detection – wavelength 295 nm.

RESULTS AND DISCUSSION

Studies were carried out on dimers synthesised from the tocopherol standards by oxidation with *p*-benzoquinone in benzene. In this way the generated dimers have the chemical structure identical with that of dimers occurring in the plant and animal material. At first individual homologues were tested and then conditions for separation were elaborated for the mixture of all tocopherols and dimers. Dimers of tocopherols were separated using the methods presented in detail in the experimental part of this paper.

Fig. 1 presents schemes of separation of tocopherols and their dimers by the adsorption column technique. $CaHPO_4$ was the adsorbent playing the role of the immobile phase whereas the proper separation was carried out using the changing gradient of the mobile phase (2). All homologous tocopherols and their dimers were fully separated

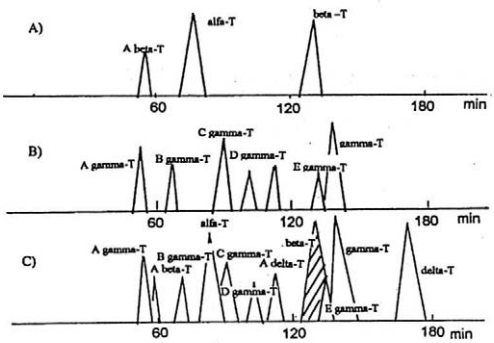

Figure 1. Separation of tocopherols and their dimers (column method) A, B – separation of the oxidized mixture of α-, β- and γ-tocopherol and their dimmers and C – separation of the oxidized mixture of homologous tocopherols and their dimers

within three hours. Quantitative determination of the separated compounds was performed by the spectrophotometric measurement of the intensity of the colour resulting from the Emmerie-Engel reaction. During separation of the mixture of tocopherols and their dimers only beta-T and the dimer of gamma-T were not separated in full – these compounds emerged as a single band.

Application of the HPLC with the mobile phase isooctane and 2-propanol allowed to separate individual tocopherols with their dimers; however, separation of the mixture of all homologous tocopherols was not obtained during a single application (6).

However, the obtained results concerning separation and determination can be made use of when only single tocopherols occur in the studied samples. The longest retention time in the applied method was that of delta-T – it amounted to 8.83 min. So the unquestionable advantage of this technique – apart from precise separation of individual tocopherols and their dimers – is short time of the separation not exceeding 10 min.

Fig. 2 presents a chromatogram obtained by application of the HPLC with the reversed phases (7). Maximum separation time was about 23 min. for dimers of alpha-T and the shortest retention time –2.48-min. – belonged to delta-T.

Applying the HPLC with the reversed phases Koskas *et al.* (3) obtained separation of only one dimer of alpha-T using the mixture of methanol and water (85:15v/v), whereas Cillard *et al.* (1) succeeded in separating three dimers of alpha-T using the LiChorsorb Si 60 column and the solvent system n-heptane: isopropanol 99.85:0.15 (v/v).

The results obtained permitted drawing the following conclusions:

- all methods applied allowed precise separation of tocopherols and their dimers

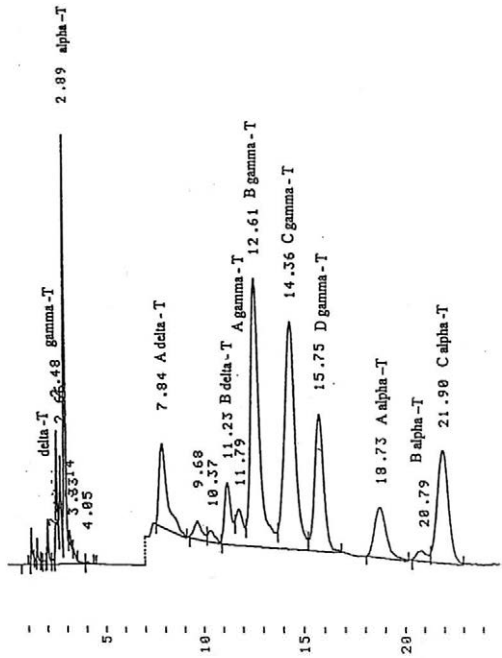

Figure 2. Separation of oxidized mixture of tocopherols and their dimers by HPLC (reversed phases), mobile phase: methanol-dichloromethan (80:20, v/v), flow rate: 1,8 ml/min

- the longest separation time was characteristic of the column chromatography
- application of HPLC with reversed phases allowed simultaneous isolation of the homologous tocopherols and their dimers from the mixture in the shortest time.

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Degradation of Tocopherols in Rapeseed Oil with Rosemary Extract under Different Conditions

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Abstract

Tocopherol degradation during oxidation of rapeseed oil containing rosemary extract at different conditions (storage at 60°C, heating at 150°C, and heating in the Oxipres apparatus at 100°C with higher pressure of oxygen) was followed. In all cases, α-tocopherol was degraded faster than γ- and δ-tocopherol. Rosemary antioxidants efficiently protect tocopherols naturally present in rapeseed oil. The degradation of antioxidant constituents of rosemary extract, carnosic acid and carnosol, was followed, too. Carnosic acid was decomposed faster than tocopherols. Carnosol was degraded nearly together with α-tocopherol.

Keywords: tocopherol; rapeseed oil; degradation; antioxidant; rosemary; carnosic acid; carnosol

INTRODUCTION

The most widely applied natural antioxidants are tocopherols (1). But, if tocopherols are active as antioxidants, they lose their vitamin E activity. Therefore, for the application of antioxidants in industrial practice it is good, if these compounds protect not only polyunsaturated fatty acids, but also tocopherols. As for natural antioxidants, rosemary and sage extracts have found the most frequent applications (2, 3). The principal antioxidants in these herbs are carnosol and carnosic acid. The antioxidant activity of crude extracts depends on the content of these compounds (4). Antioxidant activities of rosemary or sage extracts in fats and oils are comparable with those of BHA or BHT (3, 4).

In this study, the effect of rosemary extracts on the degradation of tocopherols naturally present in rapeseed oil was investigated. The degradation of carnosic acid and carnosol was followed, too.

EXPERIMENTAL

Material. Refined rapeseed oil I was produced in Setuza a.s., Ústí nad Labem, Czech Republic. The peroxide value was 0.95 meq/kg, the acid value 0.03 mg KOH/g, and the content of tocopherols was 518.5 mg/kg. Refined rapeseed oil II was produced in Setuza a.s., Ústí nad Labem, Czech Republic, too. The peroxide value was 0.30 meq/kg, the acid value 0.16 mg KOH/g, and the content of tocopherols was 458.9 mg/kg.

Rosemary extract I (obtained by extraction with ethanol) was prepared in the Department of Milk, Fats and Food Hygiene, Slovak Technical University in Bratislava, Slovakia. The content of carnosol was 4.02% and that of carnosic acid 14.28%. Rosemary extract II (obtained by extraction with ethylacetate) was prepared in the Department of Human Nutrition and Food Technology, Agricultural University in Poznań, Poland. The content of carnosol was 3.71% and that of carnosic acid 25.01%.

Procedure. The antioxidant activity of rosemary extract I was tested in rapeseed oil I during storage at 60°C in the dark, and during heating at 100°C using the Oxipres apparatus (Mikrolab Aarhus, Denmark; the initial oxygen pressure was 0.5 MPa). The activity of rosemary extract II was tested in rapeseed oil II during heating at 150°C. In all cases, losses of carnosol and carnosic acid (except heating test at 150°C) and of individual tocopherols were determined in optimum time intervals. During storage at 60°C peroxide value (5) was determined and during heating at 150°C polymerized TAGs (6) were followed.

Analytical methods. The content of tocopherols was determined using reversed phase HPLC: the pump was LCP 4000.1, Ecom Prague; injection port: Rheodyne, USA; column: Separon SGX RPS 5 μm, 4 mm × 250 mm, Tessek Prague; injected volume: 20 μl; preparation of samples: dissolved in methanol (standards of tocopherols) or acetone (samples of oils and fats – 10 g of sample to 100 ml of acetone); the mobile phase was a mixture of acetonitrile: methanol (1:1, v/v) with $LiClO_4$ (0.02 mol/l) and NaCl (0.005 mol/l); flow rate: 1 ml/min; the electrochemical detector: HP 1049 A, Hewlett Packard, equipped with a glassy carbon electrode; the detection potential: 1.05 V; signal treatment: chromatography station CSW 1.6, DataApex,

Czech Republic. The same apparatus was used for the determination of carnosic acid and carnosol: column: Hypersil ODS 5 μm, 4.6 mm × 200 mm, Hewlett Packard; injected volume: 20 μl; preparation of samples: dissolved in methanol; the mobile phase was a mixture of acetonitrile: 0.5 % (m/m) phosphoric acid – with EDTA – 1 mmol/l (65:35, v/v) with NaCl (5 mmol/l); the detection potential: 0.8 V.

RESULTS AND DISCUSSION

In oils without rosemary extract, tocopherols decomposed relatively fast. For example, during storage at 60°C, the content of α-tocopherol decreased to 50% at a peroxide value of about 20 – 30 meq/kg. During heating at 150°C, total tocopherols were consumed at the content of lipid polymers of about 6%. In all cases, α-tocopherol is decomposed faster than γ- and δ-tocopherols, in the same way as they show their ability to be donors of the phenolic hydrogen for lipid peroxy radicals (1). Similar results were obtained at frying conditions (7). During the storage at 60°C, γ- and δ-tocopherols started their decomposition at about the moment when the content of α-tocopherol decreased to 50%. During heating at 150°C, γ- and δ-tocopherols are decomposed from the beginning, but more slowly than α-tocopherol.

Rosemary extracts inhibited the degradation of tocopherols in all the experiments. Figs 1 and 2 show the degradation of α- and γ-tocopherols (δ-tocopherol is presented in a low amount) together with rosemary antioxidants in rapeseed oil I at 60°C and at 100°C. In both cases, carnosic acid is decomposed faster than carnosol, which is decomposed nearly together with α-tocopherol; γ-tocopherol and δ-tocopherol are decomposed as last. Rosemary extract

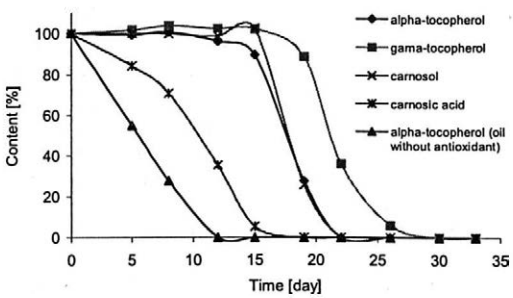

Figure 1. Changes of tocopherols, carnosic acid and carnosol contents during storage at 60°C

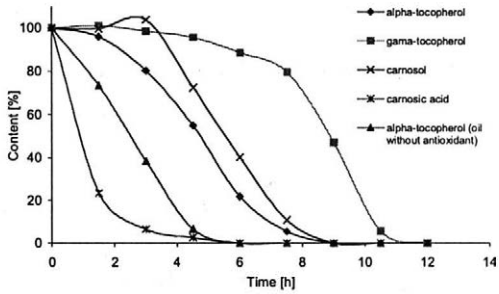

Figure 2. Changes of tocopherols, carnosic acid and carnosol contents during heating at 100°C (using the Oxipres apparatus)

prolonged induction period of α-tocopherol three times at 60°C and about two times at 100°C. Induction periods of γ- and δ-tocopherols were prolonged less than this of α-tocopherol, but also significantly.

Therefore, rosemary extract can be used for stabilization of tocopherols in vegetable oils, which are important sources of these vitamins, during storage and also during frying. Similar stabilization effect was obtained for diludine (a synthetic dihydropyridine antioxidant) (8).

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Impact of Disinfecting Treatment on Ascorbic Acid Content in Minimally Processed Vegetables

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Abstract

To assure the microbiological safety of minimally processed refrigerated vegetables, a disinfecting washing is included into the technological process. However, a negative impact of this step on nutritive value of vegetables could be expected. Hydrogen peroxide, peroxyacetic acid and natrium hypochlorite were used as active antimicrobial agents in the washing bath for processed cabbage, carrot, onion and Chinese cabbage. Significant decreases in the ascorbic acid concentration have been determined after the sanitizing treatment, nevertheless, considerable losses in water washed vegetables have been found after 5-days cold storage as well.

Keywords: minimally processed vegetables; ascorbic acid; antimicrobial treatment

INTRODUCTION

Fresh, row vegetables have high natural loads of diverse microorganisms, spoilage organisms and soil pathogens included. Characteristics of vegetable tissue such as high water and nutrient contents and a neutral pH enable the development of almost any type of microorganism. Wounded tissues provide favorable conditions for microbial growth; therefore the minimally processed vegetables (MPV) should be considered highly perishable.(1)

In the recent past, there was detected the widely used temperatures below 6°C are not sufficient to control the growth of some psychrotrophic types of pathogenic and spoilage microorganism. Washing of the cut vegetables with a disinfectant can considerably decrease the microbial load (2, 3). Various forms of active chlorine or hydrogen peroxide are used around world, no disinfectant is permitted in the Czech Republic for the MPV production. A negative impact on some nutrients, especially on ascorbic acid, could be expected due to the increased rate of metabolic processes as well as the oxidizing antimicrobial agents. The aim of the paper was to compare the efficacy of different disinfectants and investigate the changes in ascorbic acid content in the minimally processed vegetables after washing.

EXPERIMENTAL

Materials. Vegetables: cabbage, carrot, onion, Chinese cabbage.

Washing medium: distilled water, hydrogen peroxide (500mg/l), Persteril (peroxyacetic acid 500mg/l), sodium hypochlorite (100mg/l)

Methods. Sample preparation: clean, peeled, cut, pre-refrigerated vegetables were washed 10 s in the washing medium, then 10 min dried et ambient temperature. Samples of 200 g were packed in the PE bags and stored 5 days at 2–5°C.

Microbiological analysis: counts of the total mesophilic organism (CFU/g of TMO), fungi (F) and coliforms (C) were determined using the standard methods

Ascorbic acid: HPLC method was applied – Ostion LGKS 0800H$^+$, 250 × 4 mm, 5mM-H_2SO_4, UVD 254 nm, external calibration.

RESULTS AND DISCUSSION

The results have shown a similar antimicrobial efficiency for hydrogen peroxide and active chlorine, a slightly higher effect was detected for peracetic acid. As demonstrated for cabbage (Fig. 1), the antimicrobial treatments resulted in only one/two-log reduction of microbial loads in comparison to vegetables washed with water only. The five-days cold storage led to re-growth of microbes, so that the counts increased about one log again, however, the differences between the water-washed and disinfected samples remained at the same value approximately.

Retention of ascorbic acid (AA) after the antimicrobial treatment was found from 50 to 75% of the initial content (Fig. 2). Within the five-days cold storage, the losses of

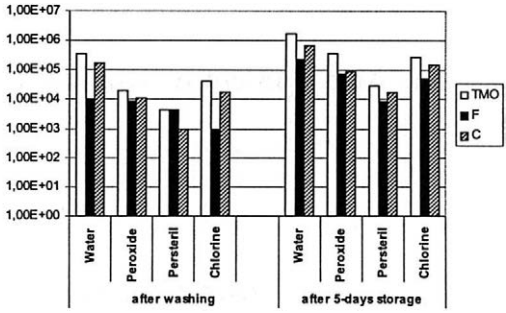

Figure 1. Counts of total mesophilic, fungi and coliforms microorganisms in cabbage after the washing and cold storage (CFU/g)

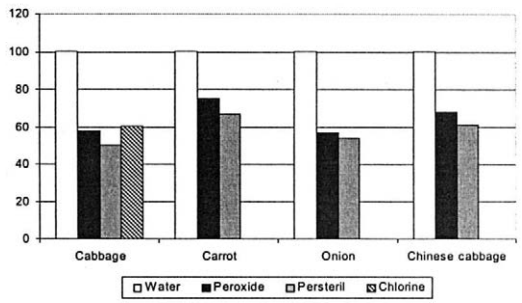

Figure 2. Retention of ascorbic acid in minimally processed vegetables after disinfecting treatment (%)

AA in the treated samples increased, but a significant decreasing was observed also in water-washed vegetables (Fig. 3). The highest losses were determined after peracetic acid application, the use of the hydrogen peroxide and hypochlorite led to a comparable decrease.

It could be concluded that the disinfecting treatment of fresh cut vegetables leads to significant losses in ascorbic acid; however, within the storage period considerable changes in AA take place in non-treated vegetables as well. Justification of antimicrobial treatment of the minimally processed vegetables is that improved process control is required to minimize the risk of microbial foodborne illness. The results demonstrate the need for improved washing or surface pasteurization methods, which are antimicrobial effective enough but friendly to nutrients.

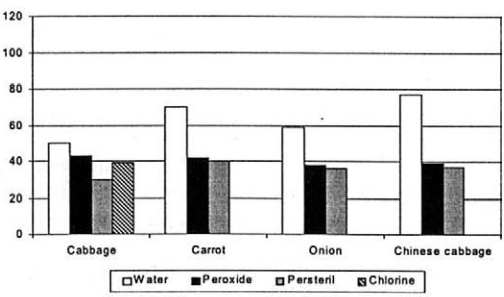

Figure 3. Retention of ascorbic acid in minimally processed vegetables after 5-days storage (%)

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Changes of Natural Antioxidants – Ascorbic Acid, Polyphenols and Anthocyanins in Apples, Potatoes and Plant Berries during their Storage

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Abstract

Apples and potatoes are significant sources of polyphenols and ascorbic acid as antioxidants with benefit effects on human health. They are closely connected in antioxidant net work because ascorbic acid reduces oxidized quinones back to polyphenols. Changes in their content were determined in three apple varieties and four potato varieties during their storage. It was found significant varietal dependency, in lesser extent year of cultivation. During cold storage at +5°C for six months content of ascorbic acid and total polyphenols significantly decreased. Ascorbic acid added to thickened extracts of elderberries and red grapes skins caused higher degradation of red anthocyanins during their storage period for 167–194 days due their reduction to colourless leucoanthocyanidin forms.

Keywords: apples; potatoes; elderberry; red grape; stability; polyphenols; ascorbic acid; anthocyanins

INTRODUCTION

Ascorbic acid (AA) and total polyphenol content (TP) are important plant antioxidants and scavengers of free radicals. They inhibit oxidation of low-density lipoproteins, lower cholesterol levels, decrease fragility of blood vessels and increase their permeability, decrease heart coronary risk, etc. (1). One of the richest sources of polyphenols and ascorbic acid in human nutrition are apples (*Malus pumilla* Mill.) and potatoes (*Solanum tuberosum* L.) that contain their abundant complex (2). Anthocyanins from elderberries and red grape skins are appreciated as natural food colorants and their stability is attracting interest (3).

EXPERIMENTAL

Material. In 1997 and 1998 years was investigated ascorbic acid content (AA) and total polyphenol content (TP) in three apple varieties (Idared, Gloster and Ontario) and in four potato varieties (Lukava, Désirée, Nicola, Rosella) cultivated under equal conditions on the experimental plot of Czech University of Agriculture in Prague-Suchdol.

Methods. AA content in apples was determined by titration method with 2,6-dichlorphenolindophenol and TP content spectrophotometrically with Folin-Ciocalteau's reagent. In two years' trials AA and TP contents were estimated immediately after harvest in September and then monthly during storage for six months at +5°C. The TP content was determined in centrifugates from peeled potato tubers or apples with skin with Folin-Ciocalteau's phenol reagent (Fluka Chemie AG) after addition of 20% Na_2CO_3 solution spectrophotometrically on the Spekol 11 spectrophotometer at $\lambda = 765$ nm against blank.

AA content was determined in peeled cooked tubers (about 50 g) in the 2% oxalic acid solution after addition of 2.5 M sodium acetate by comparison with the standard solution of AA (1 mg/mL). The samples were measured polarographically on the EKO-TRIBO Polarosensor polarograph.

Anthocyanin stability. Effect of addition of AA (50 mg/100g) on the stability of anthocyanins in thickened extracts of elderberries (*Sambucus nigra* L.) and red grape skin residues (*Vitis vinifera* L.) adjusted with sucrose to 65% refractometric dry matter was investigated. Anthocyanin content was determined spectrophotometrically at $\lambda = 535$ nm. Stability was investigated during storage of thickened and adjusted concentrates for 167–194 days at 4°C in refrigerator.

RESULTS AND DISCUSSION

Statistically high significant differences were determined both in AA and TP contents during storage period – there was observed meaningful AA and TP decrease (Fig. 1 and 2). The highest antioxidant content showed in both investigated years early variety Ontario (av. 12.05 mg/kg

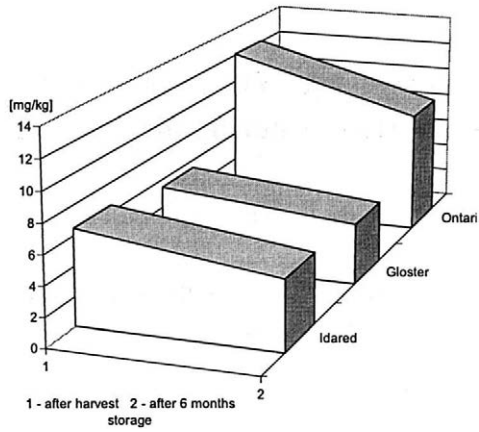

Figure 1. Average content of ascorbic acid in apples

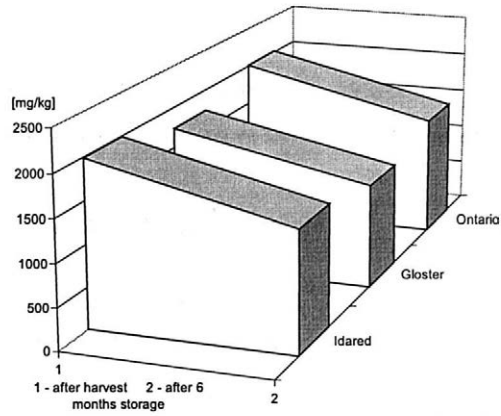

Figure 2. Average content of total polyphenols in apples

AA, 2011.5 mg/kg TP), the lowest semi-early variety Gloster (in average 5.45 mg/kg AA, 1738.0 mg/kg TP). In this context the variety Ontario is the most favourable. The highest decrease of AA content was in variety Ontario (–30.05%$_{rel}$), the lowest in variety Gloster (–22.93%$_{rel}$); in TP contents differences were negligible (–27.14 and –27.82%$_{rel}$). In spite of this decrease were in Ontario variety both values after six months storage the most favourable (8.45 mg/kg AA, 1466.5 mg/kg TP). Results were statistically evaluated with *F*-test and *t*-test. There could be seen differences among varieties and years of cultivation, but these differences were under the level of statistical significance showing only apparent tendencies. While varietal differences in decreasing of AA content during storage were greater (in interval from –22.93%$_{rel}$ in Gloster variety to –30.05%$_{rel}$ in Ontario variety), decreasing of TP contents during storage was nearly the same (about –27 %$_{rel}$ of initial content).

Potatoes are relatively rich source of polyphenol antioxidants and ascorbic acid that is with polyphenols very closely combined in antioxidant network (4). On TLC chromatograms the most manifested phenolic compounds was chlorogenic acid (2–6×10^{-4} M) and L-tyrosine (1–2×10^{-3} M). Caffeic acid and other phenolics were present only in minor amount (5). Caffeic acid may be a product of hydrolysis of chlorogenic acid. During the storage of four potato varieties for six months decreased original AA content statistically significantly to $^1/_2$–$^1/_3$ of original amounts (Fig. 3). There were found varietal differences (in Désirée variety the lowest decrease to 80% of 23.73 mg/100g, in Rosella variety the highest to 49% of 16.00 mg/100g). TP content showed analogical running during storage (Fig. 4). The highest decrease was determined in Lukava variety (–49.8% of original amount 74.8 mg/100 g) and the lowest in Désirée variety (–17.9% of original amount 62.0 mg/100 g).

Ascorbic acid affected stability of anthocyanins negatively decreasing anthocyanin content in elderberries in average by 0.45% during storage for 194 days and red grape stampings by 9.24% during storage for 167 days in comparison with control (Fig. 5). AA apparently reduced during long term storage period anthocyanins to colourless leucoanthocyanidin forms.

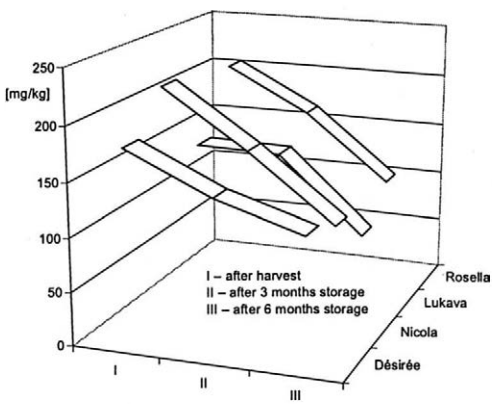

Figure 3. Average content of ascorbic acid in potatoes

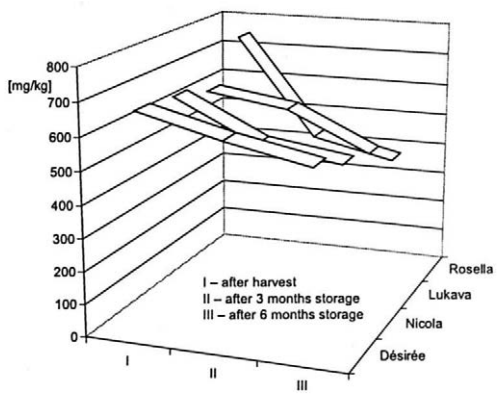

Figure 4. Average content of total polyphenols in potatoes

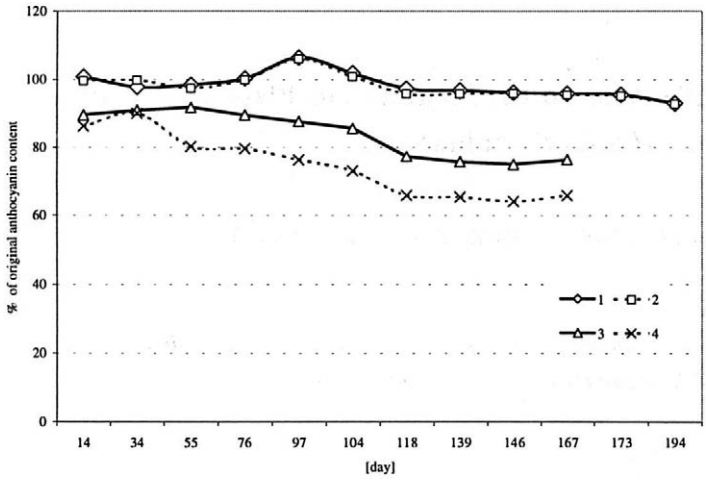

Figure 5. Changes in anthocyanin content during storage. 1 – Elderberries control, 2 – Elderberries + AA, 3 – Red grape skins control, 4 – Red grape skins + AA

Acknowledgment: This work was supported by research project of the Faculty of Agronomy of Czech University of Agriculture in Prague MSM 41200002.

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The Effect of Ferulic Acid on the Structure of Plant Cell Wall and its Dermination

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Abstract

Ferulic acid plays the significant role in the plant cell wall because forms the bond between polysaccharides and proteins. Therefore the determination of ferulic acid has a great importance. It is known that sugar beet pectin and spinach pectin contain ferulic acid. To determine ferulic acid dry sugar beet pulp was used as samples. The ferulic acid was determined by UV/VIS, IR, NIR- spectroscopy. In case of the IR and NIR-spectroscopy measurement the fine powder with defined size of particles was used. The acid extract was prepared for UV/VIS-spectroscopy. Before the UV/VIS measurement the pH of extract was adjusted to value 10. To release ferulic acid from esteric bond the hydrolysis at alkaline medium (pH = 12.5) took place. The maximum of absorbance non-hydrolysed extract was at 372 nm. The maximum of absorbance of hydrolysed extract was at 345 nm. The same value was obtained by measurement of the ferulic acid standard under same conditions. The content of ferulic acid in sugar beet pulp was in the range from 0.3% to 0.7%. The results of UV/VIS- spectroscopy is compared with special regions of NIR-spectra and with height of band (1516 cm^{-1}) of IR-spectra. The results of all methods are in good congruence between obtained data.

Keywords: ferulic acid; sugar beet pulp; NIR; IR; UV-VIS; spectroscopy

INTRODUCTION

Ferulic acid (3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid) is a phenolic compound with summary formula $C_{10}H_{10}O_4$. It occurs in nature mainly in *trans* configuration. The *trans* isomer ferulic acid is covalently bound to cell walls polysaccharides of plant. Ferulic acid is released after treatment of cell walls polysaccharides by alkali, that means ferulic acid is esterified on cell wall polysaccharides. The maximum absorbancy of ferulic acid changes with pH. According to the study of Fry (1), the maximum of absorbancy ferulic acid and methyl ferulate, ethyl ferulate, hydroxy- methyl ferulate at pH = 10 is describe in Table 1.

Table 1. Maximum of absorbancy of ferulic acid and its esters (1)

Compound parameter	Max. absorbance pH = 10 (nm)
Ferulic acid	343
Methyl ferulate	366
Ethyl ferulate	369
Hydroxy-ethyl ferulate	371

It was found that solutions of separated isomers (*cis* and *trans*) could be preserved as such by keeping in total darkness (2). The darkness prevent light-induce isomerization of ferulic acid. Solutions of ferulic acid prepared under normal laboratory lighting were equilibrium mixture of the *cis* and *trans* isomers (1).

Cross-linking of polysaccharides has influence on the structure of plant cell wall. Dimerization of ferulate esters is a pathway for cross-linking polysaccharides chains. Ralph and Quideau (4) describe two disparate mechanisms forming dimmers of ferulic acid: Photochemically induce [2+2]-cyclodimerization and oxidative coupling effecting by the action of peroxidases to produce dehydrodimers (4, 7).

EXPERIMENTAL

Materials

As a sample was used sugar beet pulp. Sugar beet pulp was obtained from sugar-factory Opava-Vávrovice. Harvesting time was autumn 1994. Ferulic acid standard was pursued from Fluka AG, purum 98%, Germany. The common chemicals (hydrochloric acid and sodium hydroxide) were pursued from Lachema, a. s., Czech Republic.

Methods

Liquid – solid extraction of dry sugar beet pulp (3, 6). Dry sugar beet pulp was grinding on crusher 1 minute. 1 g

of sample was placed into a flat-bottom flask and 100 ml of 0.2 M were added and kept 30 minutes at laboratory temperature. Afterward the flask was put into a water bath 85°C heated for 60 minutes Warm suspension was filtrated.

UV-VIS spectroscopy. The pH of solid-liquid extract was changed by using 0.2 M sodium hydroxide on value 10 and resulted in solution A. Solution B was prepared by hydrolyses of extract under condition pH = 12.5 at room temperature, one hour. The hydrolyses was finished by adding 0.2 M solution of hydrochloric acid to obtain pH = 10. Solution A and B was measured on UV 4 Unicam UV/VIS-spectrometer. The range of wavelength was from 190 nm to 500 nm. The content of ferulic acid was calculate from calibration curve at wavelength 345 nm (Software: Vision 3.31).

IR-spectroscopy. The fine powder with particles size as small as 78μm of sugar beet pulp was obtained and measured on the IR-spectrometer Nicolet 740, (Nicolet, Analytical Instrument, Madison, USA). The Kubelka-Munk model (6) was used for evaluation of spectra. Software Omnic E. S. P. 5.0 was used.

NIR-spectroscopy. The fine powder with particles size as small as 78 μm of sugar beet pulp was measured on Avatar 360 N, Nicolet, s.r.o., NIR-spectrometer. Software Omnic E.S.P.5.0, TQ Analyst 5.0.

RESULT AND DISCUSSION

The alkali hydrolyses has been used to release ferulic acid from sugar esters. The maximum of absorbancy of the extract before hydrolyses was 372 nm. After hydrolyses the maximum of absorbancy of the extract shifted to the wavelength 345 nm, which is in agreement with the optimum of free ferulic acid. This optimum is at 345 nm. The spectra of non-hydrolysed and hydrolysed extract there are on Fig. 1. The content of ferulic acid was in range from 0.3 to 0.7. The 30 samples of sugar beet pulp was measured. Correlation coefficient between UV/VIS-data of the non-hydrolyse and the hydrolyse extract was 0.84.

By IR-spectroscopy was measured the powder samples with the defined size of particles. The DRIFT- technique of measurement was used. The ratio of height of bands 1516 and 831 cm^{-1} was compared with UV/VIS-data of non-hydrolysed and hydrolysed extract.

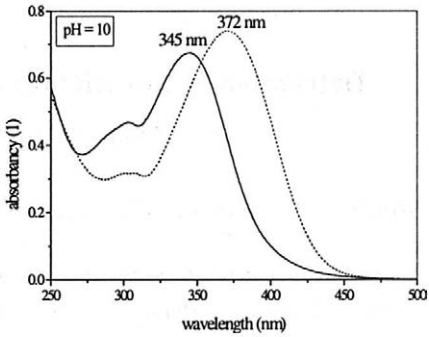

Figure 1. UV/VIS-spektra of non-hydrolysed and hydrolysed extract

The chosen regions of NIR-spectra were compared with data obtained by UV/VIS-spectroscopy by using software TQ analyst 5.0. The best correlation with UV/VIS-data of non-hydrolysed extract was in range 9890–7270 cm^{-1} (correlation coefficient 0.92). The UV/VIS -data of hydrolyse extract correlated with regions from 9875 to 7226 cm^{-1} in the best way (correlation coefficient 0.86).

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Determination of Biologically Active Compounds in Wines using Overpressured-Layer Chromatography

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Abstract

Twenty five hungarian wine varieties were studied regarding biologically active compounds such as resveratrol, biogenic amines, total polyphenols and free amino acids. Simple and efficient OPLC methods were used for separation and quantification of first two compounds and amino acid analyser was used for determination of free amino acids. There were significant differences between red and white wines only in the case of resveratrol and of total polyphenol content. Negative correlation was found between resveratrol of beneficial activity and tyramine content.

Keywords: wines; resveratrol; polyphenols; biogenic amines; free amino acids; OPLC

INTRODUCTION

According to recent investigations, wines generate different beneficial biological effects on the human organism. This is especially valid for the red wines. Resveratrol, a stilbenic phytoalexin present in fresh grapes and wines, has a pronounced biological activity. Red wine and its constituent, resveratrol has cardio-protective effect, because it reduces e.g. the susceptibility of low-density lipoprotein (LDL) to lipid per-oxidation (antioxidant effect), as well as shows a cancer chemo-protective activity (1, 2).

Biogenic amines are normal constituents of foods. During storage or fermentation microorganisms with decarboxylation of free amino acids produce them. Some biogenic amines (e.g. histamine, tyramine) manifest harmful effect on human health. Therefore, consumption of foods and beverages (e.g. wines) rich in biogenic amines can cause different inconvenient symptoms (e.g. headache, diarrhoea) (3).

It is obvious that the chemical composition of wines may be responsible for these activities; therefore, the comparative study of chemical components of wines is an actual task.

The aim of this work was to study correlations among resveratrol, total polyphenol, free amino acid and biogenic amine content of wines.

EXPERIMENTAL

Determination of resveratrol was accomplished by the BS 50 Personal OPLC Chromatograph (OPLC-NIT Ltd., Budapest, Hungary) based on the method by Kátay *et al.* (4).

Total polyphenols were determined by spectrofotometric method using the Folin-Ciocalteu reagent (5).

Biogenic amines were determined by OPLC method according to Kovács *et al.* (6).

Determination of free amino acids was solved by automatic amino acid analyser (BIOTRONIK LC 3000) using the ninhydrin reagent.

RESULTS AND DISCUSSION

17 Hungarian red and 8 white wines were investigated. Table 1 shows the content of biologically active compounds in red and white wines.

The average contents of resveratrol in red wines and white wines were 2.24 mg/dm^3 and 0.32 mg/dm^3, respectively. The amount of resveratrol in red wines was about one magnitude order higher than in case of white wines.

The average content of total polyphenols in red wines was 1422 mg/dm^3, and in white wines was 256 mg/dm^3. The amount of total polyphenols in red wines was about six times higher than in case of white wines.

The average contents of biogenic amines in red and white wines were 26 mg/dm^3 and 25 mg/dm^3, respectively. There was no significant difference between the total amount of biogenic amines in red and white wines. The concentrations of individual biogenic amines were slightly different in the two types of wine. The main biogenic amines found in wines were putrescine, tyramine and agmatine (Fig. 1).

The average content of free amino acids in red and white wines were 527 mg/dm^3 and 468 mg/dm^3, respectively. The

Table 1. The content of biologically active compounds in red and white wines (mg/dm³)

		Resveratrol	Total polyphenols	Total biogenic amines	Total free amino acids
Red wines	Rubintos	4.90	864	24.74	346.48
	Vranac	4.83	974	19.91	317.07
	Kekfrankos	4.46	1310	20.67	362.30
	Kekoporto	3.74	773	23.64	358.36
	Turan	2.41	2481	11.16	481.04
	Alicante Bouschet	2.35	2527	14.36	199.95
	Titan	2.26	2663	10.51	518.64
	Blauburger	2.02	1142	69.31	505.26
	Pinot noir	1.96	1124	14.47	643.83
	Biborkadarka	1.85	2098	42.96	479.34
	Cabernet sauvignon	1.59	1187	13.13	639.81
	Medina	1.36	942	16.13	380.10
	Cabernet franc	1.34	559	27.90	1143.74
	Merlot	1.33	1484	11.26	719.13
	Zweigelt	1.00	1383	37.86	605.20
	Kadarka	0.88	686	66.37	683.66
	Karmin	0.61	1971	19.12	568.23
White wines	Rajnai rizling	0.50	248	32.66	568.04
	Furmint	0.43	252	19.32	386.03
	Chardonnay	0.37	240	23.70	827.79
	Olasz rizling	0.32	209	16.80	348.23
	Harslevelu	0.32	222	19.63	353.64
	Cirfandli	0.29	356	50.73	488.40
	Zenit	0.21	240	20.49	420.31
	Sauvignon blanc	0.15	277	18.86	347.78

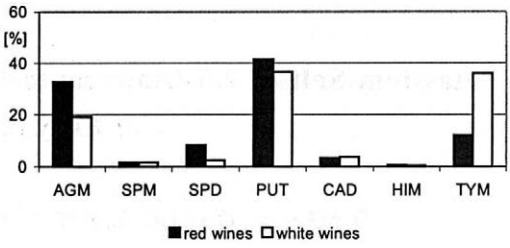

Figure 1. The ratio of biogenic amines in red and white wines (AGM – agmatine, SPM – spermine, SPD – spermidine, PUT – putrescine, CAD – cadaverine, HIM – histamine, TYM – tyramine)

main amino acid found in wines was proline (>50% from the total free amino acid content).

Based on the statistical analysis it was found negative correlation between resveratrol and tyramine content. Further investigations are needed to clarify the relation between the beneficial and non-beneficial component.

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Potassium Salt of 2,5-Dimercapto-1,3,4-Thiadiazole as Potential Inhibitor of Enzymatic Browning

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Abstract

The purpose of this study was to investigate the possibility of monopotassium salt of 2,5-dimercapto-1,3,4-thiadiazole (DMTD) as potential inhibitor of enzymatic browning of fresh-cut fruits and vegetables. The synthesis of monopotassium salt of 2,5-dimercapto-1,3,4-thiadiazole is presented. The oxidation of phenolic compounds, chlorogenic acid (CA) and 3,4-dihydroxy phenilalanine (L-DOPA), catalized by mushroom polyphenol oxidase (PPO), was investigated in the presence of DMTD. Also DMTD was evaluated on the cut surface of apples (Jonagold variety), as browning inhibitor. Inhibition of PPO activity in model solutions of phenolic compounds was measured by spectrophotometer, and the enzymatic discoloration on the cut surface of apples was measured by the tristimulus colorimeter. DMTD was effective as an inhibitor of oxidation of phenolic compounds used in assay as well as inhibitor of browning on cut surfaces of apples. For the comparison, as inhibitors of enzymatic browning, ascorbic acid (AA), and benzoic acid (BA) were used too.

Keywords: polyphenol oxidase; enzymatic browning; inhibition; potassium salt of 2,5-dimercapto-1,3,4-thiadiazole

INTRODUCTION

Browning of raw fruits and vegetables, after tissues damage, during postharvest handling and processing, is one of the main causes of quality loss. Enzymatic browning represents a serious problem for the food processing industry, especially with recent restrictions in the use of sulfites in raw fruits and vegetables and other products (1, 2). Enzymatic browning has a deleterious effect on fresh-cut fruits and vegetables and limit product quality and shelf-life (3, 4, 5).

In order to prevent browning of raw fruits and vegetables, many investigations has been done to develop the method for eliminating or retarding the process (6), as well as to find alternatives to sulfites (7, 8).

The objective in the present study was to evaluate the performance of DMTD as potential PPO inhibitor.

EXPERIMENTAL

Material. Mushroom polyphenol oxidase (PPO; EC 1.14.18.1) product T-7755, with activity of 3000 units/mg of solid (Sigma Chemical Co., St. Louis, MO, U.S.A.) as a dry powder was dissolved in phosphate buffer (pH 6.5, 47 mM).

PPO substrates, chlorogenic acid (Sigma) and L-DOPA (Sigma) were prepared as 2.5 mM solutions by disolving in phosphate buffer (pH 6.5).

Synthesis of monopotassium salt of 2,5-dimercapto-1,3,4-thiadiazole (DMTD). The compound was prepared by dissolving of 0.6 g dimercapto-thiadiazole in 4.6 mL of n-KOH, and than evaporated in vacuum to dryness. Crystalline product (0.8 g) was dissolved in 12 mL of ethanol and recrystallized at room temperature. Yild of 0.31 g of yellow product in the form of the long fine needles was get. The product has m. p. above 270°C.

Analysis: Calculated for $C_2HN_2S_3K$ (188);

Theoreticaly: C, 12.77; H, 0.56; N, 14.89; S, 51.06; Found: C, 12.03; H, 0.60; N, 13.45; S, 49.31 (Fig. 1).

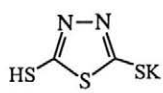

Figure 1. Structure of monopotassium salt of 2,5-dimercapto-1,3,4-thiadiazole (DMTD)

Activity assay. The enzyme activity was assayed spectrophotometrically using CECIL 2000 (CECIL INSTRUMENTS , England) spectrophotometer. Reaction mixture, in a total volume of 3 mL, included L-DOPA (2.5 mM), mushroom PPO (16 μg/mL) dissolved in phosphate buffer (pH 6.5, 47 mM) and certain concentration of DMTD solutions.

Before measurements, reaction solution (PPO substrate and DMTD in phosphate buffer) were thermostated for 20 min at 25°C. The enzyme solution was added in treatment solution before measurement.

The appearance of the brown colour, in the case of the L-DOPA was measured at 475 nm as a function of time (for 10 min), and in the case of the chlorogenic acid at 420 nm. The enzyme activity was calculated from the initial part of the curve of the absorption vs reaction time (ΔA/min) (4). Measured values were transformed into % inhibition.

Apple preparation for browning measurements. Apples (Jonagold variety) were purchased from the local growers and stored at 4 °C until needed. Before samples preparation apples were held at least one hour at room temperature. To prepare samples, apples were washed with water, peeled and cut into dices (1 cm × 1 cm × 1 cm) with a sharp knife. 100 g samples were immediately immersed for 120 sec in treatment solution (DMTD, 0.01, 0.05 and 0.1%). The excess solution was than blotted with adsorbent tissue and samples were packed in plastic boxes, covered with plastic film to prevent evaporation, and stored at 4°C.

The degree of browning of apple samples was monitored by reflectance measurements immediately after cutting, treatment with DMTD solutions ("0" time), and during storage of apple samples (untreated and treated) on day 1, 4, 8 and 12. L*, a*, and b* values were measured by the tristimulus colorimeter Minolta CR-300 (Minolta Camera Co., Osaka, Japan) using the averaging mode with fifteen replications. Based on the measured data the calculation of effectiveness of each inhibitor was performed by equation:

$$DE_{ab} = [(DL^*)^2 + (Da^*)^2 + (Db^*)^2]^{1/2}$$

RESULTS AND DISCUSSION

To determine whether or not monopotassium salt of 2,5-dimercapto-1,3,4-thiadiazole (DMTD) can be used as potential PPO inhibitor, model solutions with phenolic compounds CA and L-DOPA with mushroom PPO were used. Apple dices as a cut surface system were used to find out whether DMDT has anti-browning activity.

Results of DMDT efficiency to prevent an oxidation of phenolic compounds are presented in Table 1 and 2. It is evident that DMTD was more effective in prevention of oxidation than ascorbic acid (AA) and benzoic acid (BA). For the 50% inhibition of oxidation of L-DOPA, 13 μM of DMTD was used. At the same conditions, for the 50% inhibition of L-DOPA, 232 μM of AA, and 875 μM of BA were used. For the 96.54% of inhibition of oxidation of L-DOPA, 0.05 mM of DMTD was needed.

Table 1. The effectiveness of different inhibitors on PPO activity in solutions of L-DOPA

Substrates	Inhibitors	I_{50} (mM)*
L-DOPA	DMTD	0.013
	Benzoic acid	0.875
	Ascorbic acid	0.232

* I_{50} = concentration of the inhibitors that causes 50% inhibition of PPO activity

In the case of CA, as PPO substrate, the most effective as inhibitor of PPO activity was again DMTD.

Table 2. The effectiveness of different inhibitors on PPO activity in solutions of chlorogenic acid

Substrates	Inhibitors	I_{50} (mM)*
Chlorogenic acid	DMTD	0.051
	Benzoic acid	0.658
	Ascorbic acid	0.414

* I_{50} = concentration of the inhibitors that causes 50% inhibition of PPO activity

Monopotassium salt of 2,5-dimercapto-1,3,4-thiadiazole (DMTD) showed considerable effectiveness as an inhibitor of oxidation of phenolic compounds CA and L-DOPA.

Table 3 summarizes some results on colour parameters L*, a* and ΔE, of enzymatic browning of apple dices by treatment by DMTD.

From the data it can be seen that DMTD was very effective as an inhibitor of discoloration of cut surface of apple dices. The most effective was 0.1% DMTD. Fresh-cut apples had good colour after 8 days, and some even after 12 days.

Table 3. Effect of monopotassium salt of 2,5-dimercapto-1,3,4-thiadiazole, as potential browning inhibitor, applied to apple dices

Treatment	Day	Colour parameters		
		L* value [1]	a* value [1]	ΔE[2]
Control	0	80.01 (0.84)	−3.18 (0.46)	
	1	76.22 (1.16)	−1.66 (0.66)	5.13
	4	76.32 (1.10)	−0.81 (0.66)	5.57
	8	47.47 (1.44)	−0.43 (0.61)	6.62
	12	75.28 (1.00)	−0.27 (0.51)	6.18
0.01% DMTD	0	79.61 (0.88)	−3.59 (0.60)	
	1	77.66 (0.73)	−2.79 (0.86)	2.46
	4	76.60 (1.77)	−1.36 (0.92)	4.03
	8	4.4 (2.61)	−0.47 (1.37)	6.91
	12	71.06 (2,63)	1.00 (1,28)	10.99
0.05% DMT	0	80.54 (0.93)	−3.74 (0.39)	
	1	78.74 (1.20)	−3.49 (0.32)	2.04
	4	77.62 (1.91)	−2.73 (0.43)	3.39
	8	76.85 (1.02)	−2.51 (0.59)	3.95
	12	75.05 (2.01)	−1.64 (0.59)	5.95
0.10% DMTD	0	80.96 (1.75)	−3.77 (0.65)	
	1	80.23 (2.20)	−2.68 (1.58)	1.71
	4	80.07 (1.79)	−2.05 (1.28)	2.56
	8	4.4 (2.19)	−1,21 (0.92)	3.33
	12	79.56 (1.29)	−1.07 (0.98)	3.84

[1]L and a values (means of 15 replicates)
Value in parentheses are standard deviation (SD)
[2]Total colour change

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Analysis of Metabolites of Cyanidinglucosides

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Abstract

With the increased significance of a potential beneficial role of flavonoids in human health, there is a growing demand for research on their absorption, metabolism, and excretion. Previous studies on the metabolism of flavonoids (1) have demonstrated that ingested flavonoids are absorbed through the intestinal tract, the absorption ratios being in the range of 4 to 58% depending on the type of flavonoids, and then the majority undergoes structural modifications such as methylation, sulfation, and glucuronidation (2, 3) before finally being excreted in urine and bile. This study focuses on the metabolism of cyanidin-3-glucoside and cyanidin-3-sambubioside as the main constituents of the *S. nigra* extract. Data will be shown on the kinetics of urinary excretion of cyanindin metabolites.

Keywords: elderberry; cyanidin-3-glucuronide; metabolism of anthocyanins; urinary excretion; oxidation of anthocyanins

INTRODUCTION

Relatively little is known about the metabolic fate of anthocyanins despite their widespread occurrence and sizeable daily intake, estimated at 180–215 mg/day (5). It is unknown to what extent anthocyanins or their aglycones are absorbed following ingestion. Early studies with experimental animals indicated urinary excretion of less than 2% of oral anthocyanins from grapes (6). Cyanidin, the most widespread anthocyanidin, is resistant to catabolism by intestinal bacteria, an unusual finding in view of its hydroxylation pattern, which, when present in other flavonoid compounds, e.g. flavonols, predisposes those compounds to bacterial ring fission.

In this study we work with the anthocyanins of elderberry, mainly cyanidin-3-glucoside and cyanidin-3-sambubioside. After oral administration of anthocyanins of elderberry to humans (freeze dried elderberry extract), different kinds of metabolites are detected in the urine. The compounds are separated by preparative high-performance liquid chromatography, structural analysis is carried out by mass spectrometry.

Therefore a HPLC method was established, which separates the different substances in urine.

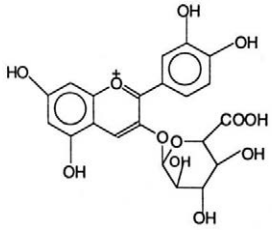

Figure 1. Structure of cyanidin-3-glucuronide

For reference the cyanidin-glucuronide was synthesized via the TEMPO method (4).

EXPERIMENTAL

The oxidation of the anthocyanins of elderberry to the glucuronides was carried out under aqueous conditions with 2.5 equiv. of NaOCl as oxidant at about 10°C in the presence of catalytic amounts of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) as radical starter. With the reaction product (cyanidin-3-glucuronide) the HPLC-method was validated.

For structural analysis of metabolites, urine was collected continously over a 24-h-period, for time profile analysis of the urinary metabolites urine samples were collected at 3-h intervals for 24 h after the ingestion of freeze dried elderberry juice.

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Coloured Secondary Products Formed in Amino Acid (Peptide) – Isothiocyanate Systems

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Abstract

The work is focused on advanced reactions in binary amino acid (peptide)/isothiocyanate (ITC) aqueous systems. The primary reaction products, i.e. *N*-allylthiocarbamoyl (ATC) amino acids and/or ATC-peptides, yield 3,5-substituted 2-thiohydantoins (2-thioxo-imidazolidin-4-ones) under H_3O^+ and OH^- catalysis, respectively. The latter compounds can be then transformed in neutral and preferably alkaline media to consecutive products, some of them are coloured. The compounds responsible for colour formation were identified as dimers of 5-unsubstituted 2-thiohydantoins (corresponding to parent amino acids containing C_α methylene group) linked with double bond, i.e. derivatives of 4,4´-dioxo-2,2´-dithioxo-5,5´-diimidazoli-dinylidene. The oxidative dimerization of 5-substituted 2-thiohydantoins leads to the non-coloured analoga, i.e. derivatives of 4,4´-dioxo-2,2´-dithioxo-5,5´-diimidazolidine. The pH optimum for the dimers formation from ITCs and most amino compounds tested is about 8. The reaction rate of dimerization itself is proportional to OH^- concentration. Two diastereomers of the dimers are usually formed. Moreover, the yellow thioxo forms are preferred only in acidic media and readily enolize with increasing pH to give red coloured structures.

Keywords: isothiocyanates; amino acids; peptides; dimerization; colour

INTRODUCTION

Degradation products of glucosinolates are the major contributors to the desirable flavour of cruciferous vegetables and condiments, such as radish, horseradish, mustard, and watercress (1). However, no food discoloration or colour development caused by the occurrence or reactions of any glucosinolate degradation product has been ever mentioned in literature.

Isothiocyanates (ITCs), the main pungent compounds of the above foodstuffs, are strong electrophilic agents and react easily with many nucleophiles including amino acids and proteins. The kinetics and mechanism of the formation of the 3,5-disubstituted 2-thiohydantoins (2-THs) were thoroughly described by Drobnica *et al.* (2) and Cejpek *et al.* (3). The reactions can proceed during storage, cooking or food processing as well as in the human digestive system.

In our previous investigations (3), a complete transformation of 3-allyl-5-methyl-2-thiohydantoin (MeATH) to other products occurred within 3 and 4 weeks in allyl isothiocyanate (AITC)/alanine systems of pH 8 and 10, respectively. The conversion led to two chromatographically resolved compounds, which were quite stable in mild alkaline media and their UV/VIS spectra possessed MeATH-like character. Further investigations revealed unknown coloured compounds arising in several other ITC/amino acid systems and forced us to define reaction conditions and structural preconditions of the reactants which are essential for the formation of these secondary products.

EXPERIMENTAL

Reactants. Isothiocyanates – allyl-ITC, butyl-ITC, and phenyl-ITC (Fluka), and amino compounds – glycine, L-alanine, β-alanine, sarcosine (Merck), Gly-Gly, Gly-Ala, Ala-Gly, Gly-Gly-Gly and Gly-Gly-Ala (Sigma) were used.

Model experiments. Solutions with equimolar 5 mM concentrations of ITC and amino compound (or with synthesized [4] 2-TH only) in 0.1 M buffered aqueous solutions of pH 4, 6, 8, 10 or 12 were prepared and stored at 25 ± 1°C in closed bottles for several weeks.

Isolation of dimers. The purification of reaction mixtures was performed on C-18e SPE column and the obtained methanolic concentrates were analysed using HPLC/PDA or HPLC/MS systems. The pure target products were collected after separation of the concentrated extract by means of semipreparative chromatography (Lichrospher 100 C_{18} in LichroCart 250-10, Merck).

HPLC analyses. Nova-Pak® C18, 250 × 4.6 mm, 4 μm, and Guard-Pak™ Nova-Pak® C18 (Waters) with gradient elution (water/acetonitrile), f = 0.7 ml/min, 20 μl loop, and HPLC system consisting of Waters 2 × 515 HPLC pump and 996 PDA detector system were used. The MS detector was Perkin-Elmer API 3000 electrospray with triple quadrupole.

Structure confirmation. Structure and purity of the isolated compounds were confirmed by ^{1}H- and ^{13}C-NMR spectrometry.

RESULTS AND DISCUSSION

In studying consecutive reactions of 2-THs, several secondary products have been identified. As recent results revealed, 2-THs partly convert to their open forms – thiocarbamoyl amino acids - under alkaline and pH neutral conditions, partly are transformed to other products. Among them, allyl thiourea (ATU) have been presented as a product of the reaction of NH_3 derived from Strecker degradation of an amino acid with AITC (3). Now, it was found, that

ATU also arise to a certain extent in the solutions of pure 2-THs, probably via hydrolysis in the presence of a reducing agent with simultaneous generation of carboxylic acids (e.g. acetic acid from 5-unsubstituted 2-thiohydantoins). Also allylamine (AA) can be originated during alkali hydrolysis of 2-thiohydantoins together with the original amino acid and carbonyl sulfide (3).

However, the most interesting consecutive reaction in systems investigated has led to another range of products. Structural analysis revealed that the compounds are dimers of 2-TH coupled with single (non-coloured) or double (coloured substances) bonds through C atom in position 5 on the heterocycle (Fig. 1). It follows from it, that optically inactive α-amino acids, such as glycine or sarcosine can be precursors of the coloured structures.

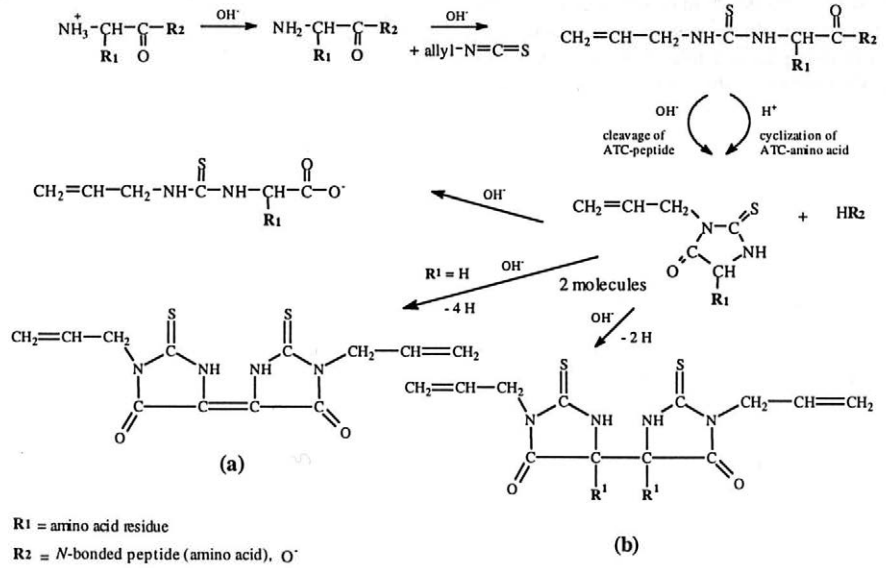

Figure 1. Formation of coloured (**a**) and non-coloured (**b**) dimers from AITC and amino acid (peptide)

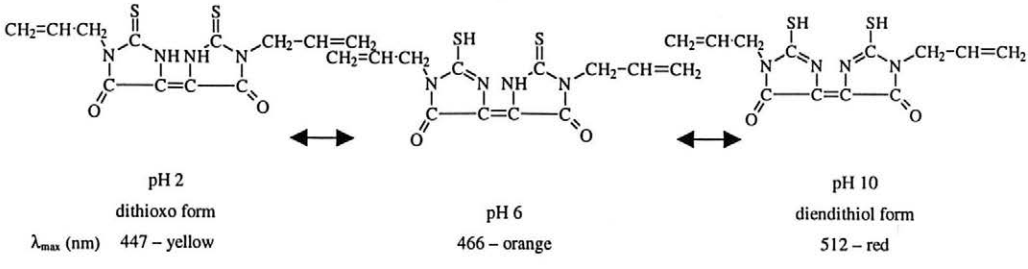

Figure 2. Some characteristics of tautomeric forms of 3,3′-diallyl-4,4′-dioxo-2,2′-dithio-5,5′-diimidazolidinylidene

The optimum for the dimers formation from ITC and amino acid (peptide) is mostly about pH 8, though in the case of alanine containing system corresponds rather to pH 10 being close to its $pK_a(NH_2)$. The rate of dimerization itself is proportional to growing pH. The dimers show the best stability at pH 8 and somewhat lower at pH 10. Much faster elimination from solutions proceeds in more alkaline pH (probably by oxidation) and in acidic media partly by precipitation, partly by further degradation.

Reaction rates leading to the dimers formation depend mainly on the character of amino acid residue and are higher for N-terminal amino acids than for free ones. The maximum yield of the dimer responsible for intensive coloured AITC/glycine pH 8 solution is less than 1% and for ATH solution about 3%. Most experiments were carried out with AITC, but also other ITCs – butyl-ITC and phenyl-ITC – have reacted with different rates of 2-TH formation and dimerization.

The dimers are formed in two diastereomers, which can give three different tautomers in dependence on pH. The coloured dimers then change colour from yellow to red when pH is changing from acidic to alkaline (Fig. 2). In AITC/sarcosine systems only one isomer was observed.

The knowledge of premises and conditions suitable for the dimers formation can be beneficial for yield evaluation of the derivatization procedures used in Edman's sequencing of peptides and/or HPLC determination of amino acids. The dimerization can participate on changes in sensory profile of ITCs containing foodstuffs. The effect of food discoloration seems to be not too significant because of low concentrations of coloured dimers at pH conditions being standard for foods and competitive ITCs reactions in the complex matrices.

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Reactions between Glucosinolate Products and Thiol Groups in Food Components

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Abstract

HPLC, MECC and NMR have been used to study the myrosinase catalysed degradation of indol-3-ylmethylglucosinolates and sinalbin in the presence of compounds containing free thiol-groups, such as cysteine and glutathione. The indol-3-ylmethylglucosinolates were found to form various thioethers as the main products, whereas *p*-hydroxybenzylisothiocyanate formed from sinalbin reacted with thiol groups upon formation of both thioethers and dithiocarbamates. Structures of formed compounds have been identified by NMR and MS.

Keywords: glucosinolates; HPLC; myrosinase; indol-3-ylmethyl; sinalbin

INTRODUCTION

Preparation, storage and mastication of food result in cell disruption and mixing of cell constituents, which may lead to various types of reactions. When glucosinolate are degraded in plant material, the compounds formed react with various types of functional groups in the food, such as thiol groups in proteins, peptides, and amino acids. Intermediates formed during hydrolysis of indol-3-ylmethylglucosinolates or sinalbin are highly reactive indol-3-ylmethyl isothiocyanates, which easily release the isothiocyanate ion and a potential free or bound carbonium ion (1–3). Both isothiocyanates and carbonium ions are electrophiles and thus reactive toward nucleophiles as thiol groups and for sinalbin and similar glucosinolates, the reaction between products of autolysis and thiol groups can both be thioethers and dithiocarbamates (3, 4) (Fig. 1). These reactions between thiol groups and hydrolysis products of glucosinolates have thus been considered as important areas, which call for specific attention. The present study is our contribution to the state-of-the-art in this field comprising use of hyphenated techniques based on HPLC, micellar electrokinetic capillary chromatography (MECC), diode array detection and NMR (5). A range of thioethers and dithiocarbamates has been produced, isolated and their structure confirmed (Fig. 1).

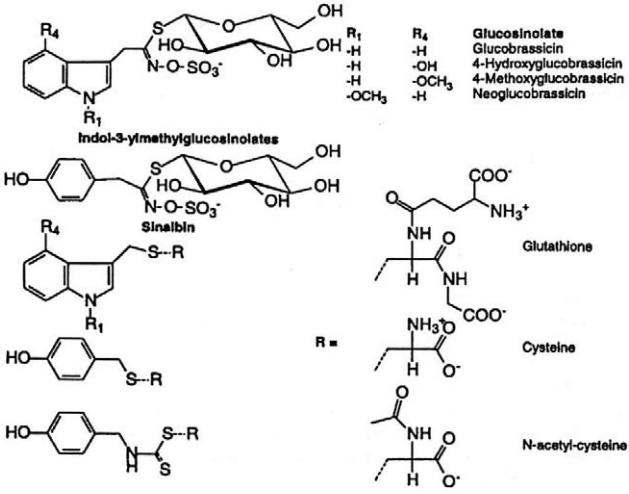

Figure 1. Structures of initial compounds and compounds formed after myrisonase catalysed degradation of indol-3-ylmethyl-glucosinolates and sinalbin

EXPERIMENTAL

Material. Reagents were of analysis grade. Pure myrosinase and sinalbin were purified from *Sinapis alba* L. Other glucosinolates were purified from *Brassica napus* L. seedlings (6, 7).

Formation of thioethers. Glucosinolates were degraded at pH 5.4 (acetate buffer, 50 mM) using myrosinase. The buffers contained approx. one equivalent of either cysteine, N-acetyl-cysteine or glutathione. As control, the glucosinolates were degraded in buffers free of compounds containing free thiol groups.

HPLC method. Degradation products were separated on a Gilson HPLC using an YMC Basic column (250 × 4.6 mm, 5 m particles) and gradient elution. Eluent A consisted of 25 mM KH_2PO_4/K_2HPO_4, pH 6.5 and 10% ACN. Eluent B was 100% ACN. Initially, the mobile phase was 100% A. After 3 min isocratic run, the mobile phase was linearly changed to 67% B in 18 min, and then returned to initial conditions. Flow rate was 1.5 ml/min and detection was performed at 217 nm.

MECC method. MECC analyses were performed on an HP[3D] CE (Hewlett-Packard, Waldbronn, Germany) equipped with a 645 × 0.05 mm I.D. fused-silica capillary. Detection was performed at 227 nm. The buffer consisted of 35 mM cholate, 50 mM taurine, 100 mM Na_2HPO_4, and 1% 1-PrOH. pH was adjusted to 7.3 with H_2SO_4. Temperature was set at 30°C.

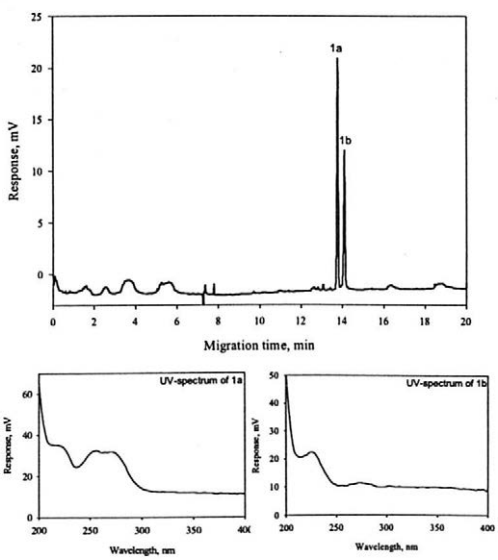

Figure 2. Electropherogram of products formed during degradation of sinalbin. N-acetyl-cysteine was added to the buffer. 1a = S-(4-Hydroxybenzylthiocarbamoyl)-N-acetyl-L-cysteine. 1b = S-(4-Hydroxybenzyl)-N-acetyl-L-cysteine. For experimental conditions see the experimental section

RESULTS AND DISCUSSION

Myrosinase catalysed degradation of sinalbin. Initially, myrosinase catalysed degradation of sinalbin lead to formation of the unstable *p*-hydroxybenzylisothiocyanat, which subsequently reacted with free thiol-groups upon formation of both thioethers and dithiocarbamates (Figs 2 and 3). Both the thioether and dithiocarbamate formed by reaction with N-acetyl-l-cysteine were identified from ^{1}H-NMR (Fig. 3) and their UV-spectra (Fig. 2). S-(4-Hydroxybenzylthiocarbamoyl)-N-acetyl-L-cysteine had characteristic 8_{max} at approx. 225, 255 and 275 nm whereas S-(4-Hydroxybenzyl)-N-acetyl-cysteine only had 8_{max} at 227 and 275 nm. The same 8_{max}'s observed for the cysteine and glutathione adducts, were nearly identical.

Degradation of mixtures of the indol-3-ylmethylglucosinolates gave a simultaneous formation of the respective thioethers in the presence of compounds with free thiol groups, like glutathione (Fig. 4). A number of other degradation products were also formed, among which various combinations of oligomeric compounds, nitriles etc. are expected to be formed.

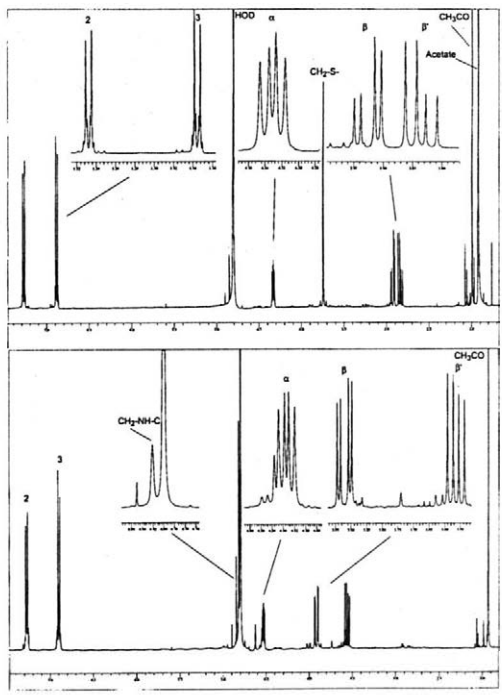

Figure 3. ^{1}H-NMR spectra of S-(4-Hydroxybenzyl)-N-acetyl-cysteine (left figure) and S-(4-Hydroxybenzyl-thiocarbamoyl)-N-acetyl-cysteine (right figure). Spectra were recorded at 400 MHz in D_2O

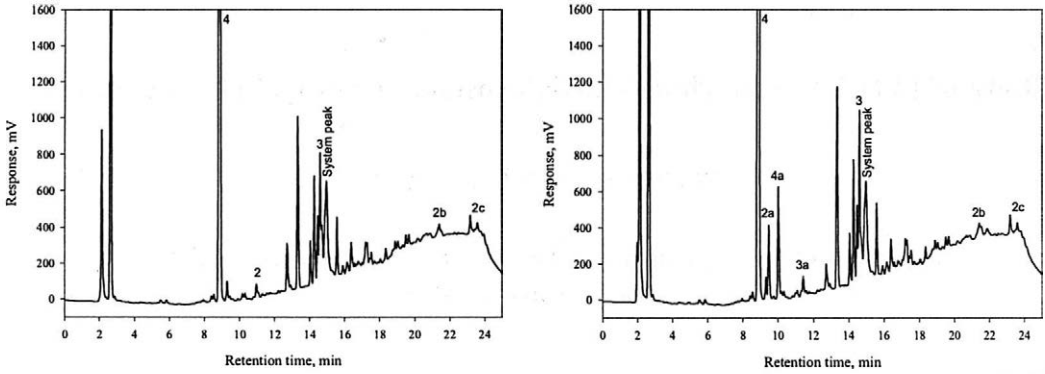

Figure 4. Chromatogram of degradation products formed from indol-3-ylmethylglucosinolates. Left figure: without glutathione, Right figure: with glutathione. 2a = S-(indol-3-ylmethyl)-L-glutathione, 2b = Di(indol-3-yl)methan, 2c = 2,3-Bis(indol-3-ylmethyl)indole (L-TRI), 3 = N-methoxyindol-3-ylmethanol, 3a = S-(N-methoxyindol-3-ylmethyl)-L-glutathione, 4 = 4-methoxyglucobrassicin, 4a = S-(4-methoxyindol-3-ylmethyl)-L-glutathione

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Study of (2*R*)-2-Hydroxybut-3-Enylglucosinolate (Progoitrin) Degradation

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Abstract

Degradation of (2R)-2-hydroxybut-3-enylglucosinolate (progoitrin) under various conditions is reported in this study. Progoitrin is a precursor of 5-vinyl-1,3-oxazolidine-2-thione (goitrin), which exhibits strong goitrogenic effect. Two model systems simulating either enzymic or non-enzymic degradation of isolated progoitrin were employed to quantify the yield of 5-vinyl-1,3-oxazolidine-2-thione. The experiments involving myrosinase (EC 3.2.3.1) showed that the yield of 5-vinyl-1,3-oxazolidine-2-thione was highly dependent on the pH value of the medium, increased yield being observed with increasing pH. The content of 5-vinyl-1,3-oxazolidine-2-thione formed by thermal treatment of progoitrin was investigated within the scope of the second model. Relatively small amounts of 5-vinyl-1,3-oxazolidine-2-thione were observed to be released from progoitrin on incubation in a boiling water bath.

Keywords: glucosinolates; progoitrin; 5-vinyl-1,3-oxazolidine-2-thione; cruciferous plants; goitrogens

INTRODUCTION

Although the major cause of endemic goitre is a deficiency in the dietary supply of iodine (1), this is not the only cause. Some natural products consumed by man or animals contain naturally occurring goitrogens, which may also play an important role in the etiology of goitre. The literature contains a lot of reports and suggestions that relate endemic goitre with consumption of cruciferous plants (plants of the family *Brassicaceae*, e.g. cabbage, cauliflower, broccoli, Brussels sprouts, turnip, rape etc.). The goitrogenic potential of cruciferous plants is mostly attributed to glucosinolates.

Glucosinolates are a class of sulphur-containing secondary plant metabolites typically occurring, together with the enzyme myrosinase (EC 3.2.3.1), in *Brassicaceae* members (2). Glucosinolates are easily decomposed during culinary processing, either enzymic or non-enzymic (mostly thermal) mechanisms being involved in the degradation. The enzymic hydrolysis is induced by cell disruption (e.g. cutting) when glucosinolates and myrosinase come into contact. Depending on the nature of the parent glucosinolate, the pH value and the presence of various compounds, a range of bioactive products is released. Some of them act as chemoprotective agents (3), others exhibit toxic effects including antithyroid activity (4). The most potent goitrogen was found to be 5-vinyl-1,3-oxazolidine-2-thione (5-VOT), also called goitrin. 5-VOT is formed from (2R)-2-hydroxybut-3-enylglucosinolate (progoitrin) – the major glucosinolate of rapeseed, which occurs also in edible cruciferous plants. The mechanism of its formation involving myrosinase mediated release of D-glucose, spontaneous rearrangement of the aglycone and final cyclization of unstable isothiocyanate is shown in Fig. 1.

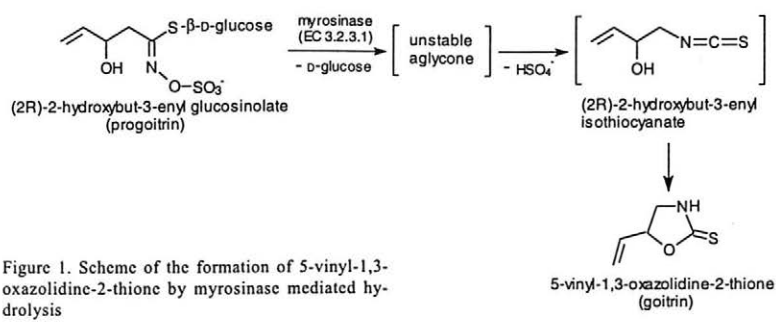

Figure 1. Scheme of the formation of 5-vinyl-1,3-oxazolidine-2-thione by myrosinase mediated hydrolysis

The main aim of this study was to isolate progoitrin and investigate the amount of 5-VOT released from progoitrin under various experimental conditions.

EXPERIMENTAL

Chemicals. All chemicals used were of analytical grade. 4,4-dimethyl-1,3-oxazolidine-2-thione (4,4-DMOT), being used as internal standard in HPLC analysis, was synthesized by the condensation reaction of 2-amino-2-methylpropan-1-ol with carbon disulfide (5).

Isolation of progoitrin. Progoitrin was isolated from high-glucosinolate rapeseed (*Brassica napus* cv. Korina) following a previously reported procedure (6) based on extraction into boiling aqueous methanol, purification of extracted glucosinolates by aniontropic alumina column chromatography and separation of progoitrin from the other glucosinolates by passage through Sephadex G-10. After recrystalization, yellowish crystals of isolated progoitrin (85 % purity) were obtained.

Degradation of progoitrin. The reaction mixtures were prepared by mixing of progoitrin (2 mg in 0.1 ml water), phosphate-citrate buffers (2 ml; pH value ranging from 3 to 7) and myrosinase preparative (5 U in 0.2 ml water) isolated from white mustard seeds (7). Following the addition of 4,4-DMOT and dichlormethane (10 ml), the reaction was allowed to proceed at room temperature for 120 min. Then the mixture was centrifuged, organic phase was evaporated under a stream of nitrogen, the residue was dissolved in acetonitrile (30%, v/v), filtered through a 0.45-μm membrane filter (Milipore) and analyzed using the HPLC system described below. Thermal degradation of progoitrin was carried out using the same procedure except of no addition of myrosinase. The aqueous solutions of progoitrin were incubated at 100°C. The aliquots, taken in 15, 30, 60 and 120min intervals, were extracted by dichlormethane, centrifuged and analyzed.

HPLC/UV analysis. Analysis of goitrin was carried out on a Constamentric 3200 high-performance liquid chromatograph (Thermo Separation Products, Riviera Beach, USA). An aliquot amounting 20 μl was injected on Waters Nova Pak C18 reverse-phase column (250 × 4.6 mm I.D., 4 0181m). A two-component solvent system consisting of 10% acetonitrile in water (A) and acetonitrile (B) was used. Constant flow rate of 1 ml/min was employed with the following solvent program: 100% A and 0% B for 8 min, a linear gradient to 5% A and 95% B at 15 min, held for 7 min, returned to the starting ratio of solvents and equilibration for 10 min. Eluted compounds were detected by the variable-wavelength UV detector set at 241 nm.

RESULTS AND DISCUSSION

The study was conducted to evaluate the effect of pH value and effect of high temperature (both factors important during vegetable processing) on the yield of 5-VOT from its precursor progoitrin. The modified HPLC method of Quinsac *et al.* (8) with the addition of structurally related 4,4-DMOT as internal standard was proved to be a rapid and reliable tool for determination of 5-VOT in reaction mixtures.

Two model systems were arranged to investigate the effect of the above mentioned factors on progoitrin degradation. The first model system, consisting of progoitrin and myrosinase in buffer solutions, simulates homogenization of fresh vegetables (e.g. by chopping, chewing in the mouth cavity). The effect of pH value (ranging 3-7) on the yield of 5-VOT was investigated. The obtained results indicated that the yield of 5-VOT arising after enzymatic hydrolysis was strongly dependent on the pH value of the medium – it increased with increasing pH value. An interval of pH values ranging from 3 to 5 was critical, as the yield of 5-VOT increased dramatically from 6% (pH 3) to 86% (pH 5) of the total progoitrin content in reaction mixture. In contrast, the yield of 5-VOT was relatively stable in solutions of pH > 5 (90–98%). This is in agreement with the generally known fact that enzymic glucosinolate breakdown at neutral pH tends to favour isothiocyanate production (5-VOT is formed by cyclization of unstable (2R)-2-hydroxybut-3-enyl isothiocyanate), while low pH leads to nitriles (9).

Since cruciferous vegetables are also consumed after thermal processing, the second model system simulates the effect of thermal treatment (boiling in water) on progoitrin degradation. The results showed that progoitrin is relatively stable to this type of thermal treatment and only small amounts of 5-VOT (up to 1% of the total progoitrin content) were released under such conditions.

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Strecker Aldehydes Arising from *S*-alk(en)yl-L-cysteines

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Abstract

S-Methyl- and *S*-allyl-L-cysteine and the corresponding sulfoxides naturally occurring in alliaceous and brassica vegetables were oxidized in model solutions with either glyoxal or $K_2S_2O_8$. Oxidation of methiin led to 2-methylthioacetaldehyde and 2-allylthioacetaldehyde was formed from alliin. The same compounds also arised during cooking of vegetables. Mass spectra, retention time data and sensory characteristics of these Strecker aldehydes are presented.

Keywords: strecker degradation; strecker aldehydes; *S*-alk(en)yl-L-cysteines; *S*-alk(en)yl-L-cysteine sulfoxides; methiin; alliin; 2-methylthioacetaldehyde; 2-allylthioacetaldehyde

INTRODUCTION

The oxidative decarboxylation of amino acids to carbonyls with one less carbon atom called Strecker degradation is an important reaction generating flavour-active components in many foods. It provides mainly so-called Strecker aldehydes and subsequently alcohols, acetals, acids, esters, heterocyclic and other compounds. The Strecker aldehydes of basic sulfur amino acids, cysteine and methionine, belong to key aroma components of many fresh and processed foods (1). Hydrogen sulfide, vinylamine and the corresponding Strecker aldehyde (2-mercaptoacetaldehyde) are obtained during the course of the Strecker degradation of cysteine. Analogously, methionine gives rise to 3-methylthiopropanal (methional), which releases methylthiol and yields propenal.

In addition to cysteine and methionine, foods of plant origin contain several other sulfur amino acids, in particular, *S*-alkyl- and *S*-alkenylcysteine sulfoxides and the respective desulfo amino acids. Free amino acid *S*-methyl-L-cysteine sulfoxide, sometimes trivially called methiin, appears to have very wide distribution in plants as it occurs in many genera of the *Brassicaceae* and *Liliaceae* family to which many important vegetables (such as cabbage, broccoli, garlic, onion) belong. *S*-allyl-L-cysteine sulfoxide (alliin) is a well-known free amino acid of the genus *Allium* (*Liliaceae*) and is mainly associated with garlic. Several other *S*-alk(en)yl-L-cysteine sulfoxides have been identified (2). *S*-alk(en)yl-L-cysteines and their sulfoxides are precursors of powerful and unusual flavours of many alliaceous and brassica vegetables and precursors of various biologically active substances. They have attracted the attention of food chemists, technologists, physiologists and pharmacologists for many decades. More then 30 different volatile products have been identified as the degradation products of methiin; about 60 volatile products arise from alliin and more then 50 volatiles from propiin (2). The Strecker aldehydes corresponding to the mentioned *S*-alk(en)yl-L-cysteines and their sulfoxides have been detected only in traces by a few research groups and did not attract much attention. Therefore, the aim of this work was to identify the Strecker aldehydes arising from various *S*-alk(en)yl-L-cysteines and/or their sulfoxides either in model experiments or in natural materials and describe their sensory properties.

EXPERIMENTAL

Material. *S*-alk(en)yl-L-cysteines and their sulfoxides have been synthesized by alkylation of L-cysteine with alk(en)yl halides (3, 4) and obtained as a racemic mixture of (±)-isomers. Pure stereoisomer of alliin, (+)-*S*-allyl-L-cysteine sulfoxide, was a gift from TNO, Zeist, NL. Glyoxal-hydrate trimer (Sigma Chemical Company, St. Louis, USA) and potassium peroxodisulphate ($K_2S_2O_8$) from Lachema, Brno, CZ were commercial products. All the other chemicals used were commercial products of highest available quality.

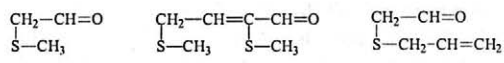
Model Reactions. S-alk(en)yl cysteines or their sulfoxides (5 mmol) and potassium peroxodisulphate or glyoxal (5 mmol) dissolved in 500 ml of water were boiled in the Likens-Nickerson apparatus for 1 hr and the distillate extracted with 100 mL of diethyl ether. The solvent was stream of nitrogen and analyzed by GC/FID and GC/MS (5).

Sample preparation. Fresh garlic (*Allium sativum* L.) or fresh broccoli (*Brassica oleracea*, var. *asparagoides* DC.) from the local market (100 g each) was crushed in 100 ml of boiling water and heated in the Likens-Nickerson apparatus as described above.

RESULTS AND DISCUSSION

S-Substituted L-cysteines and their sulfoxides (methyl and allyl derivatives) naturally occurring in vegetables belonging to the genus *Allium* (*Liliaceae*) and *Brassica* (*Brassicaceae*) were oxidized in model solutions with either glyoxal or $K_2S_2O_8$. The arising volatiles were isolated and analyzed.

Oxidation of *S*-methyl-L-cysteine or its sulfoxide led to the corresponding Strecker aldehyde (2-methylthioacetaldehyde, Fig. 1) in both cases. The reaction was complex and the amino acid was not only decomposed by the Strecker degradation but also by other pathways. The highest yield of the Strecker aldehyde formed from methiin oxidized with glyoxal (0.23 mg from 5 mmol, i.e. 755 mg of methiin) while much lower yields were achieved from methiin oxidized with $K_2S_2O_8$ (< 0.2 mg). 2,4-Bis(methylthio)-2-butenal (Fig. 1) arising by aldolization and dehydration of the Strecker aldehyde was identified in deoxymethiin/glyoxal systems as the minor reaction product. 2-Methylthioacetaldehyde was also identified as a constituent of cooked broccoli volatiles (cca 0.05 mg/100 g fresh weight).



2-methylthio acetaldehyde | 2,4-bis(methylthio)-2-butenal | 2-allylthioacetaldhyde

Figure 1. Structure of the Strecker aldehydes and related products from methiin and alliin

Analogously, oxidation of *S*-allyl-L-cysteine or its sulfoxide led to 2-allylthioacetaldehyde (Fig. 1). This Strecker aldehyde was the major volatile product in both systems (its yield was 58 mg from 5 mmol of alliin in systems containing glyoxal and 0.2 mg when $K_2S_2O_8$ was used). Minor sulfur-containing reaction products were diallylsulfide,

Table 1. Mass spectra, retention time data and sensory properties of aldehydes derived from methiin and alliin

Compound	2-methylthioacetaldehyde	2-allylthioacetaldehyde
m/z (%)	90 (M^+, 63), 63 (4), 62 (4), 61 (100), 47 (17), 46 (16), 45 (29), 35 (26), 27 (23)	116 (M^+, 35), 98 (5), 87 (27), 85 (9), 73 (33), 72 (47), 59 (7), 53 (10), 47 (18), 45 (54), 41 (100), 39 (31), 29 (5), 27 (5)
RT (min)	7.6	12.4
R.I.	1266	1493
Odour description	burnt, roasty, pungent, with sulfury notes, unpleasant	fresh, green, grassy, pleasant
Odour threshold value (water, 20°C)	0,1 mg/L	0,2 mg/L

Table 2. Mass spectra and retention time data of aldehydes derived from different *S*-alk(en)yl-L-cysteines

2-Alk(en)ylacetaldehyde	RT (min)	R.I.	m/z (%)
2-ethylthio	9.4	1347	104 (M^+, 67), 75 (100), 61 (20), 59 (15), 47 (91)
2-propylthio	12.0	1460	118 (M^+, 58), 90 (4), 89 (61), 75 (12), 74 (27), 61 (74), 59 (18), 55 (9), 47 (73)
2-isopropylthio	9.9	1361	118 (M^+, 54), 89 (58), 75 (26), 74 (55), 59 (28), 55 (36), 47 (79), 43 (100)
2-butylthio	14.0	1545	132 (M^+, 17), 114 (4), 103 (9), 88 (12), 61 (100), 57 (8), 55 (19), 47 (17)
2-(2-butenyl)thio	15.0	1577	130 (M^+, 11), 112 (4), 87 (11), 86 (53), 59 (5), 55 (100), 53 (12)
2-(3-butenyl)thio	15.3	1589	130 (M^+, 1), 102 (49), 101 (24), 89 (31), 88 (33), 87 (25), 86 (39), 85 (18), 73 (8), 67 (37), 61 (100), 59 (41), 55 (61), 54 (31), 47 (29)
2-isobutylthio	12.0	1460	132 (M^+, 54), 103 (54), 89 (12), 88 (30), 61(65), 59 (11), 57 (100), 55 (27), 47 (27)
2-pentylthio	16.2	1630	146 (M^+, 23), 117 (19), 102 (9), 87 (13), 70 (9), 69 (65), 61 (100), 55 (17)
2-isopentylthio	15.1	1573	146 (M^+, 15), 118 (2), 117 (2), 102 (8), 86 (12), 70 (42), 69 (27), 61 (100), 55 (35), 43 (41)
2-prenylthio	17.5	1673	144 (M^+, 10), 100 (51), 85 (11), 69 (86), 67 (12), 59 (5), 53 (11), 45 (11), 41 (100)
2-hexylthio	19.1	1745	160 (M^+, 25), 142 (4), 131 (30), 117 (14), 116 (7), 87 (24), 83 (45), 61 (100), 60 (20), 56 (20), 55 (74), 43 (52)
2-methylthiomethyl	23.2	1915	136 (M^+, 14), 121 (7), 93 (27), 92 (17), 61 (100), 59 (6), 46 (12), 45 (31)

diallyldisulfide, diallyltrisulfide, 2-vinyl-1,3-dithiine, 3-vinyl-1,3-dithiine and 2-thiophenecarbaldehyde. 2-Allylthioacetaldehyde was also a constituent of cooked garlic volatiles (about 0.1 mg/100 g fresh weight). Mass spectra of 2-methylthioacetaldehyde and 2-allylthioacetaldehyde, their retention time data and sensory characteristics are summarized in Table 1.

Odour threshold values of either 2-methylthioacetaldehyde or 2-allylthioacetaldehyde were sufficiently low and both compounds may contribute to the typical aroma of the respective vegetables because their measured concentrations may be over their threshold values (depending on the amount of water, vegetable and some other conditions).

For comparison, 12 additional *S*-alk(en)yl-L-cysteines, i.e. *S*-ethyl-L-cysteine, *S*-propyl-L-cysteine, *S*-isopropyl-L-cysteine, *S*-butyl-L-cysteine, *S*-(2-butenyl)-L-cysteine, *S*-(3-butenyl)-L-cysteine, *S*-isobutyl-L-cysteine, *S*-pentyl-L-cysteine, *S*-isopentyl-L-cysteine, *S*-prenyl-L-cysteine, *S*-hexyl-L-cysteine and *S*-methylthiomethyl-L-cysteine, were oxidized and the corresponding Strecker aldehydes isolated and analyzed. Their mass spectra and retention time data are listed in Table 2.

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Pectin Esterase Activity of Potatoes

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Abstract

Pectin esterase (PE) activity in potatoes is supposed to affect the texture properties of cooked tubers. De-esterification of the cell wall and middle lamella pectins due to PE activation enable the calcium-pectate-gel formation, which can increase the firmness of tissues. Four potatoes cultivars of different cooking type were compared and changes in the PE activity within the long time storage were investigated. No direct correlation between the PE activity and cooking behavior of potatoes could be deduced from the results.

Keywords: pectin esterase; PE activity; cooked potatoes; texture

INTRODUCTION

Pectic polysaccharides affect strongly the texture of cooked potatoes. The gel-forming pectic network limits the porosity of the cell wall and controls the cell wall thickness; the pectins of middle lamellae play an important role in maintaining tissue integrity by connecting the adjacent cells. Due to thermal treatment, the pectic material is solubilised as a result of the chemical and enzymic degradation. The enzymic degradation is thought to start by the action of pectin esterase (PE), which decreases the degree of esterification and prepares a more readily hydrolysed substrate for polygalacturonase (1). Native potato PE is activated by heating, with optimum temperature at 55–60°C. De-esterified pectin allows the calcium-pectate gel formation, which contributes to the tissue integrity and firm texture. Chemical degradation of pectin takes place according to the β-eliminative mechanism next to the esterified galacturonic unit. This reaction is decelerated with decreasing esterification, thus PE counteracts the pectin degradation and solubilisation (2).

Positive effect of the pectine esterase on the texture of preheated potatoes has been proved (3) and many data on the PE activity in plant tissue have been published. However, no information was found regarding the relation of PA activity to the cooking type of potatoes.

EXPERIMENTAL

Material. Four potatoes cultivars of different cooking type were chosen: Nicola (A-B, non-mealy), Agria (B), Panda (C-D), Javor (C, mealy).

Potatoes were grown at the Potato Research Institute in Havlíčkův Brod. The tubers were harvested in full maturity at the beginning of October 1998,1999 respectively and stored at 6°C and 95% humidity.

Methods. Pectin esterase activity and degree of esterification: Gas chromatographic method was used for measurement of released methanol after its conversion to methyl nitrit (4). Fractionation of pectin: Successive extraction by acetate buffer, EDTA-oxalate and HCl was performed; the pectin fractions were precipitated by ethanol (5). Pectin determination: Spectrophotometric method based on reaction of the galacturonic acid with m-hydroxydiphenyl was used (6). Degree of acetylation: Capillary isotachophoresis method was applied for the acetate determination after the pectin hydrolysis.

RESULTS AND DISCUSSION

In both of the harvest seasons, significant differences in the PE activity between the cultivars were determined (Fig. 1). These differences were observed over the whole storage period, although the measured activity decreased

Table 1. Degree of esterification (DE) and degree of acetylation (DA) of pectin fractions

	Nicola		Agria		Panda		Javor	
Fraction	DE	DA	DE	DA	DE	DA	DE	DA
1	4.0	0.1	12.2	0.2	1.9	0.2	12.8	0.03
2	45.3	24.4	58.4	43.2	82.1	14.0	96.7	51.7
3	7.1	0.5	7.7	11.4	12.4	2.1	34.8	1.7

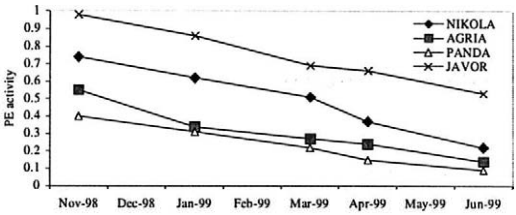

Figure 1. Pectin esterase activity of different cooking type potatoes

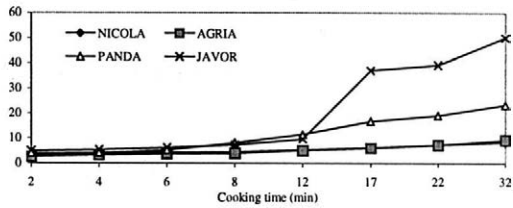

Figure 2. Transfer of pectin (as galacturonic acid) into cooking medium

with the storage time. The highest and lowest PE activities were found for cv. Javor and Panda respectively, both the mealy cooking types. This observation contradicted the expected correlation between the PE activity and mealiness and consistency of potatoes.

To obtain some additional information about the substrate, the pectin fractionation was carried out and pectin leakage into the cooked medium was investigated. The highest content of pectin was determined for cv. Javor, with dominated 2. fraction (Fig. 2). This fraction, abundant in all studied cultivars, was found highly esterified and acetylated. The increased content of highly esterified pectin could explain the high PE activity of cv. Javor. Pectin of the 2. fraction is supposed to originate mainly from the middle lamellae and its solubilization and leakage result into decreasing of the intercellular contacts and eventually cell sloughing. Investigation of the pectin transfer into the cooking medium confirmed the higher amount of released pectic material for the mealy cultivars in comparison to the non-mealy potatoes (Fig. 3). Similar results were reported previously (7).

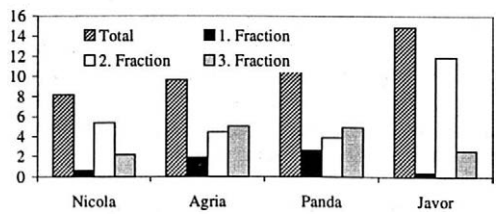

Figure 3. Fractionation of potato pectin polysaccharides

In conclusion, no direct correlation could be deduced for the PE activity and cooking behavior of potatoes. Nevertheless, the pectin esterase is thought to play an important role in the cell wall development. There has been proposed the pectin matrix in the cell wall of mealy potatoes has thicker and/or less porous structure than that of non-mealy cultivars (8). Thus, it could be supposed that pectin esterase affects the cooking behavior indirectly.

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Bioavailability of Dietary Iron: The Dialysability of Non-Heme Iron in *In Vitro* Protein Digested Preparations of Pork Meat following Different Heat Treatments

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Abstract

Four selected heat treatments of pork meat together with raw meat were investigated for the effects of the meat factor on enhancing iron bioavailability during the digestion. The obtained results indicate a correlation between the ability to reduce iron and enhancement of iron bioavailability. Furthermore, the enhancing effects on bioavailable iron was found to be pronounced in the meat sample heat treated at 120°C. Due to pepsin digestion, both 80 and 100°C heated meat samples revealed increase in iron dialysability. After total digestion (pepsin + pancreatic enzymes) iron bioavailability decreased by a factor 10.

Keywords: dietary iron; non-heme iron; meat

INTRODUCTION

The ability of various meat sources to enhance iron bioavailability and absorption is a well-known phenomenon (1). Specific but yet unknown constituents of the meat are believed to account for the enhancing properties in relation to the mechanism of the improved iron absorption. These components are often referred to as "meat factor" and may function by formation of complexes with iron or by participating in redox reaction resulting in increased solubility and bioavailability of iron. Peptides and/or amino acids, especially those with thiol groups (cys), liberated during digestion of the meat are able to chelate iron and thereby prevent precipitation of iron caused by environmental circumstances in the intestinal lumen (2). This study aims to examine the effects of heat treatments of meat on the functionality of the meat factor by investigation of iron dialysability and ability to reduce iron during *in vitro* protein digestion of selected meat samples and proteins.

EXPERIMENTAL

Material. Porcine meat (*Musculus longissimus dorci*), raw and heat treated at 4 selected temperatures (60, 80, 100, and 120°C) for 1 hour in sealed stainless-steel cans were used in this study together with haemoglobin (Hb) and bovine serum albumin (BSA).

In vitro protein digestion (IVPD) was performed as described elsewhere (3).

In vitro dialysis (cut off 6000–8000 Da) was performed for 4 hours, pH 6.1.

The Iron assay measures ferrous iron and total iron content in the samples, the dialysate and the retentate (4). The method has been modified and adapted to micro-titer plates. This iron assay can be used to differentiate between ferri and ferro iron, which is essential for the studies of iron bioavailability.

RESULTS AND DISCUSSION

Iron bioavailablity studies. Results from investigation of the effects of different pre-treatments of the meat on iron bioavailability during *in vitro* digestion are presented in the Figs 1–3. The results are given as percentage of dialysable ferrous iron calculated on basis of the total amount of recovered iron (Eq. 1), which in general is believed to be a good approximation of iron bioavailability (5, 6). In the start samples of IVPD (Fig. 1), great variations in dialysable iron were observed. The effects of 60 and 80°C heated porcine meat on iron dialysability were lower dialysability than obtained for the raw meat sample. Raising the

$$\%D - Fe(II) = \frac{Conc.\ Fe(II)_{dialysate} \times V_{total}}{Amount\ iron\ included} \times 100\%$$

Equation 1. Measurement of amount of bioavailable iron in IVPD samples

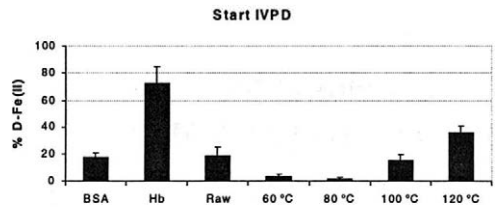

Figure 1. Bioavailability of iron (% of total iron included) in heat treated meat samples (60, 80, 100 and 120°C) and in reference protein samples (Hb; haemoglobin and BSA; bovine serum albumin)

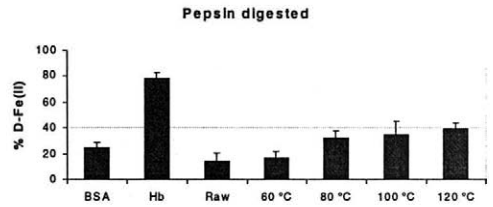

Figure 2. Iron bioavailability (% of total iron included) in pepsin digested samples (as in Fig. 1)

temperature to 100°C and 120°C resulted in increased iron dialysability. The meat heated at 120°C had appreciable effects on iron bioavailability, which indicates that the meat proteins are denaturated in a matter that facilitates solvating iron or reduce the binding of iron. The pronounced capacity of haemoglobin (Hb) with respect to the very high amounts of dialysable iron may be explained by the ability of the porphyrrin-ring system to bind iron and by the reducing properties of Hb.

After pepsin digestion (Fig. 2), all tested samples, except the raw meat sample, resulted in increased iron dialysability. This elevated ferrous iron availability could be a consequence of alterations in solubility of the sample constituents or effects from peptides with special functionalities. This could for example be thiol-rich peptides, which have ability both to form complexes with iron and to transform ferri irons into ferro irons.

Total digested samples represent a degree of protein hydrolysis expected to be as found for meat passing through the digestive tract to the small intestine or the proximal part of the ileal intestine (Fig. 3). The environment in this *in vitro* digestion system results in very low iron solubility, both for ferri and ferro iron. This is probably also one of the reasons for decrease in iron dialysability for all of the tested samples after total digestion. Only the amount of dialysable iron in the total digested BSA sample was statistically different and higher (9 %) than obtained in all of the other tested samples.

Reduction capacity studies. The capacity of the different meat samples to reduce added ferri iron to ferro iron during IVPD is shown in Figs 4–6. In Fig. 4 the initial iron reduction capacity are illustrated and compared for the start samples before performing IVPD. The effect of gastric (pepsin) digestion on iron reduction capacity of the samples are presented in Fig. 5 and corresponding, Fig 6 contain the informations on iron reduction capacity in total digested samples expected to be present distal to the duct of pancreas in the small intestine.

Reduction capacity is believed to be one of the beneficial mechanisms of the "meat factor" on enhancing the iron solubility and availability (5). Comparing the results obtained in the iron bioavailability study (Figs 1–3) and those obtained in the reduction capacity study (Figs 4–6) reveals that not all reduced iron becomes "available" to dialysis. This is probably due to association of ferro iron to protein or other constituents, which are unable to cross the dialysis membrane. However, this explanation could only be true if these iron chelators are resistant toward proteolytic digestion because reduced iron is found in the total digested samples, which are not dialysable. The theory of the existence of a correlation between iron dialysability and ability to reduce iron seems to be recognised for all the tested meat samples. When the ability to reduce ferri iron increases the amount of bioavailable iron increases. However, for the total digested Hb sample the amount of reduced iron was measured to 70 percent whereas iron dialysability was very low, which may be a result from complex binding of Fe^{2+} in non-dialysable Hb.

In conclusion, these results shows that the applied preparation of the meat is crucial for the performance of the meat factor in enhancing iron bioavailability. Low heat treatment of the meat resulted in decreased iron bioavailability in relation to the effects caused by the BSA sample, both by the start samples and the pepsin-digested samples. Heat

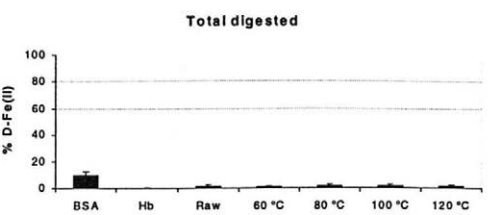

Figure 3. Bioavailability of iron (% of total iron) in meat samples and reference samples digested (total) by pancreatic enzymes following pepsin digestion

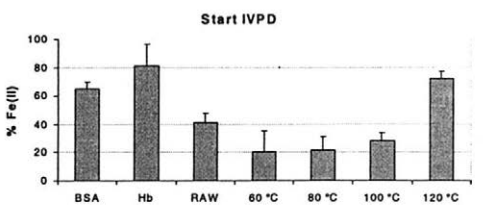

Figure 4. Amount of ferro iron produced in start samples by reduction of ferri iron added before performing IVPD analysis of Hb, BSA, raw meat and heated meat

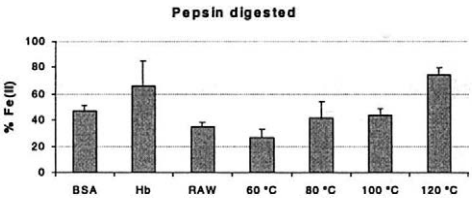

Figure 5. Ferro iron present in in vitro pepsin digested samples (as in Fig. 4) calculated as percentage of total iron included

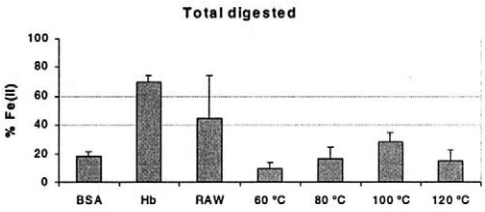

Figure 6. Ferro iron remaining in total digested samples (as in Fig. 4)

treatments of 80, 100 and 120°C resulted in increased amount of bioavailable iron in relation to the BSA sample for all of the pepsin-digested samples. Furthermore these studies strongly indicates a correlation between meat factor functionality and ability to reduce iron. A very small percentage of added iron was recovered as bioavailable iron after total digestion with pepsin and pancreatic enzymes.

Acknowledgement: This project (No. 9315013, "Improved Iron Status") with Prof. Leif Skibsted as coordinator is supported by grants from the Danish Research Council, Danish Bacon & Meat and Norma & Frode S. Jacobsen's Foundations. Special thank to Lars Kristensen who kindly donated the heat-treated meat samples and to Pia Madsen for excellent technical assistance.

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The Reaction of Citrus Pectin with Metal Cations

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Abstract

The pectate complexes with three metal cations (Co^{2+}, Cr^{3+} and UO_2^{2+}) were prepared and study using UV-Vis spectroscopy. Obtained value of *logK* at 1.9–2.1 for all cations confirm that the stability of these complexes is similar to each other. Observed changing in position of characteristic bands in diffuse reflectance The value of *n* for Co^{2+} is near the theoretical one of 0.5 and corresponds to cation: uronic unit relation of 2:1. However, Cr^{3+} and UO_2^{2+} cations can bind more then two carboxylates and form bridging complexes. UV-Vis spectra of pectin-metal complexes can be explained by the polysaccharide ligand influence on metal cation and by removing of non-organic ligands.

Keywords: metal cations; pectate; UV-Vis spectroscopy

INTRODUCTION

Pectins are plant polysaccharides that are widely used in food processing. The presence of uronic carboxyls permits them to bind heavy metal cations presented in food and therefore protect organism from toxic elements and radionuclides (2). Pectins demonstrate a strong affinity and selectivity to counterions of heavy metals. Pectates of polyvalent cations are water insoluble and can be precipitated by an addition of pectate solution into the solution of metal cation. The difference in concentration of free metal cation in solution after metal pectate precipitation and removing by centrifugation or filtration gives the quantity of cations bonded to pectate. The concentrations of free cations can be obtained on the basis of the characteristic UV-Vis bands of metal cations and used for estimation of stability constants of pectin-metal complexes. This work describes the study of interaction between citrus pectin and metal cations using UV-Vis spectroscopy.

EXPERIMENTAL

Material. Potassium pectate (Sigma chemical, USA), $CoCl_2.6H_2O$ (Lachema, Brno, Czech Republic), $CrCl_3.6H_2O$ (Fluka, Svitserland), $UO_2(NO_3)_2.6H_2O$ (Lachema, Brno, Czech Republic) were used in analysis.

Determination of logK and n. Metal pectate complexes were precipitated by the addition of the various volumes of aqueous solution (final concentration of 1.25–6.25 mM of galacturonic units) of potassium pectate to 20 ml of 0.05 M of cation salt. The volume of reaction mixture was adjusted to 40 ml by distilled water. The pellets were removed by centrifugation and the supernatants were used for absorption measurement at 512 (Co^{2+}), 432 (Cr^{3+}) and 414 nm (UO_2^{2+}) for quantification of free and bound metal cations, where:

$$[M^{2+}_{tot}] = [M^{2+}_{free}] + [M^{2+}_{bound}]$$

The stability constants K and the numbers of binding sites n were calculated according to the equation:

$$\frac{1}{r} = \frac{1}{nK[M^{2+}_{free}]} + \frac{1}{n}$$

where r is the number of bound cations per galacturonic unit:

$$r = \frac{[M^{2+}_{bound}]}{[-(GalA^-)-]}$$

UV-Vis spectroscopy. UV-Vis spectra were measured on double-beam UV-4 (UNICAM) spectrophotometer, bandwidth 2 nm, photo multiplier detector, labsphere$_{®}$ (for diffuse reflection spectra of powder samples). Spectral data were processed by "Vision" (UNICAM) and "Origin 6.0" (Microcal) softwares.

RESULTS AND DISCUSSION

The *logK* values of metal pectate complexes were 2.14, 2.01 and 1.95 for Co^{2+}, Cr^{3+} and UO_2^{2+} cations, respectively. Therefore, the stability of these complexes is similar to each other. Relatively small values of *logK* for Cr^{3+} and UO_2^{2+} may be explained by the partial hydrolysis of these cations in aqueous solutions to hydroxo complexes. Hyd-

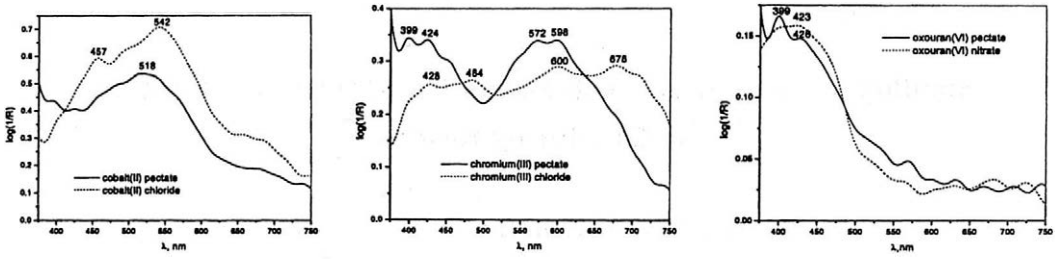

Figure 1. Diffusion refrectance UV-Vis spectra of metal pectates and corresponding non-organic metal salts

roxo and oxo oxygens can participate in the interaction of metal cation with carboxylate and therefore influence the structure and stability of metal pectate complexes.

The numbers of binding sites *n* were 0.552 (Co^{2+}), 0.423 (Cr^{3+}) and 0.367 (UO_2^{2+}). The value of *n* for Co^{2+} is near the theoretical one of 0.5 and corresponds to cation: uronic unit relation of 2:1. However, Cr^{3+} and UO_2^{2+} cations can bind more then two carboxylates and form bridging complexes.

Diffuse reflectance UV-Vis spectra of powder metal pectates of Co^{2+}, Cr^{3+} and UO_2^{2+} in comparison to solid state spectra of corresponding non-organic metal salts are shown in Figure 1. Observed changing in position of characteristic bands in the spectra of pectin-metal complexes can be explained by the polysaccharide ligand influence on metal cation and by removing of non-organic ligands (chloride or nitrate anions), which are present in original salts. Our results suggest that UV-Vis spectroscopy have some perspectives in the study of interaction between pectin and metal cations.

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Stability of Plant Sterols and Formation of Oxidation Products in Oils during Heating

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Abstract

This study was performed to study how stable plant sterols are during heating at various temperatures and which oxidation products are formed. Sterols were stable at 80°C while significant losses occurred at ≥120 °C. Sterols with a saturated ring structure (stanol) were more stable compared to Δ5-sterols, while Δ5,7-sterols were the most unstable. Detectable amounts of various sterol oxides were found after one hour of heating at 180°C.

Keywords: plant sterols; oxidation; rapeseed oil; thermal stability; sterol oxides

INTRODUCTION

Plant sterols are the most dominant compounds in the unsaponifiables of edible oils. These and other minor lipid components may have significant chemical, technological and nutritional effects on food. Plant sterols are exposed to various reactions during processing and storage of foods. The aim of this experiment was to study how stable plant sterols are during heating at various temperatures and to develop an analytical method to study which oxidation products are formed.

Experimental

Sitosterol, stigmasterol, sitostanol and ergosterol and cholesteryl oleate were added to rapeseed oil and tripalmitin at 0.1–1% level. Oil samples of 1.0 g were heated at 80, 120 and 180°C for up to seven days. Stability of sterols was measured by analyzing residual sterol contents at regular intervals. In brief, oil samples were saponified and sterols were analyzed as TMS-derivatives by capillary gas chromatography (GC) and quantified using an internal standard (1). A method for analyzing sterol oxides consisting of cold saponification, solid-phase extraction purification and separation by GC as TMS-derivatives was developed and evaluated. 19-hydroxy cholesterol was used as an internal standard. Identification of sterol oxides was performed by comparing retention and mass spectra data. Each experiment was repeated and analyses were performed in duplicate.

RESULTS AND DISCUSSION

Free sterols and sterol esters decomposed at 180°C in oils (Fig. 1). Sitostanol, a sterol with a saturated ring structure, was more stable than other sterols in both oils, while ergosterol, a Δ5,7-sterol, was the least stable one. There were no significant differences between the stabilities of stigmasterol and other Δ5-sterols and steryl esters. Moreover, natural sterols present in rapeseed oil decomposed similar to the added Δ5-sterols. Thus, the thermal stability of sterols was mainly determined by the unsaturation of the ring.

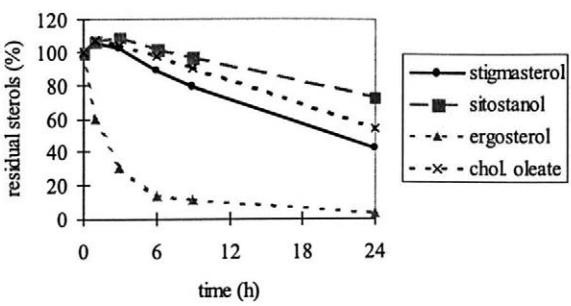

Figure 1. Stability of added sterols and cholesteryl oleate in rapeseed oil at 180°C

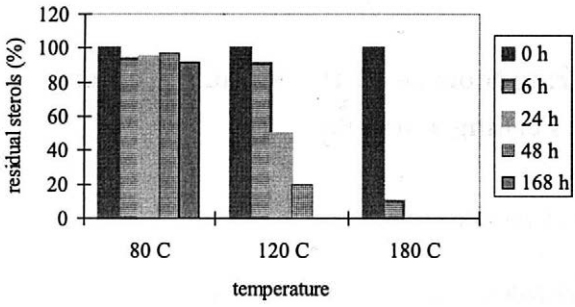

Figure 2. Stability of added stigmasterol in tripalmitin at 80, 120 and 180°C

Heating temperature had a significant effect on the stability of sterols (Fig. 2). All sterols were relatively stable at 80°C while significant losses occurred already at 120°C.

All sterols were more stable in rapeseed oil than in tripalmitin at 180°C. For example, in tripalmitin only <10% of Δ5-sterols were found after 9 hours of heating, while in rapeseed oil about 80% of them was left. The unsaturated matrix in rapeseed oil had no co-oxidating effect on sterols compared to the saturated matrix in tripalmitin at this high temperature. Better stability of sterols in rapeseed oil was not due to tocopherols present, because sterols were as stable in rapeseed oil as in tocopherol-stripped rapeseed oil.

During the sample preparation for analysis of sterol oxides, special care was taken to minimise further reactions of these products. To compensate for the losses during the procedure, an internal standard (19-hydroxy cholesterol) was added to the samples prior to saponification. Recoveries of different oxidation products of cholesterol were evaluated against the recovery of the internal standard. Detectable amounts (> 10 μg/g) of oxides of Δ5-sterols were formed already during one hour at 180°C and during three hours at 120°C. The major oxidation products of sterols formed were 7-keto, 5,6-epoxy and 7-hydroxy compounds.

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Influence of Time and Aw during Storage on the Vitamin Content of Enteral Feeding Formula

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Abstract

Two different enteral feeding formula were stored at 30°C for up to 6 months with no Aw (water activity) and Aw = 0.44. The modifications in thiamin, tocopherols (α-, γ- and δ-tocopherol) and retinols (all-*trans* and 13-*cis*-retinol) content were studied. Formula I and II stored at 30°C and Aw = 0.44 had a gradual loss of vitamin content from 1 month to 6 months. When Formula II was stored at 30°C and no Aw a very slight, but significant, reduction in vitamin content were obtained after 3 months, similar to those stored for 1 month with Aw = 0.44, and from that time onwards a gradual decrease in vitamin content was found. Aw has a noticeable influence on vitamin content during storage.

Keywords: vitamins; enteral feeding formula; thiamine; tocopherol; retinol

INTRODUCTION

The composition of commercial formulas are based on protein (in the form of amino acids or peptides), carbohydrates (in the form of glucose polimers or maltodextrins, disaccharides and oligosaccharides), different amount of fat (partially digested in mono- or diglycerides) and, most commercially available enteral feeding formula, are fortified with minerals and vitamins.

Vitamin B_1 (thiamin) is quite stable below pH 5.5 but above pH 7.0 could be destroyed very fast even at room temperature. Vitamin E (α-tocopherol, δ- tocopherol and γ-tocopherol) and vitamin A (all-*trans*-retinol and 13-*cis*-retinol) seem to be very stable under nitrogen atmosphere in a dark cold place, but they are particularly sensitive to oxidation by air in the presence of light (1). According with this information the conditions of storage of enteral feeding formulas could affect in some extent their vitamin content.

The aim of the present paper was to study the influence of different storage conditions of time (up to 6 months) and Aw (no Aw and Aw = 0.44) for 30°C on the thiamin, tocopherols (α-, γ- and δ-tocopherol) and retinols (all-*trans* and 13-*cis*-retinol) content of different enteral feeding formula.

MATERIAL AND METHODS

Samples. Two different commercial solid enteral feeding formula (Formula I and II) were purchased at a local pharmacy. The sample bags were open and freshly analysed. The remaining sample was homogeneized and stored for 1, 2, 3, 4 and 6 months. Formula I was stored at 30°C and Aw = 0.44. Some samples of formula II were kept at no water activity and other at Aw = 0.44, all of them at 30°C.

Determination of vitamins. The extraction and quantification by HPLC of thiamin, tocopherols (α-, γ- and δ-tocopherol) and retinols (all-*trans* and 13-*cis*-retinol) were carried out according to previous paper (2).

Statistical methods. Data were subjected to multifactor analysis of variance by a Statgraphics Program (Statistical Graphics System 5.0 Computer Software). The experimental model used was multiple range test and the range test was least significant differences (PL0.05).

RESULTS AND DISCUSSION

Table 1 shows proximate composition (carbohydrates, protein and lipid content) in the commercial enteral feeding formula taken from the label. The content of vitamin B_1 (thiamin), vitamin E (α-tocopherol, γ-tocopherol, δ-tocopherol) and vitamin A (all-*trans*-retinol and 13-*cis*-retinol) were determined in our laboratory and they are also collected in Table 1. Figure 1 shows the retention of vitamins for Formula I and II during storage.

When Formula I and II were stored at 30°C with Aw = 0.44, both formula behaved similarly. A slight decrease (2–5%) was observed in vitamin content after 1 month of storage. After that time, a gradual decrease took place and after 6 months decreases between 57–66% were observed for the content of thiamin tocopherol isomers and retinol isomers (Fig. 1) in both formula.

Table 1 Proximate composition and vitamin content of enteral feeding formula I and II

	Formula I	Formula II
Carbohydrates (%)	73	55
Proteins (%)	12	16
Lipids (%)	14	29
Thiamin (mg/100g)	0.611±0.049	0.362±0.003
α-Tocopherol (mg/100g)	4.104±0.087	3.976±0.077
γ-Tocopherol (mg/100g)	1.182±0.004	0.722±0.020
δ-Tocopherol (μg/100mg)	26.679±0.112	12.584±0.146
All-*trans*-retinol (mg/100g)	0.275±0.002	0.264±0.004
13-*cis*-retinol (μg/100g)	4.034±0.015	2.640±0.020

Mean values in dry matter

A 5% reduction in vitamin content was observed when Formula II was kept at 30°C for 3 months with no Aw. This result was not significantly different ($P \leq .05$) to those obtained with this formula when it was stored for 1 month and Aw = 0.44. The content of thiamin and fat-soluble vitamins decreased slightly (15–20%) after 4 months of storage at 30°C and no Aw. Larger vitamin elimination were obtained (30%) after 6 months in these conditions, but they were smaller than those observed when Formula II was stored for the same period of time at 30°C with Aw = 0.44 (60% reduction) (Fig. 1).

Little information has been found about the effect of storage conditions on the vitamin content of enteral feeding formula. For liquid enteral feeding formula stored at 4, 20 and 30°C for 3, 6 and 9 months Frias and Vidal-Valverde (3) reported that three months of storage did not produce significant changes in the content of thiamin, tocopherol and retinol isomers, but significant reductions in vitamin content were found after 6 months of storage. A reduction of 17% in tocopherol content (4) and 10% of retinol (5) takes place when the powdered milk was kept at 20°C and Aw = 0.44 for 60 days. When the storage temperature was increased to 50°C the retinol reduction was even larger (56% after 60 days) (5).

The conclusion is that the Aw and storage time play an important role in the vitamin content of solid enteral feeding formula, a result that should be taken into consideration for the packaging and storing by the industry.

Acknowledgments: This work was supported by the Spanish Comision Interministerial de Ciencia y Tecnología ALI-97-0606-C02-01.

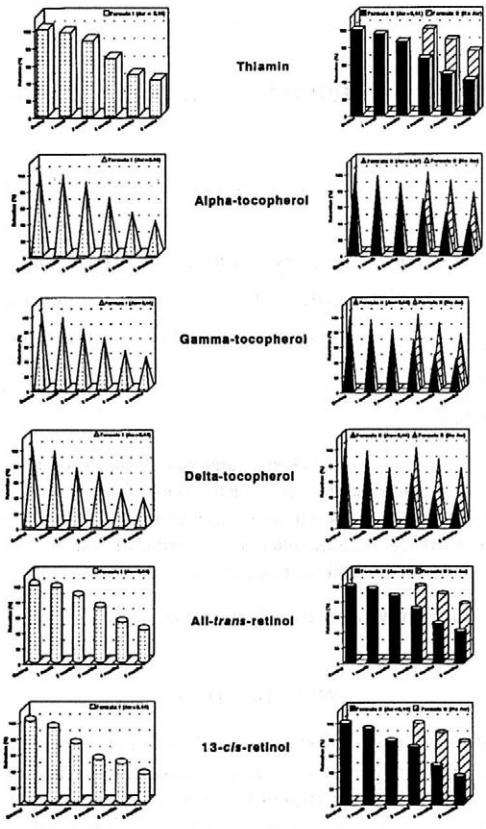

Figure 1. Effect of storage conditions on vitamin content of enteral feeding formula

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Behaviour of Resveratrol under Different Conditions

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Abstract

Trans-resveratrol in the crystal form was stable. After dissolving in ethanol 50% (v/v) changes in *trans/cis* ratio have been observed in influence to exposition to daily-diffused light or UV light, temperature, etc. Resveratrol solution exposed to the daily-diffused light isomerised to the *cis*-isomer reaching within 5 hours the equilibrium of 1:7 (mass ratio *trans/cis*) from original ratio 50:1. *Trans*-resveratrol solution was converted (in relation to UV light exposition) to *cis*-isomer from original ratio 23:1 to 2:1. UV light led to the formation of two unknown substances. The ratio *trans/cis*-resveratrol in red wine samples was relatively stable.

Keywords: resveratrol; isomeration; wine; stability; HPLC

INTRODUCTION

Flavonoids are secondary metabolites found ubiquitously in plants where they act as UV-B protectants and are involved in the regulation of pollen tube growth in the stigma(1). Antioxidants are well known to have positive influence on health. The phytoalexin *trans*-3,5,4´-trihydroxystilbene (resveratrol) was firstly reported in the skins of grapes and later in wines (2). Resveratrol is a phenolic substance occurring in several plants, especially in the vine where it is located in certain parts, including the stems, leaves and skin of grapes. Resveratrol occurs in the *cis* and *trans* isomeric forms (Fig. 1) and *cis* and *trans* glucosidic forms. The last mentioned substances are called piceid. Piceid is the resveratrol form found in the root of *Polygonum cuspidatum*. The powder from this root is used in China and Japan as a treatment for atherosclerosis and for other therapeutic purposes (2). Resveratrol has been found in more than 72 herbal species belonging into 31 genus and 12 families. Many of these are a normal part of the human diet such as mulberries, peanuts and grapes. *Cis*-resveratrol has not been reported in *Vitis vinifera*; however, it has been shown to be present in wines (3). In grapes, resveratrol synthesis is mostly located in the skin cells. In *Vitaceae* fungal infection or UV light stimulate the production of stilbene synthase and catalyse the reaction of 4-hydroxycinnamoyl-CoA and malonyl-CoA to form *trans*-resveratrol. In the grape berry, *trans*-resveratrol production is stimulated by UV light exposure, fungal infection or plant injury. The *trans*-isomer is transformed to the *cis* forms under exposition to UV light. Resveratrol requires a relatively long maceration time on the skins to be fully extracted.

Potential anticancer activity has been mentioned in relation to resveratrol furthermore, *cis*-resveratrol also shows antiaggregation properties. However, the activity of the *cis* isomer in LDL oxidation has not been studied yet (4). As a natural polyphenolic substance, it shows a wide range of biological activities such as anti-oxidizing and anti-microbial features (namely anti-mould features), the ability to absorb free radicals, effect blood sedimentation rate (agglutination of blood cells). Recently, *trans*-resveratrol has also been attributed anti-mutagen and chemo-protective features against tumor proliferation (5).

Relatively rich and rampant resources of resveratrol are the grapes of *Vitis vinifera*. Grape skin contains about 50 to 100 μg of resveratrol per g and the average concentration in red wines fluctuates between 1.5 to 3.0 mg/l.

EXPERIMENTAL

Material. Trans-resveratrol in the crystal form was synthesised by Dr. J. Šmidrkal in purity approx. 99%. Cis-resveratrol was obtained by 5-hour exposure of trans-resve-

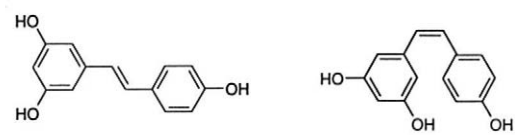

Figure 1. Structures of the *trans* isomer (A) and *cis*-isomer (B) of resveratrol

ratrol standard solution (100 mg/l) to daily-diffused light. At these conditions the 80% of trans-resveratrol was converted into the cis-isomer.

HPLC analysis. Resveratrol isomers from standard solution in ethanol 50% (v/v) were determined by HPLC method using TSP 3500 liquid chromatograph (TSP, U.S.A.) equipped by UV detector (TSP, U.S.A.) and HP 1049 electrochemical detector with glassy working electrode (Hewlett-Packard, U.S.A.), couplet to Apex Data Station (Czech Republic). The eluate was monitored at wavelength 306 nm and at 0.75 V. A Nucleosil reversed-phase column, C18-120 (250 × 4 mm), 5 μm particle size, with a pre-column (10 × 4 mm) of the same material, was used for the stationary phase. Injection was by means of Rheodyne injection valve (U.S.A.) with a 20 μl fixed loop. The isocratical elution at a flow rate of 1.0 ml/min used mobile phase (1litre) of 25% acetonitrile, 0.1% H_3PO_4 and 5 mmol NaCl in demineralized water. Spiking trans and cis resveratrol standard solution for quantification was used external standard method carried out Resveratrol identification. After each analysis, the column was re-equilibrated with mobil phase for 15 min.

RESULTS AND DISCUSSION

Behaviour of pure *trans*-resveratrol has been studied at the varying conditions. *Trans*-resveratrol in the crystal form was stable on the light and at stock temperature in the range of 0–70°C. After dissolving in ethanol 50% (v/v) significant changes in *trans/cis* ratio has been observed when samples were exposed to daily-diffused light or UV light, temperature, etc.

Resveratrol solution exposed to the daily-diffused light isomerised to the *cis*-isomer reaching within 5 hours the equilibrium of 1:7 (mass ratio *trans/cis*) from the original ratio 50:1 (*trans/cis*) (Fig. 2). The isomer equilibrium remained unchanged during samples continuing exposition to daily-diffused light as well as during standing in darkness. On the contrary, varying temperature conditions resulted in remarkable ratio changes. If the equilibrated solution was stored at 50°C the original ratio 1:7 (*trans/cis*) found at 20°C has been changed to 1:3 (*trans/cis*) (Fig. 4).

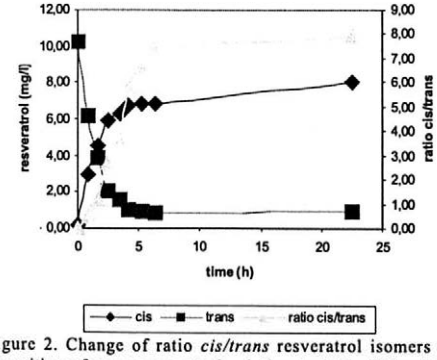

Figure 2. Change of ratio *cis/trans* resveratrol isomers during exposition of *trans*-resveratrol solution to daily-diffused light

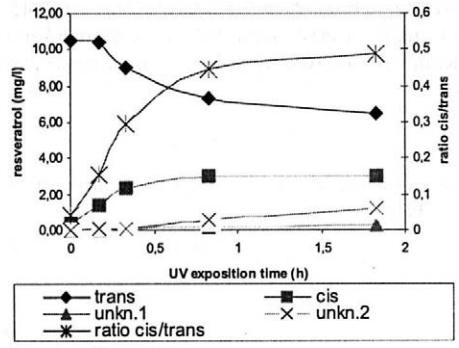

Figure 3. change of ratio *cis/trans* resveratrol isomers during exposition of trans-resveratrol solution to UV light

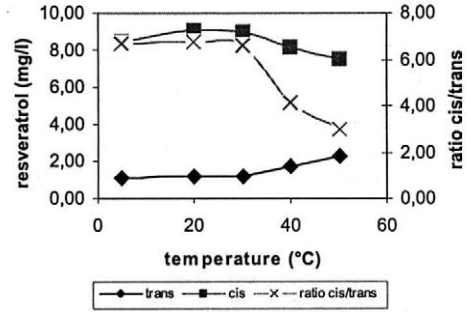

Figure 4. influence of *cis/trans* equilibrium ratio for resveratrol solution previously exposed to daily-diffused light at temperature during storing in darkness

Furthermore changes of resveratrol isomer ratio were studied in relation to UV light exposition. *Trans*-resveratrol solution was converted to *cis*-isomer from original ratio 23:1 (*trans/cis*) to 2:1 (*trans/cis*) (Fig. 3). Exposition of *trans*-isomer solution to the UV light led to the formation of two unknown substances.

The ratio of *trans*-resveratrol to *cis*-resveratrol in red wine samples remained unchanged during exposition to UV light and at varying temperature conditions. The *trans* isomer in red wine samples during exposition to daily-diffused light changed towards the higher *cis*-resveratrol content (Table 1). The final ratio of *cis/trans* isomers diff-

Table 1. Change of cis/trans resveratrol isomers ratio during exposition of red wine samples by daily-diffused light

Vineyard region	Variety of vine	Year of harvest	Ratio *cis/trans*	Ratio *cis/trans* after 2 days	Ratio *cis/trans* after 17 days
Mostecká	Svatovavřinecké	1996	1:1.8	1:1.3	2.2:1
Žernosecká	Svatovavřinecké	1998	1:4.4	1:2.6	1.9:1
Roudnická	Svatovavřinecké	1996	1:2.8	1:1.9	1.8:1
Mutěnická	Svatovavřinecké	1997	1:4.7	1:2.5	2.1:1

ers from equilibrium of resveratrol solution in ethanol 50% (v/v). This fact can be explained by presence of other phytoalexins and antioxidants and their mutual protective effect.

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E Natural Food Flavours and Colours

Increase of Red Wine Colour Stability

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Abstract

This work shows that the knowing of the reactions' factors allowed to obtain best final products. The study was focused on the chromatic quality of red wines and the way to improve it. Colour extraction enzymes were used in the winemaking process of red wines, to improve the extraction of phenolic compounds. Found results showed that enzymatic treated wines had higher levels of new pigments, higher colour intensity and more stability of colour during the storage than control wines.

Key words: red wines; colour stability; anthocyanins; extractive enzymes

INTRODUCTION

The different factors which affect the wine colour and the changes happened during the storage have been widely studied in the past. At present, it is well known that anthocyanins and the reactions, in which they take part, are the responsible of these changes. Certainly, in the last years, studies about mechanisms of reaction, in which anthocyanins and other wines compounds are involved, have enabled to isolate and elucidate the structure of some new condensed anthocyanin. Some of them are vitisin B (2), pyruvic derivatives of anthocyanins (3) and malvidin-catechin condenses (6). They seem to have a great importance in colour stability, since they are very stable pigments in a larger range of pH and in the presence of SO_2 (2). In addition, previous works (4) showed that the amount of these new pigments is proportional to the amount of phenolic substrates which can be affected by condensation processes.

Taking into account these previous studies, the aim of this work was to study if the increase of phenolic compounds, induced by the use of colour extraction enzymes, could be correlated with a higher intensity and stability of the colour of the wines.

MATERIALS AND METHODS

Tinto Fino grapes (*Vitis vinifera*) were harvested at commercial maturity 24–25 °Brix). Intact clusters were processed according to the next procedures:

Control wines (Test): The grapes, once de-stemmed and crushed, and added with 0.04 g/L of SO_2, were inoculated with 0.1 g /L of commercial yeast, *S. cerevisiae* and *S. bayanus* (Wormser Oenologie). Then, alcoholic fermentation was carried out at 25°C. After that, the wine was strained off, the grape pomace was lowly pressed, and the whole resulting wine was racked off into another tank and kept at 1°C for 48 hours to facilitate the settling process. Afterwards, the wine was centrifuged (10500 g for 10 min at 5°C) and the clarified wine was bottled and stored at 15°C in dark place for two years.

Enzymatic treated wines: In these cases, the only difference to the control treatment was the addition of pectolytic enzyme one hour (the time suggested by the producers) before the inoculation of yeast, in order to improve the enzymatic action. During this time, the grape pomace was kept at 20°C. Two different commercial preparations of pectolytic enzymes were used at the doses suggested by the producers: *Pectinase WL Extraction* (Wormser Oenologie) 0.01 g/L *(P.1)*, and *Rapidase Ex. Colour* (Gist Brocades) 0.05 g/L *(Rex.5)*.

Every treatment (control and enzymatic wines) was performed in duplicate.

Analytical procedures

Colour variables of Glories (1984): Colour intensity (I), tone (To), proportion of yellow (%Ye), proportion of red (% Rd) and proportion of blue (% Bl) were calculated from 420, 520 and 620 nm absorbance measurements.

Analysis for anthocyanin content: HPLC method (5) was used. Diode Array and Mass Spectra detectors were employed The analytical results were summarized according to the next group of anthocyanins (Acy): Acy-3-monoglucosides (*Acy3glu);* acidulated *(acyl)* derivatives (acetic and cinnamic) of Acy3glu; pyruvic acid derivatives *(Acy-pyr);* vitisin B, and malvidin-3-glucoside condensed with catechin *(Mvcat).*

Color parameters and anthocyanin contents were measured in duplicated in each sample of each wine.

Statistical analysis

ANOVA were applied. LSD Fisher-test was employed to test for statistically significant differences between samples. All the statistical analyses were carried out using Statgraphic Plus for Windows Computer Package (1995 Manugistics, Inc.).

RESULTS AND DISCUSSION

In general, total anthocyanin levels were higher in the treated wines compared to control wines elaborated in the traditional way without enzyme. In addition, treated wines showed higher colour intensity and more intense red colour. So they had less tonality values (Table 1).

According to initial data, after two years of wine storage, treated wines showed higher value of total anthocyanin, more colour intensity and, the most important, lower values of tonality (Fig. 1), due to less values of yellow

Table 1. Average content of anthocyanins in red studied wines, and chromatic parameters

	Initial						Bottled two years					
Wine	I	To	%Rd	%Bl	%Yc	Acy	I	To	%Rd	%Bl	%Yc	Acy
Test	1.31	0.46	62.70	8.45	28.84	491.1	0.27	1.08	39.24	18.52	42.19	9.57
P.1	1.50	0.42	64.12	8.41	27.46	642.4	0.91	0.59	54.44	13.60	31.96	60.70
Rex.5	1.41	0.42	64.35	8.01	27.63	566.9	0.78	0.59	54.43	13.58	31.99	48.50

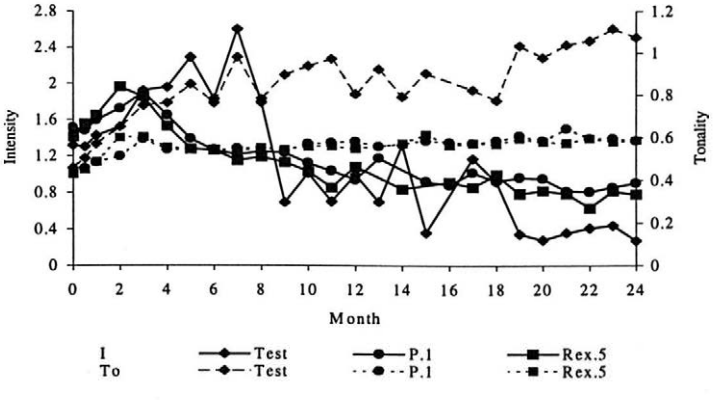

Figure 1. Evolution of chromatic characteristic of wines during theirs storage

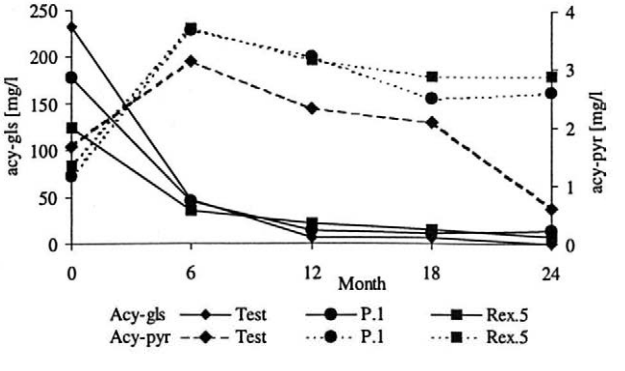

Figure 2. Evolution of anthocyanin compounds of wines during theirs storage

component and higher levels of red component (Table 1). These results point out that colour of enzymatic treated wines was more stable.

This colour stabilisation should be related to a larger levels of "new anthocyanins" formed during storage. So, higher values of pyruvic derivatives, vitisin B and malvidin-catechin, were observed in enzymatic treated wines. All of them presented a similar evolution during storage (Fig. 2). In addition, control wine showed an important decrease of levels of Acy-3glu and theirs acyl derivatives. Both groups of anthocyanins changed in the same way during the storage of wines (Fig. 2). This strong decrease of this pigment is correlated to the high loss of red component and the increase of the yellow one.

The higher level of the "new pigment" in treated wines should be explained according to the fact that in these wines there were more levels of cofactor such as catechin, (data unpublished in this paper), which were extracted in more quantity, from the grape skin, when extractive enzymes were applied.

CONCLUSIONS

These results point out the fact that previous knowledge of reaction mechanisms involved in the "new anthocyanins" formation is very interesting to plan, to adapt or to develop new technologic practices, for example use of extractive enzymes, which would make possible the production of higher quality products.

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Changes of Pigments in the Red Wine

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Abstract

Concentration of anthocyanins influences the colour of the red grape wine. Discoloration during storage is a serious problem for all winemakers and consumers. While a observing separation stability of the pigments, degradation folloving the kinetics of the first degree was identified.

Keywords: anthocyanins; beverage; identification; HPLC; stability

INTRODUCTION

Red colour in young wine is mainly due to the presence of monomeric anthocyanins and other phenolics The anthocyanins are fundamentally responsible for all of the color differences between grapes and the resultant wines. The selective and reproducible separation of anthocyanins is important not only from the analytical but also from the practical points of view, for judgment the evalution of the origin of anthocyanins in products of the most frequently occuring cultures of *Vitis vinifera*. In this work we employed the suitable conditions for the separation of the anthocyanins in the red wines by HPLC using gradient elution to determine speed of degradation (1, 3).

EXPERIMENTAL

Wine samples. The variety Frankovka was used.

Separation of anthocyanins. A chromatograph LC-10AD SHIMADZU liquid chromatogram with SPD-M10 AVP diod array detector.

Conditions for analysis. Column Biospher SIC 18, 150 mm × 4,6 mm i.d., 5 μm, No 1726, flow of the mobil phase of 0,5 ml/min, wavelenght of 520nm.

RESULTS AND DISCUSSION

By the retention times the pigments were identified as delphinidin-3-monoglucosid, cyanidin-3-monoglucosid, petunidin-3-monoglucosid, peonidin-3-monoglucosid and malvidin-3-monoglucosid. There also were acetic and coumaric acid acylated pigments recognised. It was found that anthocyanins containing two or more acyl groups are stable in weakly acidic and neutral aqueous solutions.

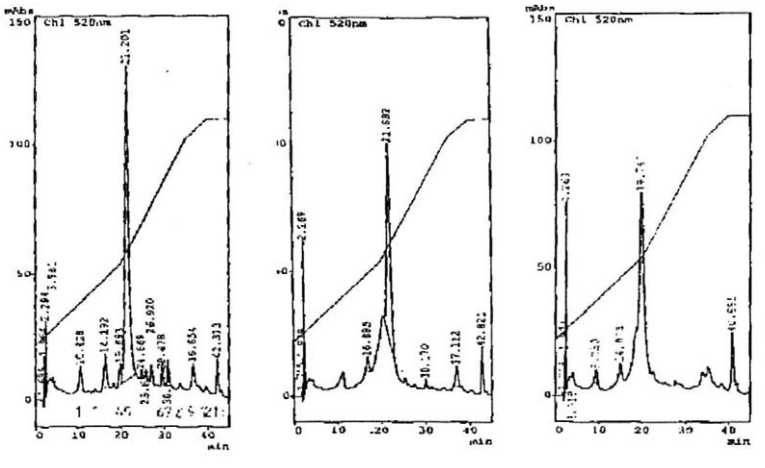

Figure 1. Chromatographic records of the antocyanins of the red wines up 2, 4 and 6 weeks of storaging (2)

Table 1. Changes of antocyanins in red wines during storage

Peak No	Rt	Anthocyanins	1 week	2 weeks	3 weeks	4.weeks	5 weeks	6 weeks
1	10.41	Dp-3-glu	4.11	3.28	4.32	4.09	3.28	3.52
2	13.9	Cy-3-glu	0.23	–	–	–	–	–
3	16.2	Pt-3-glu	5.46	5.51	4.51	4.25	5.06	5.13
4	19.7	Pc-3-glu	3.81	23.2	10.02	11.72	19.08	–
5	21.2	Mv-3-glu	73.98	56.96	70.20	68.63	62.05	73.60
6	29.5	Dp-3-glu-ac	2.26	0.31	0.35	0.32	0.32	0.19
7	30.8	Cy-3-glu-ac	2.50	1.13	0.87	0.41	0.81	0.34
8	33.7	Pt-3-glu-ac	2.14	0.20	0.22	0.09	0.18	–
9	36.0	Pc-3-glu-ac	0.63	0.27	0.47	0.41	0.31	2.83
10	36.7	Mv-3-glu-ac	4.48	4.28	3.46	4.21	3.97	4.80
11	38.0	Pt-3-glu-coum	0.31	0.09	–	0.23	0.22	0.75
12	39.6	Pc-3-glu-coum	0.48	0.11	0.27	0.29	0.29	0.74
13	42.31	Mv-3-glu-coum	3.46	4.66	4.95	5.10	4.44	8.10

Size of the rate constant is $2{,}7.10^{4}\ s^{-1}$

In these pigments, color stability appears to increase with increasing content of organic acid (cinnamic and malonic acids). During maturation of red young wine, the rednes decreases and the absorbance in the yellow/brown region rises. These changes in colour characteristics reflect the progressive displacement of the grape anthocyanins by more stable polymeric pigments, which account for up to 50% of the colour density of 1-year-old wine. These polymers are also responsible for alterations of taste and flavour.

There was the most considerable peak on all of ours chromatograms–malvidin-3-monoglucosid .In the ageing of Frankovka wine at 30°C in the dark place, the loss in amount of malvidin-3-monoglukosid during the five weeks was logarithmic with time. The rate of loss of individual anthocyanins in Frankovka depends on their structure. Malvidin-3-glucoside was disappearing more slowly than malvidin-3-acetylglukosid or malvidin-3-p-coumarylglucoside. Relative amounts of anthocyanins during storage are wrote down in the Table 1.

This study follows the work, which deals with enzyme application in the wine production for enhancement of extraction of desirable grape pigment and flavour components.

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Conversion of Betalains in the Presence of Antioxidants

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Abstract

Effect of different antioxidants on stability of betalains were measured in solution at 70, 80 and 90°C, pH 5.0. Ascorbic acid, extracts from balm (*Melissa officinalis*) and oregano (*Origanum* L.) had similar effects in improving betalains stability.

Keywords: betalains; antioxidants; stability

INTRODUCTION

Betalains is a comprehensive name for red betacyanin and yellow betaxantin pigments found in red beet. The main component of betacyanin fraction is betanin (80–90%), other components being betanidin, prebetanidin and their C_{15} isomers. The main component of the betaxantin fraction is vulgaxanthin. The absorption peak of betanin in the pH range from 3.5 to 7.0 is at 537 nm. During the heating of betanin the molecule is split, giving rise to cyclodopa and betalamic acid with an absorption peak at 420 nm. The half-life of the heat decomposition of betanin depends on the pH value.

The objectives in this study were to elaborate the method for assessing total antioxidant activity of different antioxidant preparates from plant materials, which contain mainly flavonoid compounds.

Experimental

Red beet concentrate with content of betanin 2.5 g/kg were diluted in McIlvain buffer in the pH range 4.7–5.5 and were heated in 50ml tubes in a water thermostat at 50–80°C. Individual samples were withdrawn at reselected time intervals, cooled and measured by spectrophotometry. A constant initial pigment level was used throughout each individual experiments. Extracts from balm (*Melissa officinalis*) resp. oregano (*Origanum* L.) were added to the red solution before heating. As reference material was used ascorbic acid at a different concentration (from 5 to 30 mg per litter of solution).

Degradation of betanine were measured at 50, 60, 70 and 80°C. Absorbance measurement was made against distilled water blank between 300 and 700 nm with Shimadzu 1601 UV-VIS spectrophotometer.

The percentage of pigment retention was calculated from absorbance at 537 nm for betanin. Antioxidant activity (AA) was expressed as the percent of inhibition pigment degradation relative to control.

RESULTS AND DISCUSSION

Phenolic compounds in balm (*Melissa officinalis*) and oregano (*Origanum*) are antioxidants [1,2] in model systems and real food systems, too.

Data in Fig. 1 show the effect of different levels of ascorbic acid on betanine stability in solution when held at 80°C. Maximum effective appears to be achieved when the ascorbic acid level reaches 30mg/dm^3.

Data comparing effectiveness of balm extract in stabilising betalaines solution are shown in Fig. 2. There was significant difference at the stability of betalain solution without and with addition of balm extract. The retention of betalains was after 1 h heating at 80°C 36.4% (sample without antioxidant) and 64.4% with addition of 3 ml balm extract.

The retention data (Table 1) show that at the addition of 3 ml oregano extract was similar effect as addition of 30 mg/dm^3 ascorbic acid. According to the proposed mechanism of degradation, betanin in solution is hydrolysed by heating into betalamic acid and cyclodopa-5-o-glycose, which are then further degraded (3–5). It seems that ascor-

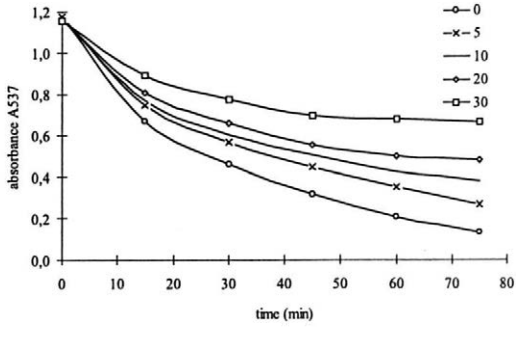

Figure 1. Antidiscoloring activity of different levels of ascorbic acid (mg/dm^3) at 80°C

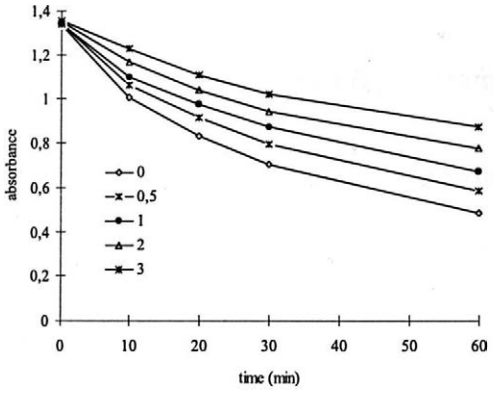

Figure 2. Antidiscoloring activity of different level of balm extract (ml/dm^3) at 80°C

bic acid and polyphenols prevent degradation not only betanin, but also betalamic acid. The strength of the antidiscoloring activity of ascorbic acid and different flavonoid concentrates may be used for expressed their antioxidant activities (6, 7).

Table 1. Retention of red beet pigments (%) in the presence of oregano extract at 80°C

	Addition of oregano extract (ml)				
time (min)	0	0.5	1	2	3
10	44.7	63.6	70.5	76.1	89.5
20	38.9	45.1	55.4	65.0	79.6
30	31.4	36.9	45.6	57.2	74.5
60	20.4	25.7	35.1	46.5	62.5

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Authenticity of Tomato Products

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Abstract

Czech food law (Decree No. 332/1997 of the law No. 110/1997) specifies the minimum tomato content in ketchup being 7 or 10% tomato solids in total refractive solid content, which is 25 or 30 for tomato ketchup and tomato ketchup labelled Prima, Extra, Special, respectively. Various analytical markers including lycopene, b-carotene, 5-pyrrolidon-2-carboxylic acid (pyroglutamic acid, PCA), glucose, fructose, sucrose, citric and malic acid, Na^+, K^+, Ca^{2+}, Mg^{2+}, formol number, etc. were evaluated. Two sets of ketchup samples were analysed. The method for the estimation of tomato content in ketchup was proposed. In spite to the limited number of calibration samples, the procedure allows to distinguish the falsified samples with tomato content lowered of more than about 1.25% of tomato solids.

Keywords: tomato ketchup; food authenticity; natural tomato solid content NTSS; falsification

INTRODUCTION

The required physical and chemical properties of tomato products are defined by the Decree 332/1997 of the Czech Food Law No. 110/1997 (1), the minimum content of soluble solids of tomato ketchup is 25 and 30 % for ketchup and ketchup Prima, Extra, Special. The minimum content of NTSS (Natural Tomato Soluble Solids) has to be 7 and 10 in ketchup and ketchup Prima (Extra, Special) respectively. The limits o the tomato content are well defined but there is not any legal basis allowing to ensure the fulfilment of the above requirements. The producers under the pressure of the distribution chains manufacture cheap products with lower tomato content. Consumers due to the ongoing economic recession are still more interested in the price of product than its quality. The main problem is in the clear requirements given in the Czech food law, which are not fulfilled.

The lowering of tomato content is the most usual way of ketchup falsification, the easiest method is the addition of hydrocolloids (usually modified starches) and sucrose. The novelised decree No. 298 does not permit the addition of colorants (ketchup with addition of hydrocolloids are pale), so some of producers add paprika extracts or other coloured ingredients (red beet).

The tomato content in ketchup could be determined according to the content of components which are related to the tomato paste used for the ketchup production. The available physical or chemical parameters are e.g. (2–6): colour of ketchup (evaluated by objective measurement, e.g. L, a, b values), lycopene and b-carotene content, glutamic acid content, 5-pyrrolidon-2-carboxylic acid (pyroglutamic acid, PCA), acids (citric acid, malic acid), Na, K, Mg, Ca, formol number and others - sugars, ammonia, fibre content, phosphates (or phosphorus). nitrogen, etc.

EXPERIMENTAL

Material

1st series of samples – 26 ketchup samples obtained in Czech food distribution chains (including 10 samples of three main producers with known tomato content).

2nd series – Tomato ketchup model samples prepared by main Czech producers (Hamé, Otma, SPAK, Kand) with defined NTSS ranging from 3 to 15% of tomato refractive solids.

Methods

PCA (5-pyrrolidon-2-carboxylic acid): HPLC Column: *OSTION LG KS 0800H*, 6.3 µm, 250 × 4 mm; Mobil phase: 5 mM H_2SO_4, flow rate 0.3ml/min, pressure 8 MPa Detection: UV 210 nm, Calibration: external standard (PCA) Sample preparation: extraction with dist. water, filtration, dilution in mobile phase.

Lycopene, β-carotene: HPLC Column: TESSEK, *Separon SGX C-18*, 10 µm, 250 × 4 mm Mobil phase: acetonitril:chloroform 85:15 (v/v), flow rate 1ml/min, pressure 3.5 MPa Detection: 470 nm, Calibration: external standard (β-carotene) Sample preparation: multiple extraction with acetone:chloroform 1:1 (v/v).

Na, Ca, Mg, K, NH_4: Capillary isotachophoresis: Leading electrolyte: 7,5 mM H_2SO_4 + 7 mM-18-crown-6 + 0,1%

Na, Ca, Mg, K, NH_4: Capillary isotachophoresis: Leading electrolyte: 7,5 mM H_2SO_4 + 7 mM-18-crown-6 + 0,1% HPMC; Terminating electrolyte: 5 mM BisTrisPropan + 10 mM caproic acid; Detection: conductivity Sample preparation: extraction with dist. water, dilution in leading electrolyte.

Glucose, fructose, sucrose: HPLC Column: *OSTION LG KS 0403(4.2 % DVB)*, 6.3 μm, 250 × 4 mm; Mobil phase: dist. water, flow rate 0.5ml/min, pressure 8 MPa; Detection: RI; Calibration: external standard; Sample preparation: extraction with dist. water, filtration, dilution in mobile phase.

Citric acid, malic acid: Capillary isotachophoresis; Leading electrolyte: 10 mM HCl + 5.5 mM BTP + 0,1% HPMC; Terminating electrolyte: 5 mM acetic acid; Detection: conductivity; Sample preparation: extraction with dist. water, filtration, dilution.

Formol number: According to RSK (4) (raw data without correction to ammonia were processed) (4).

RESULTS AND DISCUSSION

To evaluate the calibration sets (with known tomato content) the both series of samples were processed using regression analysis, the significant values of correlation coefficient of NTSS and selected chemical markers (at $p < 0.05$, $n = 10$ and 12, 1st and 2nd series respectively) were obtained for the following markers only: PCA (0.94); NH_4(0.69); K (0.98); Ca (0.84); Mg (0.98), malic acid (0.89), citric acid (0.98) and formol number (0.96).

Low values of correlation coefficients of carotenoids pigment were probably caused (in addition to limited number of samples) by different degree of chemical degradation of pigment in samples (cis/trans isomeration, autoxidation, etc.) and also by general properties of analysed samples (tomato pastes with different pigment content was used by producers, etc.). Parameter sugar content has usually negative values of correlation coefficient, the lower level of paste is used, the higher amount of sucrose (or other sugar) is added. In some cases acids, especially citric acid can be used as acidulant in ketchup prescription.

Calibration data were also processed using multiple regression method. Various combinations of markers were tested in the both series of samples with multiple R values ranging from 0.900 to 0.99. For the estimation of tomato content in ketchup the following parameters were chosen: In *the 1st series* – lycopene (mg/100g of dry matter (DM)), PCA content (mg/100 DM), potassium (mg/100 g DM), malic acid (mg/100 g), citric acid (mg/100 g), formol number (ml 0.1M NaOH/100ml). In *the 2nd series* – PCA (mg/100g of dry matter), potassium (mg/100 g DM), calcium (mg/100 g DM), magnesium (mg/100 g DM), malic acid (mg/100 g DM), citric acid (mg/100 g DM), formol number (ml 0.1M NaOH/100ml).

Multiple regression equation with the selected parameters was used to estimate tomato content in the set of tomato ketchup samples obtained in the Czech market in Autumn 1999, in 8 samples of 26 analysed the lower content of NTSS were found, in 4 samples the NTSS was less than 50% of the value given by the Decree No. 323/1997. Verification of the procedure was done using the 2nd series of samples. The set of 12 samples (remaining 4 was excluded as outliers, the producers probably did not declare the correct content of NTSS in samples) was always divided into two groups, the calibration equation was calculated for 9 of the samples and the obtained equation was used to estimate the NTSS content in remaining 3 samples. The results of NTSS estimation compared with the declared NTSS content are given in the Table 1.

Table 1. Verification of the procedure (estimated and declared tomato content)

Sample No.	Tomato content (% of DM) estimated	declared	NTSS (%) estimated	declared	declared –estimated
c1	11.78	13.27	2.66	3.00	–0.34
c2	21.62	26.32	5.75	7.00	–1.25
c3	20.48	33.78	6.06	10.00	–3.94*
c5	31.87	29.00	8.29	7.54	0.75
c6	35.43	31.23	9.21	8.12	1.09
c7	19.70	14.84	5.00	3.77	1.23
c8	13.48	11.89	3.29	2.90	0.39
c10	16.23	16.94	5.14	5.37	–0.23
c11	23.66	22.36	7.52	7.11	0.41
c12	18.55	19.11	5.21	5.37	-0.16
c13	24.95	25.85	6.86	7.11	-0.25
c14	75.43	69.71	15.69	14.50	1.19

*value of PCA was outlaying, out of the course of calibration curve

The method for the estimation of tomato content in ketchup was proposed. In spite to the limited number of calibration samples, the procedure allows to distinguish the falsified samples with tomato content lowered of more than about 1.25% of tomato solids. In the case the calibration set will be improved according to the varying composition of processed tomato paste, the procedure could be useful tool to ensure the fulfilment of the requirements of the Czech food law for the tomato ketchup.

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Stability of New Red Colourant

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Abstract

New red colourant – Arpink red (carmine derivative, microbial source) was examined for the possible food use. The effect of pH, temperature and light was studied in model solutions. Applications in both soft and alcoholic beverages were tested as well as in dairy products. Good colouration stability in dairy products was found. High light sensitivity was detected in model beverages.

Keywords: natural food colourants; colour stability

INTRODUCTION

Colour is considered as a major attribute in evaluating the appearance of food; first visual impressions have a significant influence on overall acceptability or preference of food. In the search for natural colourants the microbial, animal and plant sources have been investigated. Although microbes offer a variety of colourants in relatively rapid, easily controlled continuous production, the stability of pigments after extraction and application poses the limit question of exploitation. Only few components have been successfully used in food production (1).

Whereas the need for yellow/orange colourants is successfully supplied by carotenoids, the demand for red pigments is remaining. A new red microbial colourant, an exogenous metabolite of *Penicillium oxalicum* var. *Armenicana* (2) has been investigated. The pigment was identified as an anthraquinone derivative (carmine analogue); its probable structure is given in Fig. 1. The purified pigment extract conforming to hygienic requirements was applied in model soft as well alcoholic beverages and dairy products and the colour stability has been studied.

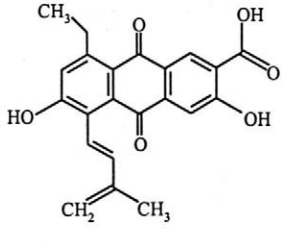

Figure 1. Chemical structure of new red colourant

EXPERIMENTAL

Materials

Colourants: 1. Arpink red – purified liquid extract of new colourant, 2. Carminic acid 50% powder (Dr. Marcus), 3. Enocyanin 3% powder (Dr. Marcus), 4. Rote-Beete-Saft powder (Dr. Marcus).

Mediums: Model soft drinks: citric acid 300 mg/l, sucrose 100 g/l or Aspartame 150 mg/l, sorbate K 200 mg/l, water to 1l. Model alcoholic drinks: ethanol 200 ml/l, sucrose 100g/l, water to 1l. Dairy product: milk, yoghurt.

Storage conditions: 1. 25°C, on daylight, without direct sunshine exposition and 2. 16°C, in the dark

Methods

Perkin-Elmer Lambda 11 Spectrophotometer was used for absorption spectrum measurements.

Sensory evaluations of the colour acceptance were provided.

RESULTS AND DISCUSSION

Both the aqueous and ethanol solutions of the new pigment have a pleasant raspberry-red colour, almost pH independent in the 4–8 range. Two absorption maxima have been determined in the visible spectrum, at 500 and 420 nm approximately. Within the storage time the ratio of the maxima changes slightly on behalf of the yellow factor (Figs 2 and 3).

No destructive effect of pasteurisation (20 min at 80°C) on Arpink coloured model drinks was found, not even in sucrose solutions. On contrary, low photostability of the new dye has been detected. Keeping both water and ethanol model drinks on daylight led to a significant decolou-

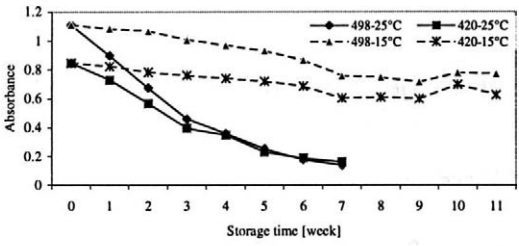

Figure 2. Absorbance changes in the model soft drink during storage at 25°C on daylight and at 15°C in the dark

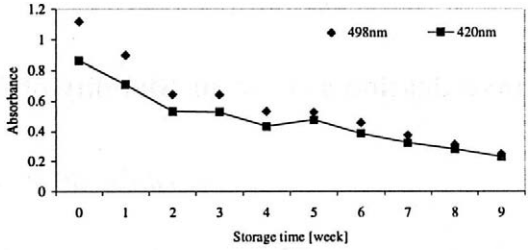

Figure 3. Absorbance changes in the model alcohol drink during storage at 25°C on daylight

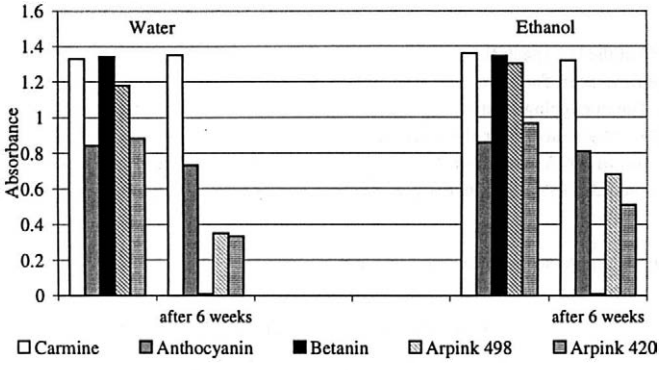

Figure 4. Comparison of some coloring preparations and their stability

ration, whereas a good colour stability was found when stored in darkness. (Figs 2 and 3) A direct exposition on sunlight leads to a very rapid degradation of the pigment. A very good colour stability was found in dairy products kept in a refrigerator (6°C, dark).

A comparative essay of tinctorial powder of some commercial colouring formulations and the new Arpink red colour was performed. To achieve the same sensorial value, the following concentrations of colours should be applied: Arpink red 0.33 g/l, Carminic acid 0.17 g/l, Enocyanin 1.222 g/l, Rote-Beete-Saft 3.0 g/l. Six weeks storage of both water and alcohol solutions of these colourants at ambient conditions has shown a very good stability of anthocyanins and carmine preparations, whereas betanine sample was totally decoloured. A moderate stability of Arpink red solutions was found within this storage time (Fig. 4).

It could be concluded, that the studied new colourant could be successfully recommended for some dairy products. For beverages application, the further investigation in pigment stability improving is needed.

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Investigation of Flavour Stability of Beer by the Absorption Integral (AI)

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Abstract

The absorption integral (AI) is defined as the area of the UV spectrum of a beer destillate between 240 and 310 nm and is applied as a fast method to predict the flavour stability of fresh beer. The influence of substances already known as beer ageing indicators were evaluated for their contribution to the AI. The not ageing related substances contributing to the AI such as acetaldehyde, acetoin, 2-phenyl-ethanol, 4-vinyl-phenol and 4-vinyl-guaiacol did not change very much in beers from different breweries. The components responsible for the ageing related part of the AI were 5-methyl-2-furfural, 2-acetyl-5-methylfuran, 2-furfural, 2-furfuryl-methanol, 2-acetyl-furan and nicotinic acid-ethyl ester. The increase of the AI in ageing beers was caused especially by 2-furfural.

Keywords: beer; absorption integral; off-flavour; ageing related substances; carbonyls

INTRODUCTION

The flavour stability of beer becomes more and more important. During the storage of beer under bad conditions (e.g. too high temperatures) the concentration of beer ageing components (mainly carbonyls) changes significantly (1, 2). These substances are products of the Maillard reaction, Strecker degradation, oxidation of alcohols and isohumulons as well as of lipid degradation and aldol addition (1–4). Till now it has been not possible to find the main substances which are responsible for the ageing flavour of beer. But there are a lot of indicator substances which are changing their concentration according to changing of flavour. Most of the methods to detect these changes are time and money consuming procedures such as gaschromatography, ESR and chemiluminescence. As a fast method the measurement of the absorption integral (AI), the area below the curve of the UV spectrum of a beer destillate between 240 and 310 nm, is used to predict the flavour stability of fresh beers (5, 6).

The goal of this work was to identify the AI relevant substances and to detect their contribution to the AI by GC and HPLC.

EXPERIMENTAL

Materials. 8 beers from Austria, 2 from Hungary and 4 from Rumania were analysed after 14 days at 10°C and after artificial ageing after 3 days at 45°C.

Methods. The AI was measured in the steam destillates. 200 ml of cooled beer (8°C) were mixed with antifoaming agent, and 100 ml of it were distilled within 280 seconds. Spectra were recorded with a UV/VIS spectrometer (*Lamda 12 by Perkin Elmer*) between 200 and 350 nm, the photometric evaluation was performed by the softwares *UV WIN LAB 2.0* and *Spectra Integration*.

Nonvolatile substances were analysed by GC/FID (7), volatile substances by headspace GC/FID (8), 4-vinylphenol and 4-vinylguajacol by a modified MEBAK method (9), ageing substances according to *Lustig* (3) and acetoin by a MEBAK method too (10).

RESULTS AND DISCUSSION

The substances specified in Table 1 have been recognized as AI related aroma compounds. The individual con-

Table 1. AI related aroma compounds

Substance	Abbreviation	Ageing relevant	Factor/concentration
5-Methyl-2-furfural	5M2F	yes	0.023/ppb
2-Acetyl-5-methylfuran	2A5MF	yes	0.023/ppb
2-Furfural	F	yes	0.037/ppb
2-Furfuryl-methanol	F-OH	yes	0.065/ppm
2-Acetyl-furan	2AC	yes	0.025/ppb
Nicotinic acid-ethyl ester	NICO	yes	0.005/ppb
Acetaldehyde	ACET	no	0.034/ppm
Acetoin	ACOI	no	0.016/ppm
2-Phenyl-ethanol	PH-OH	no	0.067/ppm
4-Vinyl-phenol	4 VP	no	3.950/ppm
4-Vinyl-guaiacol	4 VG	no	5.410/ppm

Table 2. Concentrations and percental distribution of AI related substances during forced beer ageing process

Days	5M2F [ppb]	2A5MF [ppb]	F [ppb]	F-OH [ppm]	2AF [ppb]	NICO [ppb]	ACOI [ppm]	ACET [ppm]	PH-OH [ppm]	4VP [ppm]	4VG [ppm]
0	5	4	8	1.49	13	8	7.5	5.0	25.5	0.01	0.15
1	5	5	56	1.49	13	10	7.5	4.8	25.8	0.01	0.15
3	5	6	192	1.55	15	15	7.5	4.6	25.5	0.01	0.15
5	4	7	286	1.55	16	21	7.5	4.8	25.0	0.01	0.15
14	6	13	607	1.69	17	46	7.5	4.8	25.5	0.01	0.15
	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
0	3	2	7	2	8	1	3	4	42	1	20
1	2	2	32	1	5	1	2	3	27	1	12
3	1	1	53	1	3	1	1	1	13	0	6
5	1	1	59	1	2	1	1	1	9	0	5
14	0	1	74	0	1	1	0	0	6	0	3

substances in definite concentrations expressed by a factor related to ppm or ppb.

4-Vinyl-phenol and 4-vinyl-guaiacol make a large contribution to the AI which can also be recognized at top-fermented beers containing high amounts of these substances correlated with high AIs compared to bottom-fermented beer. It can be assumed that other not ageing relevant substances contribute to the AI; these compounds were investigated by a forced ageing process (11).

After thermal treatment (14 days) 74% of the AI are caused only by 2-furfural whereas the AI contribution of 2-phenyl-ethanol and 4-vinyl-guaiacol, the ageing relevant main components, decreases to 36 or 17% respectively (11).

The absolute concentrations of not ageing related AI aroma compounds do not change significantly and therefore cannot increase the AI. It is a consequence of this fact that a comparison of AIs of beers from different breweries is only possible if the contribution of not ageing related substances to the AI is similar (11).

The concentration of 2-furfural increases much more during ageing than the other components, and therefore it is first responsible for the rise of the AI. So 2-furfural does not contribute much to the off-flavour it can be considered as an indicator substance of beer ageing and thermal stress. The measurement of the AI in beers to predict flavour stability can be applied for fresh beer as well as for thermal influenced beer.

The examination of bottled beers from various breweries revealed that approximately 80% of the described AI related substances in fresh beers and 85% in aged beers were claimed by the described substances (11).

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Aroma Compounds in Yoghurt

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Abstract

Acetaldehyde, diacetyl and ethanol are compounds that are critical to the aroma and flavour of fermented milk products. In this work twelve commercial brands of plain yogurt were stored within 10 days on two different temperature levels and every two or three days changes in acetaldehyde, diacetyl and ethanol were established. At the same time sensory evaluation was carried out too. The aim of the study was to investigate the relationship between sensory evaluation and acetaldehyde, diacetyl and ethanol, in the term of linear regression. Expert panel's ratings were regressed against concentrations of aroma compounds for both temperature levels and linear relationship among them were determined. Consequently very accurately predicting equations were obtained using regression analysis. Coefficients of correlation were generally high (from –0.8164 to –0.9990). The most pronounced relationship expressed as correlation coefficients between expert panel's ratings and contentrations of each individual compounds was observed with ethanol ($r = -0.9990$) in yogurt sample stored at +4°C. The least linear correlation coefficient have been obtained between expert panel cs ratings and diacetyl ($r = -0.8164$) in sample stored at +20°C.

Keywords: yoghurt; acetaldehyde; lyzinoalanine

INTRODUCTION

The most widely used and recognized fermented milk is yoghurt, a coagulated product obtained by specific lactic acid fermentation, through the action of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (1).

Except that nutritional and health-promoting aspects, the flavour properties of yoghurt are appreciated by consumers. Generally lactic acid gives fermented milks their slightly tart taste. The other characteristic flavours and aromas are additional results of LAB metabolism. Thus more than 100 chemical compounds have been isolated from yoghurt and fermented milks but only a few (acetaldehyde, ethanol, diacetyl, acetone and butanone-2) have a high impact on the desired product flavour (2–4).

Acetaldehyde provides the characteristic aroma of yoghurt. The *Streptococcus* may form acetaldehyde from lactose vie pyruvate, but only trace amounts are formed via this route by *Lactobacillus delbrueckii* subsp. *bulgaricus*. Optimum flavour and aroma is obtained between 23 and 41 ppm acetaldehyde. Diacetil and acetoin result from methabolic activity of *Streptococcus thermophilus* and are very low, only 0.5 ppm. Diacetyl may be produced also by *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and *Lactococcus lactis* subsp. *cremoris*. The presence of diacetyl contributes to the delicate, full flavour and aroma of yoghurt and is especially important if acetaldehyde is low because it can enhance yoghurt flavour. Many starter organisms metabolise acetaldehyde to ethanol. The lack of alcohol dehydrogenase, the enzyme catalysing this reaction, in both *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* makes these starters incapable of metabolizing acetaldehyde to ethanol, which means that acetaldehyde is the end metabolite (5).

The aim of this study was to show changes in concentration of these typical flavour compounds and sensory evaluation occuring during storage as a function of duration and temperature in depot.

EXPERIMENTAL

Material. Twelve commercial brands of plain yogurt purchased from market of Zagreb, were stored within 10 days on two different temperature levels (+4°C and +20°C). Every two or three days changes in acetaldehyde, diacetyl and ethanol were established. At the same time sensory evaluation was carried out too.

Methods. Acetaldehyde and ethanol content was determined by aldehide dehydrogenase and alcohol dehydrogenase method (6). Diacetyl was measured by modification of the Hill's colorimetric method (7). Sensory analysis was conducted by five member panel, using a scoring system with weighted factors in the 20-point scale (8). The data were analysed by correlation and regression analysis (9).

RESULTS AND DISCUSSION

Sensory evaluation were regressed against the concentrations of aroma compounds for both temperature levels. Statistical parameters obtained from regression analysis were summarized and presented in Table 1. Regression analysis determined linear relationship among them. Coefficients of correlation were generally high (from –0.8164 to –0.9990). The most pronounced relationship expressed as correlation coefficients between expert panel's ratings and

Table 1. Statistical parameters from regression analysis

	Samples	T [°C]	Aroma compounds					
			acetaldehyde		diacetyl		ethanol	
			r	R^2	r	R^2	r	R^2
Sensory analysis	S-1	+4	0.958	0.919	–0.849	0.721	–0.986	0.971
			$Y = -19.51 + 1.30x$		$Y = 6.12 - 0.26x$		$Y = 38.02 - 1.81x$	
		+20	0.952	0.906	–0.867	0.752	–0.996	0.992
			$Y = -8.09 + 0.73x$		$Y = 3.66 - 0.14x$		$Y = 25.40 - 1.18x$	
	S-2	+4	0.966	0.933	–0.926	0.858	–0.970	0.940
			$Y = -26.78 + 1.97x$		$Y = 8.75 - 0.41x$		$Y = 15.50 - 0.77x$	
		+20	0.947	0.897	–0.938	0.880	–0.957	0.916
			$Y = -8.82 + 1.02x$		$Y = 4.52 - 0.18x$		$Y = 12.52 - 0.62x$	
	S-3	+4	0.905	0.819	–0.932	0.868	–0.956	0.915
			$Y = -20.22 + 1.50x$		$Y = 12.99 - 0.58x$		$Y = 59.81 - 2.77x$	
		+20	0.897	0.804	–0.992	0.984	–0.988	0.976
			$Y = -4.57 + 0.66x$		$Y = 3.65 - 0.11x$		$Y = 8.41 - 0.67x$	
	S-4	+4	0.980	0.961	–0.884	0.781	–0.985	0.970
			$Y = -51.43 + 3.97x$		$Y = 9.99 - 0.48x$		$Y = 11.77 - 0.58x$	
		+20	0.905	0.818	–0.816	0.667	–0.955	0.913
			$Y = -12.40 + 1.98x$		$Y = 4.57 - 0.20x$		$Y = 10.14 - 0.52x$	
	S-5	+4	0.937	0.878	–0.980	0.960	–0.877	0.769
			$Y = -107.81 + 7.04x$		$Y = 7.38 - 0.36x$		$Y = 14.65 - 0.77x$	
		+20	0.996	0.992	–0.966	0.933	–0.994	0.988
			$Y = -32.49 + 2.89x$		$Y = 2.06 - 0.07x$		$Y = 5.45 - 0.26x$	
	S-6	+4	0.956	0.914	–0.927	0.860	–0.915	0.838
			$Y = -1.08 + 0.68x$		$Y = 3.78 - 0.14x$		$Y = 3.18 - 0.12x$	
		+20	0.998	0.995	–0.979	0.958	–0.986	0.972
			$Y = -2.65 + 0.80x$		$Y = 3.59 - 0.13x$		$Y = 4.52 - 0.19x$	
	S-7	+4	0.919	0.845	–0.964	0.929	–0.994	0.988
			$Y = -5.71 + 0.56x$		$Y = 2.36 - 0.10x$		$Y = 20.19 - 0.97x$	
		+20	0.828	0.686	–0.949	0.900	–0.961	0.923
			$Y = -2.05 + 0.35x$		$Y = 1.59 - 0.06x$		$Y = 12.88 - 0.58x$	
	S-8	+4	0.873	0.761	–0.981	0.963	–0.969	0.939
			$Y = -10.00 + 0.99x$		$Y = 5.26 - 0.21x$		$Y = 23.27 - 1.10x$	
		+20	0.924	0.854	–0.996	0.993	–0.958	0.917
			$Y = -7.58 + 0.86x$		$Y = 3.27 - 0.11x$		$Y = 17.93 - 0.84x$	
	S-9	+4	0.930	0.865	–0.963	0.928	–0.972	0.944
			$Y = -9.58 + 0.86x$		$Y = 5.90 - 0.23x$		$Y = 9.17 - 0.38x$	
		+20	0.944	0.891	–0.934	0.871	–0.963	0.928
			$Y = -5.03 + 0.64x$		$Y = 3.71 - 0.12x$		$Y = 8.20 - 0.32x$	
	S-10	+4	0.942	0.887	–0.975	0.951	–0.973	0.946
			$Y = 1.12 + 0.19x$		$Y = 2.91 - 0.10x$		$Y = 2.88 - 0.10x$	
		+20	0.955	0.912	–0.974	0.949	–0.997	0.994
			$Y = 0.38 + 0.24x$		$Y = 2.80 - 0.10x$		$Y = 3.22 - 0.13x$	
	S-11	+4	0.986	0.973	–0.971	0.943	–0.999	0.998
			$Y = -38.22 + 2.98x$		$Y = 1.94 - 0.06x$		$Y = 9.30 - 0.44x$	
		+20	0.981	0.963	–0.906	0.822	–0.990	0.980
			$Y = -14.41 + 1.80x$		$Y = 1.71 - 0.05x$		$Y = 6.78 - 0.32x$	
	S-12	+4	0.931	0.868	–0.847	0.717	–0.886	0.785
			$Y = -24.55 + 1.85x$		$Y = 8.86 - 0.39x$		$Y = 14.35 - 0.69x$	
		+20	0.936	0.876	–0.947	0.897	–0.986	0.971
			$Y = -19.71 + 1.58x$		$Y = 7.02 - 0.30x$		$Y = 11.41 - 0.56x$	

concentrations of each individual compounds was observed with ethanol ($r = -0.9990$) in yogurt sample stored at +4°C. The least linear correlation coefficient have been obtained between expert panel's ratings and diacetyl ($r = -0.8164$) in sample stored at +20°C. Consequently very accurately predicting equations were obtained.

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Changes of Aroma in Shell Eggs

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Abstract

New possibilities for the determination of eggs freshness were studied. The volatile compounds of eggs (resp. egg yolks) and their changes during storage were followed. We Two methods for extraction of volatiles were compared: static headspace (solid phase microextraction-SPME) and extraction according to Likens-Nickerson by simultaneous distillation-extraction (SDE) with diethylether as organic solvent. The extracts were analyzed by GC/FID. About 50 volatiles applying SDE extraction were proved, from which more then 30 were identified by GC/MS. These include aldehydes, alcoholes, acids and esters. Using headspace technique lower concentration of volatiles were obtained due to the analysis of unheated eggs yolks. The changes of volatiles during storage of shell eggs using above mentioned methods were studied.

Keywords: quality of eggs; changing during storage; volatile compounds; Solid Phase Microextraction; SPME; Simultaneous Distillation-Extraction

INTRODUCTION

Freshness of eggs is in tight connection with the quality and, therefore, belongs to the most important criteria, which are necessary to follow to protect the consumer and to lower the economical losses of producers. Most of the criteria employed have an empirical or semiempirical character. These methods are based on physical (e.g. pH measuring, index of refraction, hydrometric method) and chemical (chemical composition – 1, 2) properties. In present time the measuring of the Haugh units (HU) is most used in commercial practise. The HU results from the weight of shell egg (W) and the height of egg thick white (H). It is defined by the relationship (3):

$$HU = 100 * \log (H - 1.7 * W^{0.37} + 7.6)$$

The higher value of HU responses to better quality of eggs, if other characteristic are good.

If the significant changes of volatiles concentration during ageing of eggs occur, it would be possible to use these parameters for the quality determination (age and storage condition) of shell eggs.

So far the most studies have dealt with the analysis of volatiles of heated eggs. MacLoad and Cave (4) studied a suitable method for preparation of samples for analysis of volatiles. In the end they used a simultaneous steam distillation-solvent extraction in modified Likens-Nickerson apparatus. Umano and Hagi (5) determined 141 volatile components in cooked whole eggs, egg yolk and egg white. According to them whole eggs contain nitriles, alkylbenzenes, ketones, pyrazines, pyrroles and pyridines as major components. Cooked egg yolk has large numbers of aldehydes and pyrazines, while the major components of cooked egg white are ketones, pyrazines and nitriles.

EXPERIMENTAL

Material. The eggs of Lohmann hen hybrid line from cages (Vejprnice, Czech republic) were stored 0–12 days at 35°C. One day of this storage of eggs corresponds approximately to three days of storage under chilling conditions. The eggs were analyzed at the beginning of storage experiment and than, on fourth, seventh, and twelfth day of storage. Together with determination of volatiles HU values were determined using TSS EQS.apparatus

After breakage of eggs, egg yolks were separated from egg whites and for following analysis only yolks were used.

Solid Phase Microextraction. For adsorption of volatiles of yolk the fiber PDMS/DVB (Polydimehylsiloxane/Divinylbenzene) by thickness 65 μm was chosen.

30 g homogenized yolks were weighted out to vial. The sorption carried over 60 min. in headspace at 40°C.

Simultaneous Distillation-Extraction (SDE). 75 g homogenized yolks were weighted and blended with 0.5 L water. The flask was connected to modified Likens-Nickerson apparatus heated to boiling point, boiled 60 min. and volatiles were extracted into 100 mL diethylether. Extract was evaporated to 200 μL. 1-pentanol was used as inside standard.

Gas Chromatography (GC). The gas chromatograph was a Hewlett-Packard 6890 Plus with flame ionization detector (FID) and with column HP-INNOWAX, 30m × 0,25 mm × 0,25μm. The helium carrier gas flow rate 1ml/min. The oven temperature was held at 40°C for 3 min. after which the temperature was programmed to 240°C at 3°C. Detector and injector temperatures were 250°C, only at SDE injector temperature was 220°C.

Gas Chromatography-Mass Spectrometry (GC-MS). The gas chromatograph Hewlett-Packard G1800 A with quadrupol mass spectrometer (MS) was used for identifications compounds. The helium carrier gas flow rate 1ml/min. The volatiles obtained by SPME were not identified.

RESULTS AND DISCUSSION

The volatiles in extract obtained by method SPME have not been up to now identified. Nevertheless the changes of areas of selected peaks of volatiles were monitored during storage at above given conditions. The chromatogram obtained by analysis of extract of SPME is shown on Fig. 1. Changes of peak areas of volatiles during ageing of eggs are given in Table 1. These changes show, that concentration of volatiles of unheated yolks that have been adsorbed to PDMS/DVB fiber decreased during storage time.

Table 1. The changes of peak areas of volatiles in extract obtained by method SPME

	Days of storage			
Peak No.	0	4	7	12
1	86.6	61.3	54.5	38.5
2	330.2	223.2	204.5	176.5
3	302.7	135.1	132.9	111.3
4	222.8	54.9	58.1	41.4
5	39.4	7.6	10.0	4.8

The changes of HU values are given in Table 2.

Table 2. Changes of HU values during storage

Days of storage	0	4	7	12
Haugh Units	77	56	42	35

In extract obtained by SDE in modified Likens-Nickerson apparatus 48 substances were identified. From them six substances (present in highest concentration) were chosen. The chromatogram obtained by analysis of SDE extract is show in Fig. 2. The changes of concentration of volatiles during ageing of eggs are show in Table 3.

Table 3. The changes of concentration of volatiles in extract obtained by method SDE

	Days of storage			
Compound	0	4	7	12
Phenylacealdehyde	0.0132	0.0122	0.0296	0.0198
Hexadecanal	0.0710	0.0283	0.0269	0.0501
Octadecanal	0.0309	0.0169	0.0143	0.0163
Palmitic acid	0.0317	0.0302	0.0455	0.0706
Heptadecanoic acid	0.0155	0.0326	0.0261	0.0153
Stearic acid	0.0218	0.0210	0.0312	0.0430

values shown in Table – mg substances/100 g yolk

It is obvious, that the changes of individual compounds are different. The increasing trend was observed at palmitic and stearic acids, perhaps as a result of lipid hydrolysis. The changing of other substances during storage time of eggs fluctuate. From this reasons further experiments, witch are now running are needed.

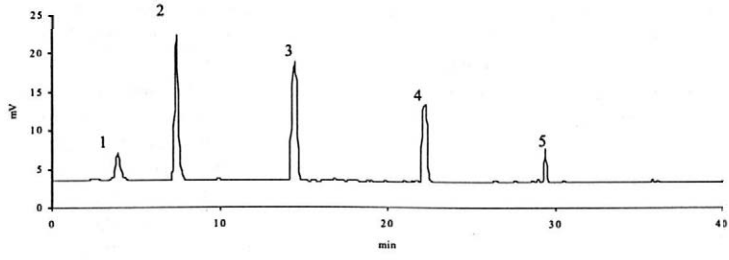

Figure 1. Chromatogram of volatiles in extract obtained by method SPME

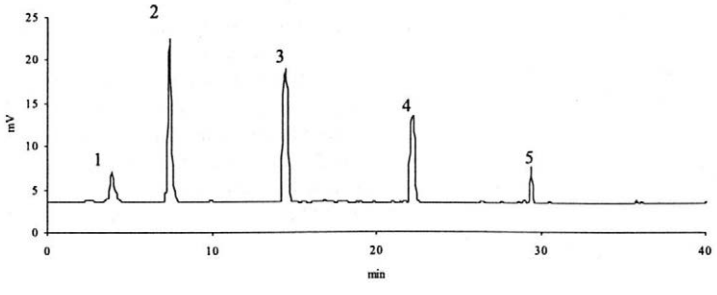

Figure 2. Chromatogram of volatiles in extract obtained by method SDE

Errata

Figure 2 on page 232 was confounded with another one during printing.

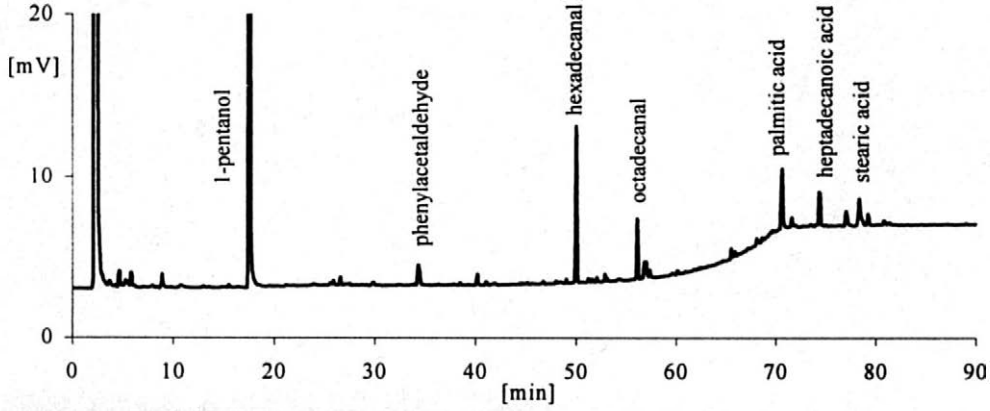

Figure 2. Chromatogram of volatiles in extract obtained by method SDE

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Changes in Composition of Volatile Aroma Active Compounds of Cooked Hen Meat during its Storage

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Abstract

The volatile aroma compounds of fresh and 24 hours refrigerated and reheated pressure cooked hen meat were isolated and analysed by GC/MS and GC-olfactometry. Flavour dilution (FD) factors of the aroma active compounds determined by aroma extract dilution analyses were used for quantification of changes in aroma of cooked hen meat during the storage period. The FD factors of compounds responsible for "warmed-over" flavour in stored meat increased significantly while the FD factors of the most potent odourants with meaty character of aroma did not changed or only slightly decreased.

Keywords: volatile aroma compounds; wormed-over flavour; autoxidation of lipids; cooked hen meat

INTRODUCTION

Dehydrated hen meat is an important constituent of convenient soups formulations. It is usually produced by drying of pressure cooked hen meat. One part of that process is several hours storing period of the cooked and deboned hen meat at 5°C in the open vessels. It is known, that during such storing period, compounds responsible for the rancid, stale so called "warmed-over" flavour (WOF) can be formed in the meat. (1–3). They arise mainly during reheating of stored meat as products of oxidative cleavage of lipids. Aroma extract dilution analysis (AEDA) of volatile compounds isolated from freshly boiled and boiled, refrigerated and reheated chicken meat revealed, that the most potent odourants contributing to WOF are carbonyls such as hexanal, 1-octen-3-one, (E,E)-2,4-nonadienal and trans-4,5-epoxy-(E)-2-decenal (4). In the study (5) it was observed, that increase of concentration of the carbonyl compounds in 48 hours stored chicken meat was moreover accompanied with a loss of meaty, sweet and chicken-like aroma compounds such as 2-furfuryl thiol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3-hydroxy-4,5-dimethyl-3(2H)-furanone, and (E,E)-2,4-decadienal.

As similar undesirable processes in cooked hen meat during its storage and consecutive decreasing of organoleptic quality of dehydrated meat can be expected, the aim of this work was to compare the composition of aroma active compounds of fresh and stored pressure cooked hen meat.

EXPERIMENTAL

Chemicals. Diethyl ether was released from peroxides and rectified. Pure compounds (see Table 1): **3, 7, 14, 16, 20, 26, 28, 29** were obtained from Bedoukian Research Incorp., USA, **4, 5, 15, 19, 22** purchased from Aldrich (Steinheim, FRG)and **11** was synthesised after (6). Compounds **6, 18** were prepared as qualitative mixed reference material by model reactions of D-Ribose, D-Glucose and L-Cystein (120°C, 60 min, pH = 5.0 and 5.8).

Cooked hen meat. The hen meat, together with skin and bones was cooked at 119°C for 60 min in an adopted commercial pressure cooker. After cooling down to room temperature, the meat was deboned, homogenised and divided into two halves. The one half was immediately used for the isolation of volatiles and the second one was stored at 4–5°C for 24 hours in sealed bags, made from polyethylene with high rate of oxygen permeability. Before the isolation of volatiles the stored meat was reheated in the sealed bags at 80°C for 20min in a water bath.

Isolation of volatiles. Volatile aroma compounds of cooked meat were isolated by extraction-high vacuum distillation as it was described in (7).

Gas chromatography - Mass spectrometry (GC/MS). GC/MS analyses were performed on Hewlett-Packard HP 5971A mass-selective detector directly coupled to HP 5890II gas chromatograph equipped with fused silica capillary column Ultra 1 (Hewlett-Packard), 50 m × 0.2 mm ×

0.33 μm. The samples were injected splitless at 250°C injector temperature. The temperature program was from 30°C (1 min) to 250°C with a gradient of 1,7°C/min. The ionisation voltage (EI) was 70 eV.

Gas chromatography (GC) and aroma extract dilution analysis (AEDA). For the measurements of retention indices and GC-olfactometry analysis a Hewlett-Packard HP5890II gas chromatograph with FID and a sniffing port was used. Samples were injected splitless on a HP-Ultra 1, 50 m × 0.32 mm × 0.5 μm column with a temperature programme from 30°C (1 min) up to 250°C with gradient of 2°C/min and on a DB-WAX (J&W), 30 m × 0.32 mm × 0.5 μm column with a temperature program from 35°C (0.5 min) up to 250°C with gradient of 4°C/min. The linear velocity of carrier gas hydrogen was 36 cm/s for Ultra 1 and 30 cm/s for DB-WAX column (measured at 143°C). The linear retention indices (RI_p) were calculated according to (8). For AEDA (9) experiments the effluent of the column Ultra 1 was splitted with a split ratio of 1:1 to the FID and the sniffing port with addition of humidified air. The analysed samples were diluted stepwise from 1:4 up to 1:256.

RESULTS AND DISCUSSION

The total ion chromatograms of volatile compounds isolated from fresh and stored cooked hen meat with indication of peaks or retention times of aroma active compounds are shown in the Fig. 1. The 29 aroma active compounds were detected by GC olfactometry (AEDA) from which the most important 17 ones were identified (Table 1). As can be seen from the chromatograms, peaks of most of the aroma active compounds are not separated from other components or are too low for quantification by current methods. Therefore the contribution of the aroma active compounds to the overall aroma of the fresh and stored cooked hen meat was expressed by means of flavour dilution (FD) factors which are proportional to the compounds concentration. As can be seen from the table, considerable increase of FD factors for hexanal, 1-octen-3-one, (E)-2-octenal, (E,Z,)-2,6-nonadienal and (E,E)-2,4-nonadienal in the stored cooked meat was observed. The compounds were created during the 24 hours storage period as products of autooxidation of fats and are known as compounds influencing the flavour of cooked meat adversely by imparting of oxidised metallic off-flavour. These results are in good agreement with the works (4) and (5. On the other side the FD factors of aroma active compounds such as 2-methyl-3-furanthiol, 3-(methylthio) propanal, 2-furfuryl thiol, 2-acetyl-1-pyrroline, 4-hydroxy-2,5dimethyl-3(2H)-furanone, 2-ethyl-3,5-dimethyl pyrazine and (E,E)-2,4-decadienal did not changed or only slightly decreased. Somewhat more significant decrease of FD factors was observed at 4-hydroxy-5-methyl-3(2H)-furanone with caramel like aroma, an unknown compound **13** with chickeny, caramel like and an unknown compound **21** with sweet, pleasant aroma. All these above-mentioned com-

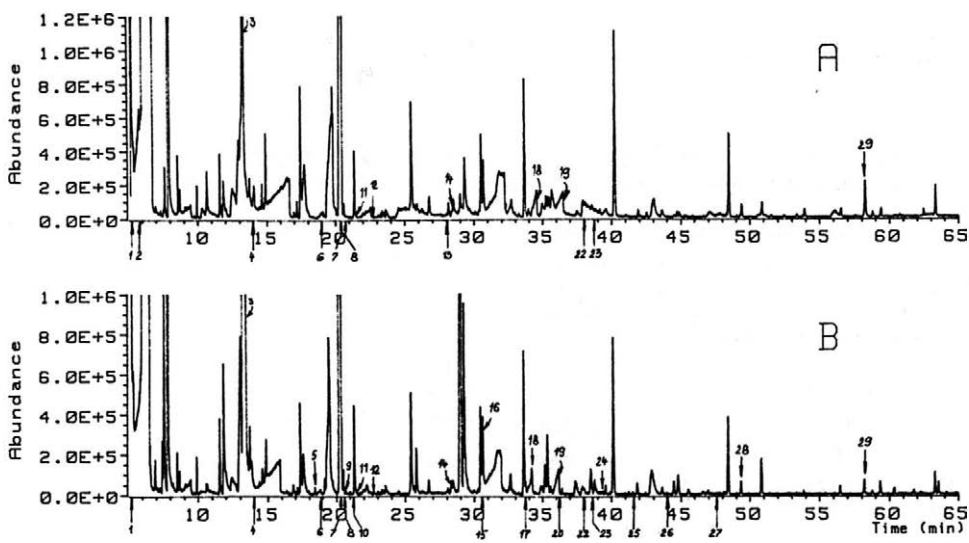

Figure 1. Total ion chromatograms of volatile aroma compounds of fresh (A) and stored (B) cooked hen meat; column Ultra 1, 50 m × 0.2 mm × 0.33 μm, the numbers of compounds correspond to Table 1

Table 1. Aroma active compounds (FD ≥ 4) of fresh and stored cooked hen meat

No.	Compound	RI_p		FD factor		Aroma character
		Ultra 1	DB –Wax	fresh	stored	
1	unknown	565	–	8	16	rotten, yeast, sweet
2	unknown	598	–	4	n.d.	onion
3	hexanal	774	1090	n.d.	64	green, herbal
4	butanoic acid	782	1645	8	4	unpleasant, rotten
5	3-methyl butanoic acid	837	1685	n.d.	4	spoiled yeast
6	2-methyl-3-furanthiol	844	1312	32	64	meaty, sweet
7	3-(methylthio) propanal	864	1465	128	128	cooked potato
8	unknown	867	–	8	8	solvent, petrol
9	unknown	869	–	n.d.	4	sweet
10	unknown	873	–	n.d.	8	gasoline
11	2-furfurylthiol	879	1447	256	256	roasted coffee
12	2-acetyl-1-pyrroline[1]	891	1349	16	16	roasty, musty
13	unknown	952	–	4	n.d.	chickeny, caramel
14	1-octen-3-one	954	1312	8	128	mushroom
15	2-ethyl-3-methyl pyrazine	977	–	n.d.	4	roasty, musty
16	octanal	979	1300	n.d.	4	fruity
17	unknown	1002	–	n.d.	4	musty, rotten
18	4-hydroxy-5-methyl-3(2H)-furanone	1007	2143	16	4	caramel, fried chicken
19	4-hydroxy-2,5-dimethyl-3(2H)-furanone	1028	2057	128	256	caramel, fried chicken
20	(E)-2-octenal	1029	1441	n.d.	8	cooked chicken
21	unknown	1056	–	16	n.d.	sweety, pleasant
22	2-etyl-3,5-dimethyl pyrazine	1058	1643	32	16	roasty, musty
23	unknown	1063	–	4	8	hydrolysed protein, spicy
24	unknown	1073	–	n.d.	4	garlic
25	unknown	1099	–	n.d.	4	rotten
26	(E,Z)-2,6-nonadienal	1123	1600	n.d.	8	cucumber
27	unknown	1163	–	n.d.	4	piny, terpenic
28	(E,E)-2,4-nonadienal	1183	1717	n.d.	16	rancid chicken fat
29	(E,E)-2,4-decadienal	1286	1827	64	32	fatty, chickeny

[1]Tentative identification

pounds, except of (E,E)-2,4-decadienal, are products of Maillard reactions and are responsible for meaty character of the aroma.

On the basis of these results it can be concluded, that it is possible partly to preserve original aroma of cooked hen meat by inhibiting of autooxidation processes of lipids either using a suitable antioxidant or by shortening of time between processes of cooking and dehydration of meat.

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F Food Processing, Additives and Contaminants

Dynamics of Reducing Sugar Changes in Scald of Rye Flour

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Abstract

The level of starch degradation in a rye scald is characterised by amount of reducing sugars. The dynamic of reducing sugar forming is analysed during the first two hours after hot water was added to rye meal. During this period continuous growth of reducing sugar is not observed -from 15th to 30th minutes it decreases. Using heat-stable enzyme preparation for a scald making in first 15 minutes rate of reducing sugar forming is much higher, but total content of reducing sugar in scald increases a little. Reducing sugar is determined by photometrycal method.

Keywords: rye flour; scald; sugar

INTRODUCTION

In Latvia scalded rye bread is traditionally used. Use of scalding improves the baking quality of the rye flour or meal and sensory qualities of rye bread. The longest technological stage in production of scalded rye bread (10–12 h) is scald making. In process of making the scald hot water was added to rye flour or meal. Starch was gelatininized and enzymes of rye flour cause partly degradation – hydrolyses of starch. One of the described factors of the starch degradation levels is reducing sugar – low-molecular dextrin, maltose and glucose. As more there are reducing sugar in the scald, as higher is the level of starch hydrolysis. The amount of reducing sugar in scald is 16–24% (1), it depends on amylase activity in the flour (2). In literature the dynamics of forming of reducing sugar is not described.

In research, the dynamics of forming of reducing sugar in the scald was analysed during the first two hours after hot water was added to rye meal. During this time the reducing sugar reached its maximum amount and the process of leavening starts. The rate of the forming of reducing sugar was also examined, if the heat-stable enzyme preparation was added to scald.

EXPERIMENTAL

Research was made in the Latvia University of Agriculture, Faculty of Food Technology.

Material

Rye meal (1998): moisture – 14.6%; Falling Number – 67.5; pH 6.54. Enzymes: Novo Nordisk A/S (Denmark) heat-stable enzyme preparations Termamyl 120L (activity – 120 KNV/g); BAN 240L and Fungamyl.

The Method of Scald Making

The first series of experiment. The scald of rye bread is made according to the following method: 3 portions hot water (90–96°C) is added to 1 portion of meal. Paste of meal and water was mixed up to homogeneous consistency. After adding hot water, the rye scald paste is let to cool down in two hours from 78-85°C to 30–33°C.

The second series of experiment. The scald was made according to traditional method, but heat-stable enzyme preparation in quantity of 0.1% from flour was added to fresh scalded meal. Produced scald paste was mixed up to homogeneous consistency and let to cool down.

Analysis of reducing sugars

Reducing sugar contains free aldehyde groups. The chemical analysis are based on free aldehyde groups reaction with $K_3[Fe(CN)_6]$ in alkaline medium. Reducing sugar was determined by photometrycal method (3). The more is reducing sugar in scald, the less is optical density.

RESULTS AND DISCUSSION

All experiments are repeated three times and the results are interpreted using method of least squares.

The first series of experiment *(scald without enzyme preparation).* The rate of reducing sugar changes is interpreted in a Fig. 1. During scald making without enzyme preparation additive, reducing sugar quantity in the first 15 minutes after hot water added increased up to 16.3% in dry matter (DM) in average. Then during the period from 15th to 30th minutes it decreased to 14.7% in dry matter in average. It is followed by steady increase of reducing sugar and after 2 hours it reached 19.0% in dry matter.

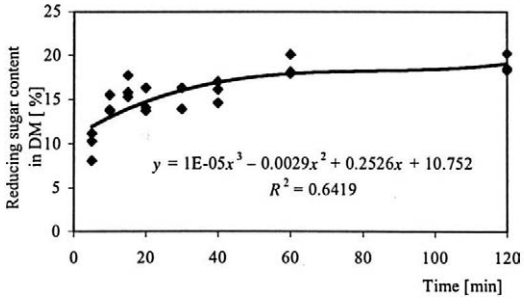

Figure 1. The rate of reducing sugar forming in the scalds without enzyme preparation

The second series of experiment *(scald with enzyme preparation).* In approximated experiments, several heat-stable enzyme preparation was used. The best results were gained with enzyme preparation Termamyl 120L. The rate of reducing sugar in the scalds with used enzyme preparation Termamyl 120L is interpreted in Fig. 2.

Making scald with enzyme Termamyl 120L almost twice higher rate of the reducing sugar growth in the first 15 minutes can be observed. The content of reducing sugars after 5 minutes is 16.3% average in DM; after 10 minutes – 20.4% and after 15 minutes – 24.1% in DM. Using ferment preparation 1 hour after adding of hot water, amount of reducing sugar reached almost its maximum (25.2% in DM).

Analyses of results. Using enzyme preparation for a scald making in first 15 minutes rate of reducing sugar forming is

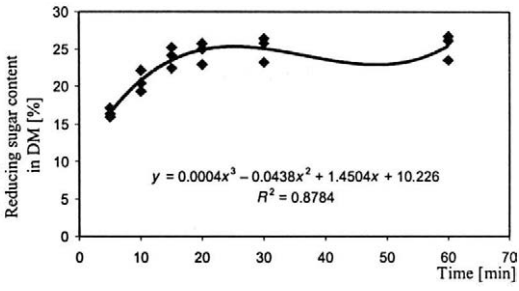

Figure 2. The rate of reducing sugar forming in the scalds with enzyme preparation Termamyl 120L

principally changed, but total content of reducing sugar in scald increases a little. It can be explained by the following, 15 minutes after adding of hot water, the temperature of scald paste is less than 70°C, which vitally lower enzyme activity. In the same time natural amylase activity is hindered by big amount of reducing sugar.

To make the scald without enzyme preparation between 15th and 30th minute after hot water was added for a scald making reduction of reducing sugar content was not observed.

The obtained results may be explained by the further reactions of chemically active aldehyde groups (for example, reversion sugar reaction or Amadori rearrangement reaction) in the first stage of a scald making. If a scald is made without enzyme additive, during the mentioned period, the rate of aldehyde groups transformation can be higher than number of aldehyde groups which were formed in starch hydrolysis.

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Changes of Selected Physical and Chemical Parameters of Beef Subjected to Electrical Stimulation and Storage

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Abstract

The influence of high-voltage electrical stimulation and storage on changes of electrical impedance, reactance, shear force and pH values of beef have been presented in this paper. Considering the changes of pH values as well as the impedance and reactance changes of the muscle examined, it was found that postmortem changes in electrically stimulated muscles occurred much faster than in non–stimulated ones. The changes of impedance and reactance, which were similar to the changes of maximum shear force of *longissimus dorsi* muscle examined during storage, indicate that the assessment as well as prediction of beef tenderness, is possible on the basis of fast impedance and reactance measurements.

Key words: electrical stimulation; beef; impedance; reactance; shear force

INTRODUCTION

Electric conductivity determining the ability of ions and polarised particles in food products to displace by applying alternating electric field shows the rate of the changes of cell walls, membrane as well as other elements of tissue structure. Thus, conductivity may be the factor both of the speed of biochemical changes occurring in meat during ripening and the index of its freshness. Pliquett *et al.* (4) stated that degradation of cell membrane is connected with wateriness as well as with optical brightening of meat tissue. This allows to distinguish three important indexes of meat quality: the condition of cell membrane, loss of cell sap, and the change of colour. Krala (3), who was studying the relation between electric properties of thigh muscles and effluent with selected factors of quality of chicken carcasses stored in a chiller, determined the index of freshness (Q) as a basis for assessment of quality of chilled chickens. Byrne *et al.* (1) pointed at the ability to assess and predict selected properties of beef: shear force, texture, tenderness, water absorption and colour by impedance and conductivity measurements.

The aim of the research was to develop a method of fast assessment of beef quality subjected to high-voltage electrical stimulation based on the changes of selected physical and chemical parameters of meat after slaughter as well as during storage.

MATERIALS AND METHODS

Longissimus dorsi (LD) muscle was experimental material removed from 8 heifer carcasses aged about 18 months 1 h after stunning.

a) Electric properties of *longissimus dorsi* muscle. LD-1 muscle was removed from left half carcass subjected to electrical stimulation with alternating current (330V, 17Hz and pulse duty factor 0.9). Muscle LD-2 was removed from right half carcass (non- stimulated). The muscles examined (LD-1, LD-2) were divided into 3 pieces about 500g each. Two of them were put into the glass containers equipped in the authorcs construction electrodes and then placed into a cooling chamber at about 2°C. In the time from 2 to 168 h after stunning the following measurements were taken: temperature, pH value, current intensity (for constant voltage of 2V and frequency from 20 to 20,000 Hz) as well as phase shift between voltage and current intensity values. On the basis of electrical measurements the impedance and reactance values were calculated.

b) Texture of *longissimus dorsi* muscle. The third piece of muscles (LD-1 and LD-2) was divided into 4 parts 125g each and stored at 2°C. During storage the pieces of muscle were being closed in hermetic metal tins and pasteurised at 72°C for 90 minutes after 6, 24, 72, 168 h after stunning.

Instrumental measurements of beef texture were conducted using an UTM Instron 4301. The shear test was made by Warner-Bratzler Meat Shear (type 2830-013) device. The speed of the head was 50 mm/min. The samples were rectangular prisms (10 × 10 × 30 mm) cut out parallel to the orientation of muscle fibres. The temperature of meat samples was about 21°C. The experiment was repeated eight times for each sample. The results were analysed by a computer Instron IX series, version 5.02 software (5, 2).

RESULTS AND DISCUSSION

The results of measurements and calculations showed that electric current destroying structure of meat during electrical stimulation caused worsening of electric conductivity of meat tissue and increased its impedance at average 20% in relation to impedance of non-stimulated muscles measured 2 h after stunning at frequency of 50Hz. Further measurements and calculations revealed that there are significant differences between the impedance changes of stimulated meat and the changes of impedance of non-stimulated meat. After about 12 h after stunning impedance of stimulated muscles samples were lower than the impedance of control samples on average 27%. In the time from 24 to 168 h after stunning, when the temperature stabilised at 2°C, impedance (measured at frequency of 50Hz) of stimulated muscles dropped at about 18 %, while non-stimulated muscles at about 38% (Fig. 1).

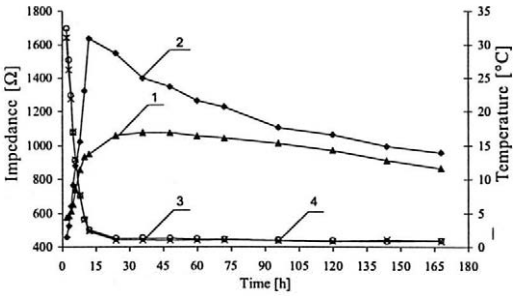

Figure 1. Changes of impedance and temperature of stimulated and non-stimulated beef in the time of storage, at frequency 50 Hz. 1 – impedance of stimulated beef, 2 – impedance of non-stimulated beef, 3 – temperature of stimulated beef, 4 – temperature of non-stimulated beef

Similarly to the impedance changes of examined uscles the reactance changes occurred. For higher measurement frequencies, both values and changes of impedance and reactance in the time of storage were lower. The results obtained acknowledge the thesis that for low frequencies, the cell membranes resistance is high and electric current flows only in extra-cellular space. The increase of frequency as well as changes of cell membrane properties during storage produce the increase of effective cross section of conductivity space. This results in decrease of total resistance (impedance) of meat tissue (3, 6).

Much faster rate of pH value decrease of stimulated muscles in comparison with non-stimulated muscles was observed during the first 12 h after stunning (Table 1).

The investigation of texture showed that from 24 to 168 h after stunning the value of maximum shear force of stimulated muscles decreased at about 21% while non-stimulated muscles at about 33% (Table 2).

Table 2. The influence of electrical stimulation and the storing time at temperature 2°C on the shear force of *longissimus dorsi* muscle drawn from heifer half carcasses 1 h after stunning

Experimental group		Stimulated samples – S		Control samples – K	
Statistical measure		$\bar{x}$	V (%)	$\bar{x}$	V (%)
Time of storing	6 h Shear force (N)	49.0	13.01	73.0	20.43
	24 h Shear force (N)	34.2	10.73	72.5	10.72
	72 h Shear force (N)	23.0	19.66	45.0	5.51
	168 h Shear force (N)	27.0	8.72	48.0	6.63

$\bar{x}$ – average value, V – coefficient of variation

CONCLUSIONS

1. The pH value of 5.54, measured 6 h after stunning, improvement of electrical conductivity of stimulated muscles, 12 h after stunning, as well as lower changes of impedance and reactance in electrically stimulated muscles, from 24 to 168 h after stunning, than non-stimulated muscles indicate that within the first 12 h after stunning the *postmortem* changes occur much faster in stimulated muscles than in non-stimulated ones.
2. The changes of maximum shear force, which are similar to the changes of impedance and reactance in the time of storage, indicate that there is ability to assess and predict beef tenderness (stimulated and non-stimulated) on the basis of fast electrical impedance and reactance measurements.

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Table 1. Changes of average pH value of beef in the time of storage

Time after stunning (h)	2	4	6	12	24	48	72	120	168
pH of stimulated beef	6.06	5.67	5.54	5.55	5.44	5.43	5.40	5.43	5.48
pH of non-stimulated beef	6.70	6.57	6.34	6.14	5.68	5.58	5.43	5.45	5.52

Changes of Selected Physical and Chemical Parameters of Raw Milk during Storage

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Abstract

Changes of electrical impedance, reactance, pH values as well as temperature of raw full fat and skim milk during storage at temperature 4°C and 20°C have been presented in this paper. Significant differences between impedance and reactance values of full fat milk and skim milk during storage at temperature 4°C and 20°C were shown. Also, the authorcs observed the changes of impedance and reactance of full fat milk and skim milk occurring parallel to the changes of pH values during storage. On the basis of the results obtained one stated that there is ability to develop a method of fast assessment of content of milk fat and its quality by measuring electrical impedance and reactance.

Keywords: milk quality; impedance; reactance; pH

INTRODUCTION

Nowadays electric current at low frequencies is applied to improve and assess raw milk quality. The using of electric current to improve quality of raw milk is known as a method of pulsed electric field (PEF). The applying of this method results in appreciable decrease in number of micro-organisms in milk. The results obtained prove that PEF method may be an alternative, non-thermal method of food preservation in future (1–3). The using of electric current for assessment of raw milk quality is difficult because the electric properties of raw milk are hardly known. Knowing these properties might be helpful to choose the parameters of electric current in the process of non-thermal milk pasteurisation (PEF) method). Also, this might be the basis to develop a method of fast assessment of raw milk quality immediately after milking as well as during storage (4).

The aim of the study was to determine the impedance and reactance changes of raw milk in dependence on frequency and the time of storage as well as to compare these changes with the changes of pH and point at the ability of practical using of the results.

MATERIALS AND METHODS

Raw, full fat milk 3.8% of fat and skim milk 0.01% of fat was the experimental material. The experimental milk was poured into the glass tanks equipped in the plate electrodes and stored at 4°C and 20°C for 96 h. In the first stage of research, to determine the optimum measurement frequency, one measured impedance and reactance of milk cooled at 4°C in the range of frequency from 20 Hz to 20 kHz. In the second stage, the changes of impedance, reactance, pH and temperature, both for full fat milk and skim milk during the time of storage, were measured for selected frequencies. To determine impedance and reactance of milk examined the following measurements were taken: current intensity (for constant voltage of 2 V) and phase shift between voltage and current intensity values. On the basis of the electrical measurements the impedance (Z) and reactance (X) were calculated using the following formulas:

$$Z = U/I \quad (1)$$

$$X = Z \sin \varphi \quad (2)$$

$$\sin \varphi = L_{y2}/L_{y1} \quad (3)$$

where: U – voltage, I – current intensity, sin – duty factor of reactive powers, L_{y2} and L_{y1} – oscilloscope readings

The measurements started six hours after milking at milk temperature about 20°C.

RESULTS

The results of the first research stage showed that the impedance and reactance values of skim and full fat milk decreased with the increase of current frequency. Simultaneously, one observed that these values were much higher for full fat milk. The greatest changes were observed in the range of frequency from 20 to 2000 Hz. Significant differences between impedance values of full fat milk and skim

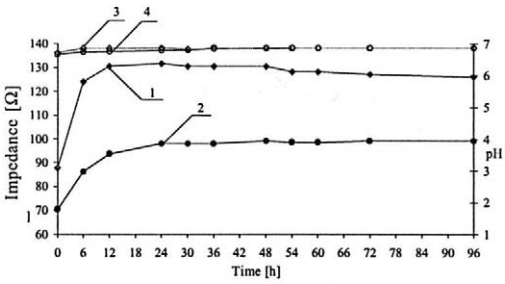

Figure 1. Changes of impedance and pH of full fat and skim raw milk in the time of storage in temperature 4°C, measured at frequency 20 Hz. 1 – impedance of full fat milk, 2 – impedance of skim milk, 3 – pH of full fat milk, 4 – pH of skim milk

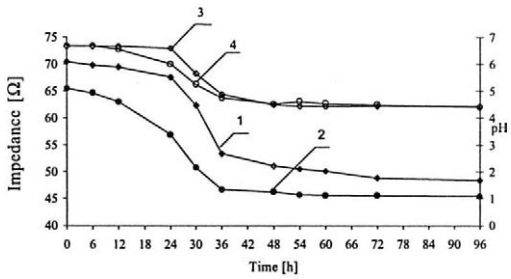

Figure 2. Changes of impedance and pH of full fat and skim raw milk in the time of storage in temperature 20°C, measured at frequency 50 Hz. 1 – impedance of full fat milk, 2 – impedance of skim milk, 3 – pH of full fat milk, 4 – pH of skim milk

milk were obtained for frequencies from 20 to 200 Hz. Also, significant differences between the reactance values were obtained for frequencies from 20 to 100 Hz and from 10 to 20 kHz. The highest differences between impedance and reactance values for full fat milk and between the values of impedance and reactance of skim milk were obtained for 20 Hz. On the basis of the results obtained one decided that in the second stage of research the measurements of milk impedance and reactance will be carried out at 20, 50, 10.000 Hz of frequency. The results obtained in the second stage ascertained the results of the first stage. The results showed that the impedance values of full fat milk stored at 4°C in the time from 12 to 96 h, measured at frequency 20 Hz, were higher than the impedance values of skim milk stored at the same temperature and at the same time at about 34 % (Fig. 1). The reactance values of full fat milk measured parallel were higher at about 100 %.

The slight (non-significant) changes of impedance, reactance, pH value and milk temperature during storage were observed. Similarly to milk stored at 4°C, significant differences between the impedance of full fat milk and skim milk as well as between the values of their reactance (measured at frequency of 50 Hz) were observed during storage at temperature of 20°C. One stated that simultaneously to decrease of pH values on average 27% in the time from 12 to 36 h, impedance value dropped at about 25% (Fig. 2). The reactance value dropped at the same time at about 38%. Also, bigger changes of impedance and pH of skim milk than changes of impedance and pH of full fat milk were observed during the time of storage from 12 to 24 h at temperature 20°C (Fig. 2).

CONCLUSIONS

1. The changes of impedance and reactance of milk examined in function of frequency testify its resistant-capacitance properties, which may affect the temperature of milk during the flow of electric current used for improvement and assessment of its quality.
2. The greatest significant changes between the impedance of full fat milk and impedance of skim milk as well as between the values of their reactance(for frequency of 20 Hz) allow to develop the fast method assessing fat content in milk based on measurements of impedance and reactance changes for this frequency.
3. The changes of impedance and reactance of milk stored at 20°C were parallel to the changes of pH value. This may indicate the possibility of using it for assessing and forecasting the quality of raw milk directly after milking as well as during storage.

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A Simplified Reaction Scheme for the Hydrogenation of Vegetable Oils

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Abstract

A new reaction scheme for the hydrogenation of vegetable oils taking into account saturation and *cis-trans* isomerization of double bonds and neglect their positional isomerization is presented. From the comparison of experimental and calculated kinetic runs, the rate constants of individual reaction steps are completed and the effects of hydrogen dispersion and oil unsaturation on the rate constants are examined. Criterion for extensive characterisation of the processes occurring in the course of hydrogenation, i.e., saturation and isomerization indices, are intended.

Keywords: hydrogenation; triacylglycerols; *cis-trans* isomerization; rate constants; kinetics; mechanism

INTRODUCTION

Horiuti and Polanyi (1) first proposed a mechanism for the hydrogenation of oleic acid catalysed with a nickel catalyst. The mechanism includes absorption of a double bond on the nickel catalyst, formation of the half-hydrogenated intermediate, formation of the saturated compound as well as positional and *cis-trans* isomerization of double bonds in fatty acids chains. The individual reaction steps are mostly reversible.

The original Horiuti-Polanyi reaction scheme involves 18 rate constants. To quantitatively inspect such a complex scheme and achieve reliable values of rate constants is a nearly unsolvable task. In this contribution an alternative kinetic scheme grounded on the explanation of the Horiuti-Polanyi mechanism is proposed. The scheme allows for hydrogenation and *cis-trans* isomerization of double bonds, whereas positional isomerization is neglected. By the simultaneous treatment of the kinetic runs for iodine value and *trans*-isomer content, the rate constants of specific reaction steps are obtained. The effects of hydrogen dispersion and oil unsaturation on the rate constants of individual kinetic steps are examined.

EXPERIMENTAL

Material. Industrial refined olive and sunflower oils with iodine values of 85.2. and 130.8 cg I_2 respectively, were used for hydrogenations catalysed with commercial nickel catalysts Nysel DM3 with Ni content 25% by wt. (Harshaw Chemie, The Netherlands). The fatty acid composition of oils determined by GLC method is as follows: palmitic acid 9.1 and 6.6% (for olive and sunflower oil), stearic acid 2.4 and 3.3%, oleic acid 78.7 and 24.2%, linoleic acid 9.8 and 65.9%.

Methods. Hydrogenations were carried out in two 250-ml glass reactors with free outlet of unreacted hydrogen. The reactors differed in hydrogen distribution. In the first type, hydrogen was introduced through a tube with inner diameter of 3 mm located at the bottom of the vessel so that the dispersion of hydrogen was poor. In the other type, with good dispersion, hydrogen was finely dispersed by bubbling it through the sintered glass bottom. A charge of 200 g of oil was used for each run, the content of the reactor was agitated by bubbling hydrogen. The flow rate of hydrogen was 75 l/h. The oil batch was heated at 180°C. After the oil reached this temperature, the catalyst was added in a concentration of 0.1% Ni/oil by wt. Hydrogenations were carried out to nearly zero iodine values and oil samples were taken from the reactor at chosen time intervals. The iodine values of filtered samples were determined by the Hanuš method (2). The *trans* isomer content was determined using a SPECORD 71 IR spectrometer (Carl Zeiss, Jena, Germany) according to the standard AOCS methods (3).

Theoretical. The simplification of the Horiuti-Polanyi reaction mechanism is based on the assumptions that the adsorption on and desorption of double bonds from the catalyst surface are not the rate-limiting steps, and that hydrogen chemisorption is so fast that its equilibrium is not affected by the hydrogen consumption in the course of hydrogenation. Initial Horiuti-Polanyi scheme shows that the isomerization and saturation of double bonds are not independent, both reactions start from the half-hydrogenated intermediate. Taking the neglect of positional iso-

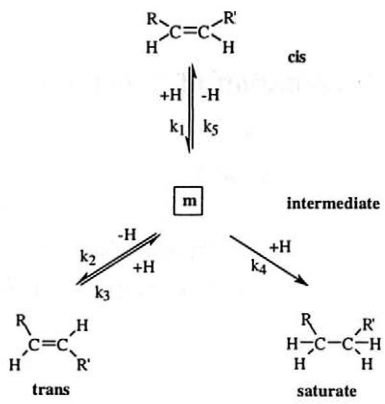

Figure 1. Simplified Horiuti-Polanyi reaction scheme

merization into account, the Horiuti-Polanyi mechanism can be reduced to the appearance as shown in Fig. 1.

The simplified reaction scheme contains only five rate constants. It can be described by the following set of kinetic equations (4):

$$dc_1/dt = - k_1c_1 + k_5m \quad [1]$$

$$dc_2/dt = k_2m - k_3c_2 \quad [2]$$

$$dc_3/dt = k_4m \quad [3]$$

where c_1 is the concentration of *cis* double bonds, c_2 is that of *trans* double bonds, c_3 means the concentration of saturated bonds formed in the course of hydrogenation, m is the concentration of the half-hydrogenated intermediate and t stands for time.

The concentration of the half-hydrogenated state can be expressed by the material balance:

$$m = c_1^0 - c_1 - c_2 - c_3 \quad [4]$$

where c_1^0 is the concentration of double bonds before hydrogenation (it is assumed that the oil contains solely *cis* double bonds before hydrogenation).

In calculations it is essential to manipulate with concentrations expressed in homogeneous units. Their expression in the moles of double bonds (*cis*-double, *trans*-double and saturated) per 100 g oil seems to be appropriate. As trielaidin contains three *trans* double bonds, the concentration c_2 is given as

$$c_2 = 3 \times (\%TI)/M \quad [5]$$

where %TI is the *trans* isomers content expressed in percent of trielaidin and M = 885.5 g mol^{-1} is the molar weight of trielaidin.

The concentration of saturated bonds formed in the course of hydrogenation can be calculated from the iodine values which indicate the advance of oil saturation:

$$c_3 = (IV_0 - IV)/M(I_2) \quad [6]$$

where IV_0 is the iodine value at the time t = 0, IV is the iodine value at time t and $M(I_2)$ = 253.8 g/mol is the molar weight of iodine.

The set of differential kinetic equations [1] through [3] is solved numerically by the fourth-order Runge-Kutta method for the initial conditions $c_1^0 = IV_0/M(I_2)$, $c_2^0 = 0$, $c_3^0 = 0$ at the time t = 0. The rate constants are obtained from kinetic runs by minimising the sums of squares of deviations between the calculated and experimental concentrations c_2 and c_3. The minimisation is carried out by the simplex method (5), the minimisation parameters are the values of the rate constants. Preliminary calculations showed that in all cases but one, the value of the rate constant k_5 was lower by about four orders than the other constants, thus indicating that the formation of the half-hydrogenated intermediate from a *cis* double bond can be treated as a simple first-order reaction. Therefore, it is put $k_5 = 0$ and the sum of squares is minimised with respect to the rate constants k_1, k_2, k_3 and k_4. The accuracy of iodine value determination is higher than that of the determination of *trans* isomers content. For this reason, in calculations the statistical weight of 2 is assigned to the kinetic curve c_3 and the weight of 1 to c_2.

RESULTS AND DISCUSSION

The experimental kinetic curves of iodine value and of *trans* isomer content are presented in Fig. 2. These experimental results were recalculated using equations [5] and [6], and the rate constants were obtained from these transformed experimental data by the non-linear least-squares method. The results of the calculations are given in Table 1.

The agreement reached between experimental and calculated curves is good which points out that the simplified Horiuti-Polanyi mechanism given by Fig. 1 adequately describes both oil saturation and *cis-trans* isomerization in the course of hydrogenation. Comparison of experimental and calculated kinetic runs for a case, which is neither the best nor the worst one is illustrated in Fig. 3.

The simplified reaction scheme follows the leading idea of the original Horiuti-Polanyi mechanism that the isomerization and saturation of oil start from the half-hydrogenated intermediate. The reactions in presented scheme are pseudo-unimolecular, the rate constants implicitly involve the concentration of hydrogen chemisorbed on the catalyst surface. Hence, their values should depend on the saturation of oil with hydrogen. The values of k_1, k_2 and k_4 should increase and k_3 should decrease with increasing hydrogen concentration in the mass of oil. As can be seen from Table 1, the values of rate constants hold this predicted trend for better hydrogen dispersion. The effect of dispersion is more pronounced, which is in agreement both with our observations and published results (6 ,7).

The values of rate constants offer the possibility to introduce criterion for a more extensive characterisation of the process of hydrogenation. An important characteris-

Table 1. Rate constants of hydrogenation of olive and sunflower oil

Type of oil	H_2 dispersion	k_1 [min^{-1}]	k_2 [min^{-1}]	k_3 [min^{-1}]	k_4 [min^{-1}]	Index saturation	Index isomerization
Olive	poor	$1.38.10^{-2}$	$9.72.10^{-2}$	$4.17.10^{-1}$	$4.59.10^{-2}$	$6.36.10^{-4}$	93.4
	good	$6.44.10^{-2}$	$1.26.10^{-1}$	$3.33.10^{-1}$	$1.08.10^{-1}$	$6.98.10^{-3}$	24.5
Sunflower	poor	$6.55.10^{-3}$	$8.57.10^{-2}$	$4.34.10^{-1}$	$4.89.10^{-2}$	$3.20.10^{-4}$	103.4
	good	$4.85.10^{-2}$	$5.69.10^{-2}$	$1.36.10^{-1}$	$8.34.10^{-2}$	$4.04.10^{-3}$	28.6

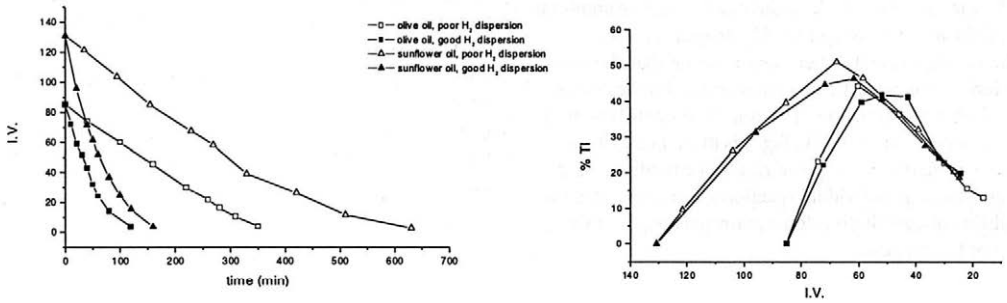

Figure 2. Dependence of iodine value and *trans* isomer on the hydrogenation of olive and sunflower oil

tic is the rate of double bonds saturation. As it is obvious from Fig. 1 this rate depends mainly on the partial rates of the half-hydrogenated intermediate formation and of the intermediate saturation. The greater the rate constants k_1 and k_4 are, the more rapid hydrogenation is. Hence the saturation index s, given as the product of both constants, provides quantitative estimation of the sensitivity of oil to hydrogenation:

$$S = k_1 k_4 \qquad [7]$$

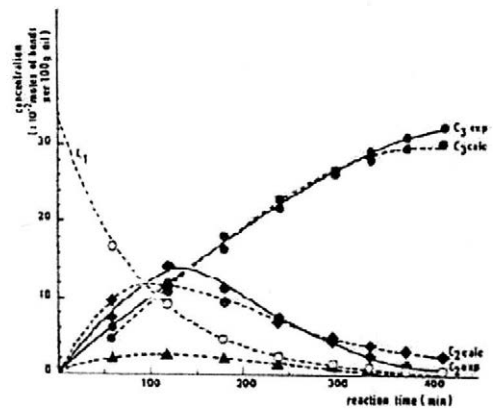

Figure 3. Experimental and calculated concentrations of saturated and isomerized double bonds in the hydrogenation of olive oil with poor dispersion of hydrogen

The formation of *trans* isomer proceeds rapidly if the rate constant k_3 is great and rate constants k_2 and k_4 are small. Then, the isomerization index i, allows us to see the tendency of oil to isomerize:

$$i = k_2/(k_3 k_4) \qquad [8]$$

This coefficient is an analogy of the specific isomerization index which gives the number of isomerized double bonds per a hydrogenated bond (8, 9). However, the specific hydrogenation index is a function of the conversion of hydrogenation (9); an advantage of the isomerization index expressed by equation [8] is that it is constant. From Table 1 it can be seen that s increases and i decreases when the concentration of hydrogen in the oil is higher.

For the same regimes of hydrogenation, the rate constants depend on oil unsaturation. In the series olive-sunflower oils the values of k_1, k_2 and k_3 decrease and k_4 remains constant or increases slightly, the index s decreases and i increases. As you can see, the reason for these alterations lies in the fact that the original Horiuti-Polanyi mechanism has been found for monoenoic oleic acid. From the oils studied here, only olive oil is monoenoic, sunflower oil includes mostly dienoic fatty acids. In the presence of nickel catalyst, the C = C bonds in polyenoic acids are hydrogenated consecutively, not simultaneously. The double bonds approach the catalyst surface gradually, one by one, which is manifested in the mentioned decrease of reaction constants in comparison with the monoenoic oil when the experimental data are treated using our reaction scheme. As it is obvious from the values of

isomerization indices, the polyenoic oils exhibit greater tendency to isomerize. It is obviously due to a greater number of reaction paths leading to the decay of the half-hydrogenated state to *trans* acids.

The catalyst activity is determined mainly by the rate of formation of the halfhydrogenated state. It is a matter of course that this rate implicitly involves the diffusion and adsorption of oil on the catalyst surface. Accordingly, any method of the diffusion, oil adsorption and the half-hydrogenated intermediate formation can be definitive for the catalyst activity. The fact that the activity is not determined by the rate constant k_4 indicates that the assumption of equilibrium chemisorption of hydrogen is correct.

The results presented here demonstrate that the reduced Horiuti-Polanyi scheme is appropriate for the description of oil hydrogenation. The principal contribution of reaction scheme illustrated in Fig. 1 is that it makes it possible to analyse the influence of reaction conditions on the rate constants of individual reaction steps and offers the possibility of quantitative description and computer simulation of the process.

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Influence of Germination and Microwave Heating on the Carbohydrates Composition of Pea

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Abstract

Effect of germination and microwave heating is the resulting way of the improvement of nutritional quality of legume products – the significant reduction of α-galactooligosaccharides. The most important influence for decreasing of a-galactooligosaccharides is during the first three days of pea germination. Combined effect of germination and the following microwave treatment and the conventional air drying to final moisture content 12–14% and the maximum temperature 80°C is recommended for improving of carbohydrate composition of pea from nutritional point of view.

Keywords: α-galactooligosaccharides; pea; germination; microwave treatment

INTRODUCTION

The problem of flatulence causing oligosaccharides (α-galactosides, raffinose family oligosaccharides – RFO) in legumes is one of the general phenomena, which have influence on the consumption and use of legumes in human and animal nutrition. Germination is one of the ways of processing, which allows reduction of antinutritional factors, such as α-galactosides and affects the nutritive value of legumes. In literature there are many papers about evaluation of the effect of germination on the composition of various grain legumes, chiefly lentils and lupine seeds (1–3). In our previous paper was evaluated that germination of pea can reduce content of a-galactooligosacharides to 17.5% of the original value (4).

EXPERIMENTAL

Material. Samples of pea (*Pisum sativum* ssp. *sativum* L.), five cultivars (Grana, Merkur, Lantra, Primus, Profi), each cultivar from three various breeding farms in Czech Republic. Samples of pea were supplied by Central Institute for Supervising and Testing in Agriculture at Brno.

Methods

Determination of dry matter content. Dry matter content (%) of grinded seeds was determined after drying at 100°C to constant weight on HA 300 Moisture Balance (Precisa, Switzerland), reproducibility 0.02%.

Extraction and assay of soluble carbohydrates. 2 g of grinded sample was homogenised in 20 ml of ethanol:water (80:20, v/v), refluxed (boiled) for 60 min. After boiling extract was diluted by demineralised water, filtered through a membrane filter 0.45 μm pore size and analysed by HPLC.

HPLC determination. The identification and quantification of monosaccharides (glucose, fructose and galactose), sucrose and α-galactooligosaccharides (RFO - raffinose, stachyose and verbascose) contents were carried out using HPLC chromatography as described by Kvasnička *et al.* (5).

Germination. Seeds were incubated in open plates in distilled water in a relation 300 g of seeds to 150 g of water at ambient temperature and pH 7.0. Times of germination were: 24, 48, 72, 96 hrs.

Parameters of microwave (MW) oven. Whirlpool MT 243/UKM 347, frequency 2450 MHz, MW power: 90, 160, 350, 500, 650, 750, 850, 1000 W, inner cavity volume 25.4 L.

Methodology of MW measurement. Before each measurement the MW oven was prewarmed by heating 2 L of water for 5 min and the absorbed power according to IEC test was determined. 200 g of wet germinated pea was heated in MW oven, power output 350 W was stopped at 80°C. Between each measurement in MW oven was break 30 min.

Drying. Microwave treated germinated pea was dried in laboratory fan assisted dry air oven at 80°C to final moisture content 12–14%.

RESULTS AND DISCUSSION

Three days of germination is for decreasing of RFO quite sufficient time (Fig. 1). Changes in composition of so-

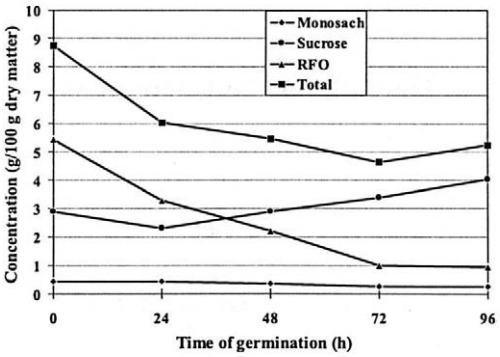

Figure 1. Changes in composition of soluble carbohydrates during germination

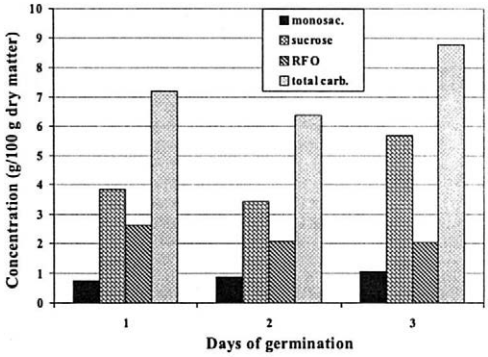

Figure 2. Changes in composition of carbohydrates after MW treatment and drying of germinated pea

luble carbohydrates of germinated pea are favourable from nutritional point of view, a-galactooligosaccharides are decreased, galactose is metabolised, and content of sucrose increases. Changes in composition of carbohydrates after MW heating and conventional drying of germinated pea (cultivar Profi, breeding farm Caslav) are demonstrated in Fig. 2. From this figure is possible to compare the changes after 1, 2 and 3 days of germination. During MW treatment and conventional drying is decrease of a-galactooligosaccharides very slow, because temperature 80°C causes inactivation of enzymes, which were prior to activated by germination. The effect of MW treatment is possible to use for heating of wet germinated seeds (moisture content 31–50%) first of all, because the moist heat is more effective than dry heat. Higher temperature of sample after MW treatment represents more economical the following conventional drying. According to these results there is possible to propose the following way of pea processing: 1) 72 hrs of germination, 2) MW heating of wet germinated seeds, stopped at 80°C, 3) conventional drying by hot air to the final moisture 12–14%, 4) grinding and milling of dried seeds, 5) preparation of meal from the modified pea flour. Combination effect of germination, microwave treatment and drying by hot air to the final moisture 12–14% and the highest temperature up to 80°C can be recommended as process to decreasing of high content of a-galactooligosaccharides and improving of nutritional quality. At both domestic and industrial scales is possible to prepare by this way the good quality pea meal or fresh salads for human nutrition uses.

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Chemical Changes during Microwave Treatment of Rice

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Abstract

The effect of microwave treatment of soaked rice is studied in this paper. The soaked rice was treated in microwave (MW) oven at the different processing microwave energy level (maximum 500 W, minimum 90 W), the initial moisture content (10 to 30%) and time of treatment. The temperature measurement of treated sample during microwave heating was carried out by means of NoEMI fiber-optic temperature system. For evaluation of microwave heating to physicochemical properties of rice were measured alkali spreading values of rice and surface structure by means the Laboratory Universal Computer Image Analysis – LUCIA G system.

Keywords: rice; water sorption; microwave treatment; image analysis

INTRODUCTION

The conventional rice parboiling process consists of soaking rice in hot water, draining the water, steaming the soaked rice to gelatinize the starch and drying of the parboiled product (1). Some steps of conventional processing of parboiled, expanded and flaked rice, as steaming and drying, can be replaced by microwave treatment.

EXPERIMENTAL

Methods

Determination of starch gelatinization temperature. Alkali spreading values is one of the simplest and quicker tests for degradation of rice grains. Six grains of rice is incubating in 10 ml of 1.7% KOH at room temperature for 23 hrs and measuring the degree of spreading using a seven-point scale as follows: 1 – grain not affected, 2 – grain swollen, 3 – grain swollen, collar incomplete and narrow, 4 – grain swollen, collar complete and wide, 5 – grain split or segmented, collar complete and wide, 6 – grain dispersed, merging with collar, 7 – grain completely dispersed and intermingled. Alkali spreading values correspond to gelatinization temperature as follows: 1–2: high 74.5–80°C; 3: high-intermediate; 4–5: intermediate 70-74°C; 6–7: low, below 70°C (2).

Image Analysis. Imaging system LUCIA G is Laboratory Universal Computer Image Analysis, product of LABORATORY IMAGING Co., Prague, Czech Republic. The main characteristics of this image processing and analysis systems there are: reliable customised solutions for scientific, biomedical, forensic and industrial imaging; true colour version; 3-chip RGB TV camera; supporting operating systems Windows 3.x, Windows 95, 98, Windows NT 4.0; supporting grabbers are Matrox Magic, Matrox Comet and VGA; wide spectrum of different objectives, microscopes, cameras and lighting systems enables the measurement of particles with different size range from several centimeters to microns; software contents large amount of tools for adjustment and arrangement the image into the form suitable for optimal computer analysis; system was applied for many measurements of particles in the food and chemical industry (3).

Parameters of microwave (MW) oven. Whirlpool MT 243/UKM 347, frequency 2450 MHz, MW power: 90, 160, 350, 500, 650, 750, 850, 1000 W, inner cavity volume 25.4 L.

Temperature measurement. The temperatures of samples during microwave heating were recorded using NoEMI fiber-optic temperature system - table-top unit ReFlex, with 2 channels, firm Nortech Fibronic Inc, Canada. The general characteristics: ultra-fast miniprobe general purpose, temperature range -40 to +250°C, response time 0.25 s, computer interface RS-232-C, data logging function, analog output 0–20 mA.

Methodology and conditions of MW measurement. Before each measurement the MW oven was prewarmed by heating 2 L of water for 5 min and the absorbed power according to IEC test was determined. 200 g of rice (in open plastic rectangular vessel, dimensions 165 × 112 mm) was heated in MW oven; power output was changed from 90 to 160, 350 and 500 W; final temperature of heating was 40, 60, 80°C; initial moisture content of rice was 11, 21 and 30%. Between each measurement in MW oven was break 30 min. The temperature of rice during microwave heating

Table 1. Characterization of microwave treatment of rice

Number of measurement	Temperature of MW heating (°C)	Original moisture content (%)	Absorbed MW energy (W*s)	Relative frequency of alkali spreading values for gelatinization temperature 70–74°C (%)	below 70°C (%)
37	original rice	11.90	0	72	28
3	40	11.89	5431	72	28
2	40	21.10	10241	72	28
15	60	11.93	13304	67	33
1	40	29.02	13965	61	39
14	60	22.68	25304	67	33
36	80	12.12	28644	78	22
13	60	28.58	34434	50	50
35	80	21.43	47124	39	61
34	80	28.79	52668	39	61

increased rapidly as a result of exposure of microwave energy, but little drying occurred during this time. After reaching of given temperature, MW power was switch off and sample of rice was retained till 1 min in the MW cavity. Then vessel with sample was mixed and cooled to ambient air outside of MW oven (1 min as well) and go back to the MW oven. This cycle of heating and mixing with cooling was repeated five times.

RESULTS AND DISCUSSION

Moistening of rice. The moisture content of original sample is one of the most important parameter for MW treatment. Various moisture contents of rice were prepared by soaking in water. The starch granules of rice must absorb water and swell and then be heated rapidly to ensure complete gelatinization, i.e. conversion of hydrogen bonding among starch micelles into hydrogen bonding with water. Fig. 1 shows the course of rice soaking (4). The excess of water was drained off on filter paper and dried 20 min on air at ambient temperature.

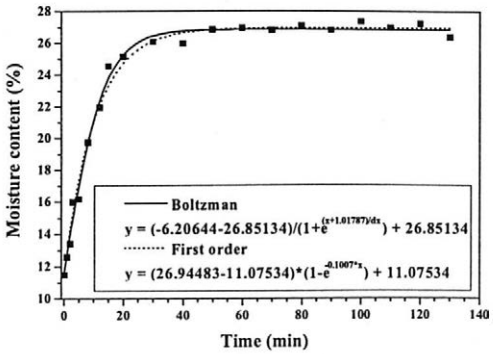

Figure 1. Course of soaking rice at temperature 25°C

Starch gelatinization temperature. As an indicator, if gelatinization temperature was reached during MW heating, alkali spreading value was used. Some chosed results, altogether with initial moisture content of rice and basic characterization of MW treatment, are summarised in Table 1 (4).

The low alkali spreading values (grain completely dispersed and intermingled), corresponding to the gelatinization temperature below 70°C, were observed for samples absorbing MW energy higher than 30 000 W*s, at temperature 80°C, moisture content 21 and 29% and at temperature 60°C, moisture content 29%. The others samples were in range of intermediate, it means the gelatinization temperature 70–74°C.

The gelatinization of rice starch is coming on quicker at MW treatment than during conventional treatment (cooking, steaming) and one of the main limiting factors for exposure times of MW treatment is the initial moisture content of rice. If it is necessary to increase the exposure time for MW treatment, the initial moisture content of the sample must be increased. The moist heat is more effective than dry heat.

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Inositol Phosphates in Raw and Extruded Cereals

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Abstract

Content of inositol phosphates in wheat, barley, rye and oat grown in 1997 and 1998 was determined, and changes of these compounds during extrusion cooking conducted in different temperatures conditions were studied. The content of inositol phosphates in cereal grains, and in their morphological elements showed high diversity within the species in two consecutive years. Extrusion cooking caused degradation of inositol phosphates in the analysed species from 8% in wheat to 31% in barley, and no links between the process temperature and degradation of inositol hexaphosphate were observed.

Keywords: cereal grains; inositol phosphates; hydrothermal process

INTRODUCTION

Cereals have a large share in a human diet and cover about 1/3 of the daily requirement for energy. Cereals are characterized by a significant nutritive value and the presence of biologically active compounds including inositol phosphates (4). Most of the food technologists and nutritionists consider inositol hexaphosphates (IP-6), as unfavourable components of human diet. The formation of insoluble complexes by these compounds with nutrients is considered as a disadvantage, making them unavailable for the organism (5, 7). However, with a proper diet, the formation of complexes may positively influence the human organism through, for example binding the metals with prooxidative properties (iron), thus preventing the formation of free radicals – the cause of many diseases, and increasing the amount of diet ballast components (1, 2).

The objective of this work was the determination of the inositol phosphates presence in grains of cereals cultivated in Poland and in their morphological elements, as well as the examination of the effect of a thermal process – extrusion cooking – on those compounds.

EXPERIMENTAL

Material. Cereal grain samples grown in 1997 and 1998 were obtained from the local plant breeding station in the North-East Poland. Wheat cv. Almari and cv. Henika, barley cv. Gregor and cv. Mobek, rye cv. Dankowskie Zlote and oat cv. Sławko were used in this study.

Hydrothermal treatment. The extrusion process was conducted using a twin-screw laboratory extruder 2S-9/5 (type 2S-9/5 Metalchem, Poland). The process was carried out on disintegrated cereal kernels of the moisture content reaching 20%. The following profiles of barrel temperatures were used: 80/100/120/120, 100/130/160/160, 120/160/200/200, 500 rpm screw speed, and 225g/min mass flow rate. The raw and extruded material was ground in a laboratory mill type WZ-1. Ground samples were stored at –30°C until chemical analysis.

Determination of inositol phosphates (IP-3–IP-6) by HPLC. Ground raw and extruded material was extracted with 20 mL HCl (0.5 mol/L) for 5 h using a BMM1 magnetic stirrer. The extract was centrifuged at 3500 g for 40 min (Centrifuge MPW-360) and the supernatant was decanted, frozen overnight (–18°C), thawed at room temperature and recentrifuged at 3500 g for 40 min. The supernatant (15 mL) was vacuum evaporated to dryness at 40°C and dissolved in 15 mL of 0.025 mol/L HCl. The samples were then transferred to the mini-columns filled with Dowex AG 1-X8 resin, from which the inositol phosphates were eluted using 2 mol/L HCl (5 × 4 mL). After the solvent had been removed by evaporation, the dry residue was dissolved in a mixture of methanol, 0.05 mol/L formic acid and 1.5 mL/100 mL TBA-OH and analysed by HPLC according to the Sandberg and Ahderinne (9) and Sandberg *et al.* (10) methods using a Shimadzu chromatograph (LC-10 AD pump, refractometric detector RID-6A, CTO 6A column oven) and a Nova-Pak C_{18} column. The mobile phase was a mixture of methanol, 0.05 mol/L formic acid (51/49), v/v) and 1.5 mL/100 mL TBA-OH. The flow rate was 0.4 mL/min. Sodium phytate (Sigma) was the external standard and the injections were made with a 20-μL loop.

RESULTS AND DISCUSSION

The qualitative and quantitative analyses of inositol phosphates were carried out on the kernels of wheat, bar-

ley, rye and oat collected from the harvests in 1997–1998. In the material harvested in 1997, only the presence of inositol hexaphosphate IP-6 (from 4.3 mg/g in rye to 12.8 mg/g in wheat) was noted (Table 1), as compared to the material harvested in 1998 which, apart from IP-6 (from 7.3 mg/g in barley to 17.6 mg/g in buckwheat), contained

Table 1. Concentration of inositol phosphates in whole-grains and their morphological elements grown in 1997

Source	Inositol hexaphosphate IP-6 [mg/g d.m.]	Source	Inositol hexaphosphate IP-6 [mg/g d.m.]
Wheat cv. Almari		Barley cv. Mobek	
– whole grain	9.90∀0.24	– whole grain	4.9∀0.46
– pericarb and testa fraction	2.13∀0.14	– dehulled grain	5.5∀0.95
– endosperm with embryo	10.82∀0.26	– hull	n.d.
		– pericarb and testa fraction	n.d.
		– endosperm with embryo	5.7∀0.36
Wheat cv. Henika		Rye cv. Dankowskie Zlote	
– whole grain	12.80∀0.17	– whole grain	4.3∀1.85
– pericarb and testa fraction	2.09∀0.17	– pericarb and testa fraction	5.7∀1.16
– endosperm with embryo	13.81∀0.37	– endosperm with embryo	4.2∀0.71
Barley cv. Gregor		Oat cv. Slawko	
– whole grain	6.9∀0.40	– whole grain	10.4∀1.31
– dehulled grain	7.1∀1.24	– dehulled grain	10.9∀0.85
– hull	n.d.	– hull	n.d.
– pericarb and testa fraction	n.d.	– pericarb and testa fraction	16.0∀2.11
– endosperm with embryo	7.6∀0.80	– endosperm with embryo	10.2∀1.11

Table 2. Concentration of inositol phosphates in raw and extruded whole-grains grown in1998

Source		Inositol phosphates [mg/g d.m.]			
		IP-3	IP-4	IP-5	IP-6
Wheat cv. Almari		tr	tr	0.1∀0.00	9.6∀0.68
after extrusion	120°C	tr	0.1∀0.00	tr	8.8∀0.21
	160°C	tr	tr	0.1∀0.02	9.2∀0.37
	200°C	0.1∀0.01	0.1∀0.02	0.1∀0.02	8.4∀0.59
Wheat cv. Henika		tr	tr	0.2∀0.00	15.1∀0.96
after extrusion	120°C	0.1∀0.01	0.1∀0.01	tr	11.3∀0.21
	160°C	tr	0.1∀0.02	tr	13.9∀0.65
	200°C	tr	0.1∀0.01	0.1∀0.00	12.4∀0.87
Barley cv. Gregor		tr	0.1∀0.01	0.1∀0.00	7.1∀0.55
after extrusion	120°C	tr	tr	tr	5.0∀0.41
	160°C	0.1∀0.01	0.1∀0.00	0.1∀0.01	5.8∀0.36
	200°C	0.1∀0.00	0.1∀0.02	tr	6.6∀0.36
Barley cv. Mobek		tr	0.1∀0.02	0.2∀0.02	7.9∀0.94
after extrusion 120°C		0.1∀0.00	0.1∀0.01	0.1∀0.01	7.2∀0.82
160°C		tr	0.1∀0.01	tr	5.9∀0.43
200°C		tr	0.1∀0.02	tr	4.0∀0.75
Rye cv. Dankowskie Zlote		tr	0.1∀0.01	0.2∀0.02	10.9∀1.20
after extrusion	120°C	tr	tr	0.1∀0.00	9.5∀0.54
	160°C	tr	0.1∀0.01	0.1∀0.01	10.1∀0.32
	200°C	n.d.	tr	tr	8.0∀0.60
Oat cv. Slawko		n.d.	tr	0.1∀0.01	10.2∀0.91
after extrusion	120°C	n.d.	tr	0.1∀0.00	8.6∀0.30
	200°C	n.d.	tr	tr	8.7∀0.50

n.d. – not detected

also trace amounts of inositol pentaphosphate IP-5 (0.1–0.4 mg/g) and inositol tetraphosphate IP-4 (0.1 mg/g) (Table 2).

Also a high diversity in the composition of those compounds within the same species in two consecutive years was shown. It proves that the content of inositol phosphates can change depending on the weather conditions and fertilization (8). The highest amount of inositol hexaphosphate was found in the endosperm with embryo fractions in wheat and barley, and in pericarb and testa fractions in rye and oat. (Table1). The accumulation site of inositol hexaphosphate in cereals is in the electron-dense aleurone particles, which are located in the outside layer of endosperm – aleurone layer (6). The IP-6 presence in pericarb and testa fractions was due to the inositol phosphates transfer to this part during separation of pericarb and testa fraction from endosperm.

Extrusion cooking caused degradation of inositol hexaphosphates (from 8% in wheat to 31% in barley); no links were observed between the process temperature and degradation of IP-6 (Table 2). The obtained results proved, that IP-6 belongs to thermostable compounds (6). Other authors also reported small degrees of IP-6 degradation during extrusion. Gualberto et al. (3) found that 25% and 13% of IP-6 was degraded during extrusion of oat and wheat, respectively, and did not find any changes in the IP-6 content during extrusion of rice. The degradation of IP-6 during extrusion caused generation of inositol pentaphosphate (IP-5), inositol tetraphosphate (IP-4) and inositol triphosphate (IP-3), but their amounts did not overcome 0.2 mg/g (IP-5) and 0.1 mg/g (IP-4 and IP-3). The presence of IP-3 was noted only in the case of extruded barley and wheat.

Inositol phosphates present in extrusion cooked cereals, commonly used as a food, can be considered as positive bioactive components.

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Modification of Flour Microstructure through the Baking Process

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Abstract

Food materials can be characterised by chemical and physical properties but also by analysing their three dimensional organisation. The microstructure gives information about the interactions among molecules and any process that modify these relationships also changes the microstructure. In this research, the modification of the microstructure through the bread-making process has been determined by using the cryo scanning electron microscopy (cryo-SEM). Cryo-SEM micrographs of wheat flour, dough and bread crumb are presented. The population of starch granules have been characterised and also their behaviour during the milling and fermentation stages have been analysed. Special attention has been paid to the microstructure of bread crumb in order to relate it to any attributes of bread quality.

Key words: Cryo-SEM; microstructure; wheat; flour; dough; bread

INTRODUCTION

Bread-making constitutes a dynamic process implying different physical, chemical and biological modifications of the flour through the mixing, fermentation and baking steps. Consequently, the initial microstructure of the wheat flour is transformed into a cohesive visco-elastic dough when mixed with water, and the foam structure after the fermentation step is shifted to an open sponge structure at the end of the baking stage. Therefore a deep analysis of the flour at the microstructure level during the process should provide a useful information to understand bread-making. Different approaches to the microstructure of cereal food samples have been done by using light microscopy (1–3), transmission electron microscopy (TEM) (1, 3–4), freeze-fracture (5), and scanning electron microscopy (SEM) (1–2, 6–8). Among them, the scanning electron microscopy (SEM) with a previous cryo-preparation of the sample, i.e. cryo-SEM, seems to be an appropriate means for identifying the physical properties and textural characteristics of the food samples. The main reasons that make cryo-SEM a very valuable tool for examination of the flour-bread transformation include its very large depth of focus and the possibility of obtaining three-dimensional images of sample surfaces with minimal preparation (7).

The aim of this study was to describe the modifications of flour macromolecules organisation from wheat flour to bread, including mixing, fermentation and baking processes, followed by cryo-SEM.

EXPERIMENTAL

Material. Commercial wheat grains from the local market were used for the analysis.

Methods. All doughs for cryo-SEM analysis and for bread-making were prepared in a Brabender farinograph (Duisburg, Germany). A straight dough process was followed for bread-making. Dough was divided in 450g pieces, hand-moulded and placed in pans for a 2.5hours proofing before baking. Breads cooled for 1hour were placed in polypropylene bags during 24 hours at 24°C, and a crumb sample from the centre of the slice was taken for a further microscopic analysis.

Cryo-SEM Analysis. A Jeol JSM-5410 SEM equipped with a CT-1500C cryo-unit (Oxford Instruments) was used. Sample was prepared as described earlier (9). Briefly, the sample was placed on the cryo-specimen holder, and cryo-fixed in slush nitrogen (T L –210°C), transferred to the cryo-unit in the frozen state, fractured, sublimated (15 minutes at –90°C) and sputter coated with gold (4 minutes 2 mbar). Wheat kernel and bread crumb samples were carefully cut before cryo-fixation in slush nitrogen. Finally, sample transferred into the microscope was observed at 15 kV and –130°C.

RESULTS AND DISCUSSION

The endosperm constitutes the great part of the wheat grain (90% of the total grain), and it is mainly formed by starchy granules and proteins. The microstructure of whe-

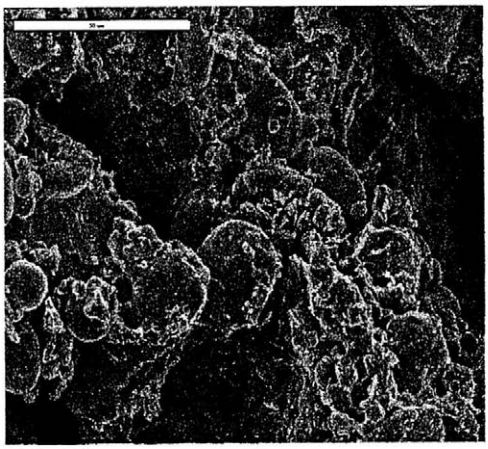

Figure 1. Cryo-SEM micrograph of wheat flour (1000×)

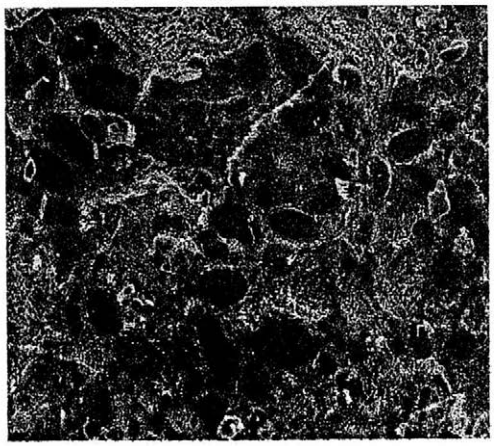

Figure 2. Micrograph of a flour-water dough (1000×)

at flour (Fig. 1) showed a compact organisation of cells disrupted as a consequence of the physical damage promoted by the milling process. Two populations of starch granules (lenticular and spherical) could be distinguished and also some deformed starch granules due to the mechanical process of milling. The proteins were also observed in the microstructure acting as cement material among starchy compounds.

The baking process produces a profound modification of the flour microstructure, since it is a dynamic system which undergoes the physico-chemical changes promoted by both the mechanical processes (mixing, rounding and moulding) and the chemical modifications due to endogenous enzyme activities and yeast action.

The addition of water and a further mixing yielded a reticular microstructure with the starch granules buried in the network of proteins and soluble solutes (Fig. 2). The network aspect was resulted of the sublimation applied for the sample performance. The two different populations of starch granules were also observed in this micrograph.

After the fermentation stage it was obtained a more dense structure than the flour-water dough, likely due to the presence of the other ingredients (yeast, salt, additives). In addition, the compounds were better distributed through the matrix as it is depicted in Fig. 3. The proteins and soluble solutes were more firmly adhered to the starch granules, which appeared as intact granules without any deformation since the fermentation occurred below the gelatinisation temperature. The α-amylase activity was patent in some starch granules, these showed a perforated zone in the centre corresponding to the hilum zone, which is the more accessible part of the granule. It is wide known that a-amylases act particularly on the gelanised starch but they are also able to hydrolyse damaged starch.

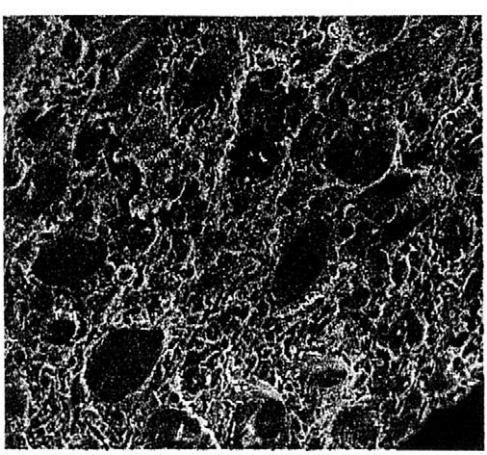

Figure 3. Micrograph of a fermented dough (1000×)

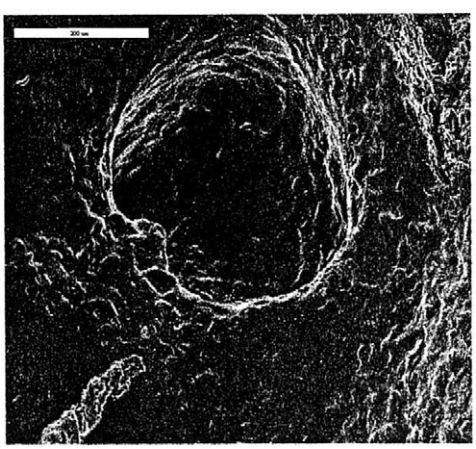

Figure 4. Micrograph of bread crumb (200×)

Dough structure becomes fixed after the baking process. In the oven the starch granules are gelatinised, proteins are denatured and soluble solutes dehydrated when water drifted to the outside part and it is evaporated. All these changes promoted by the temperature yielded an opened sponge microstructure with numerous alveolos. The alveolar surface (Fig. 4) showed a rugged appearance, in a manner of a fine film covering different structures. These were remaining starch granules partially gelatinised.

From the above, it could be concluded that cryo-SEM provides a valuable tool for following food modification through the analysis of the compounds organisation or microstructure.

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The Production of Agglomerates in Milk and White Chocolate

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Abstract

The agglomerate production during conching or storage of melted milk and white chocolate leads to deterioration of the sensory properties of products. The crystallization of amorphous lactose initiates this process and crystals of lactose together with crystals of saccharose are nuclei of agglomerates, i.e. clusters. The composition and size of clusters was analysed by X-ray diffraction, polarising microscopy, liquid chromatography and particle size analysis. The results of sensory evaluation were confirmed by polarising microscopy. Polarising microscopy shows the presence of crystalline lactose in clusters besides crystals of saccharose. X-ray diffraction analysis reveals as an unsuitable method for observing the state of sugars at low amount. This contribution compares the results of all methods mentioned above.

Key words: chocolate; lactose; saccharose; agglomerates; X-ray diffraction analysis; polarizing microscopy

INTRODUCTION

The cluster production during conching (1, 2) of milk or white chocolate is a complicated process in which lactose and moisture play the main roles and which could lead to defects of sensorial quality of chocolate. In the cluster production, the chocolate mass as well as the conching temperature, removing of moisture and mechanical energy input by conching play important roles. The cluster production occurs when moisture and well soluble sugar are present while the other hydrophilic components of solids integrate with the dissolved sugar. The amorphous lactose and saccharose are hygroscopic and they can absorb moisture from the atmosphere until the crystalline state is followed by a sharp loss of the sorbed moisture. If this moisture is not evaporated quickly it solves non-fat material and causes production of agglomerates. This phenomenon is called production of clusters. The following conching cannot destroy those agglomerates usually.

A similar phenomenon is evoked by denaturation of milk protein at the high temperatures during conching. The denaturation of the proteins and following reactions (Maillard Reaction) are most often connected[1] with the agglomeration effects in the conching process.

Therefore, there are two groups of clusters, very hard structure of crystals saccharose and lactose and soft structure of milk protein.

EXPERIMENTAL

The content of moisture in each sample was evaluated by drying at 105°C. The content of fat was evaluated by NMR (NMR spectrometer, Newport MK II Newport Instruments Ltd.). The contents of saccharose and lactose in the same samples were analysed by HPLC (Thermo Separation Products, USA).

The clusters evaluated by the sensory analysis were estimated by methods including the particle size analysis by laser diffractometer (SYMAPTEC HELOS), the roentgen diffraction analysis (RIGAKU, Bragg-Brentan) and the polarizing microscopy (optical microscope JENAPOL-Carl Zeiss Jena).

RESULTS AND DISCUSSION

Roentgen diffraction analysis. Samples of dried milk used for production of chocolate showed one characteristic peak of amorphous state. Any peaks of crystalline state were not observed, so that means dried whole milk contained only amorphous lactose.

Agglomerates in chocolate bigger than 40 μm and representative sample of chocolate mass without clusters were observed by roentgen diffraction analysis. Each sample contained saccharose in the crystalline state. No other reflections with exception of saccharose reflections were found in measured scale. The diffractogram of agglomerates was compared with the diffractogram of the mixture of crystalline saccharose and crystalline lactose. The diffractogram of the mixture showed the strongest reflections of crystalline lactose, nevertheless diffractograms of chocolate agglomerates did not display any reflections of crystalline lactose.

Polarising microscopy. Samples of saccharose crystals, lactose crystals, dried whole milk powder, nontempered

cocoa butter, dark chocolate, milk chocolate and isolated clusters from milk chocolate were studied by optical microscope. Indices of refraction of both of sugars are different, so crystals of lactose have a higher polarized colour than crystals of saccharose. Dried whole milk looked like many globules. Nontempered cocoa butter looked like many non-round particles. Polarizing microscopy revealed no differences between dark and milk chocolate.

The difference between pictures of clusters and chocolate was that fat particles were not around crystals and solids in the cluster picture. Using polarization by double refraction, crystalline lactose was found in the clusters. The size of the crystals was about 10 μm in the sample of the clusters, so the crystals were not really clusters, which interfered with a smooth taste of chocolate. This corresponds to predication that the moisture released by crystallization of amorphous lactose solves non-fat material and causes agglomeration.

Roentgen diffraction analysis revealed as an unsuitable method to observe state of sugars in chocolate if the amount of crystals is very low. Opposite to it polarising microscopy showed the presence of crystalline lactose in the chocolate clusters.

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Changes in a Composition of Concentrated Sugar-Beet Raw Juice during Processing and Storage

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Abstract

Extension of sugar beet raw juice treatment into the period when sugar campaign does not run was one of the most important aims. First, it was necessary to verify methods for thickening of raw juice and find suitable conditions under which it is possible to store raw juices without adding of any chemical additives. Raw juice concentrates were prepared on the climbing film evaporator and stored for the period of 5–7 months. Changes in a composition of stored concentrates (refractometric dry solid content, polarization, purity, pH, sucrose, glucose and fructose content) were observed in month intervals.

Keywords: storage; sugar beet; raw juice; evaporator; invert

INTRODUCTION

Besides a traditional sugar technology, sugar-beet can be processed to obtain an energy source – bioethanol. From this reason the problem of raw juice storability has risen and is solved at many research centers. Fiedler *et al.* (1) determined conditions (water activity, pH, temperature) for storability of raw thick juice from sugar beet. Limiting parameters were water activity 0.88 (equivalent to 67% refractometric dry solid), pH 6 and temperature 5°C. Tests with periodic formalin dosing were also carried out. All research is complemented with detailed results from microbiological tests. In Austria there they focused on raw juice thickening and problems connected with it, such as scale formation. Concentrate was stored under carbon dioxide in a tank with the conical head to restrict the exposed surface (2, 3). Pollach (4) also widely discussed the risk of microbial contamination in sugar factories and mentioned the recent results from storage of thick juice and its protection by spraying of sodium hydroxide solution to the surface.

EXPERIMENTAL

Thickening: Totally 15 samples of sugar beet raw juices were taken from different sugar factories and different extractors during the sugar campaigns 1997/1998 and 1998/1999. Before storage juices were thickened on a climbing film evaporator (ARMFIELD, England). Evaporation run in two stages, under the vacuum and following conditions:

steam pressure:	100–150 kPa
pressure inside the evaporating tube:	60 kPa
input juice temperature:	22°C
output juice temperature in the 1st stage:	72–74°C
output juice temperature in the 2nd stage:	74–77°C
input juice flow rate:	15–17 l/h

Storage. Concentrates were placed into 5litre or 10litre barrels and stored in a stock-room without any heating for the period of 5 months (campaign 97/98) and 7 months (campaign 98/99). Temperature in the stock-room was very close to the outdoor average temperature. No chemical additives (as e.g. formaldehyde) were added to extend the durability.

Analytical methods

- Refractometric dry solid (RDS) content (= saccharization) – measured directly on digital refractometer ABBEMAT (Dr. Kernchen, Germany).
- Polarization – determined on polarimeter SUCROMAT VIS/NIR (Dr. Kernchen, Germany) at the wave length 589 nm after precipitation of diluted sample with Herles I and II agents and filtration.
- Purity – calculated as a ratio of polarization and RDS multiplied by 100.
- Content of sucrose and of invert (= sum of glucose and fructose) – analysed on HPLC with autosampler AS 54 (Ecom s.r.o., Czech Republic), column filling: ionex OSTION LG KS 0800 Ca^{2+}, mobile phase: demineralyzed water with flow rate 0.5 ml/min, detection: refractometer RIDK 101 (Laboratorní pøístroje Praha, Czech Republic).

RESULTS AND DISCUSSIONS

Sugar campaign 1997/1998. Results (Figs 1 and 2) show very gradual increase of saccharization and invert content (= sum of glucose an fructose), confirmed by higher polarization as well. This result it is possible to explain by the way of juice storage. From the beginning of the experiment there sucrose partly crystallized and later it dissolved because of the rising temperature in a stock-room.

Another reason could be the way how samples of concentrates have been taken – concentrates were solution with very high viscosity placed in 10litre barrels so it was not possible to heat it for better sampling otherwise the experiment condition would have been changed. From these reasons saccharization changes can be consider very small.

By the comparison of total sugar amount, what was constant, it is evident that invert created by sucrose hydrolysis was not subsequently decomposed. This assumption is also approved with pH stability during the whole five months storage experiment and polarization and purity decline.

Sugar campaign 1998/1999. By comparison of all measured values it is obvious that concentrate stability is affected the most by the initial saccharization of raw juice concentrate. Initial refractometric dry solid at least 65% (Fig. 3) seems to be sufficient for avoiding of microbiological decomposition during spring months with lower temperatures (maximum 20°C). During storage under such conditions there slight sucrose inversion could be expected but not any other monosaccharide decomposition.

Seven month storage with temperature maximum 26°C without any chemical additives (as e.g. formaldehyde) is possible with concentrates with initial saccharization at least 69%. Under these conditions 2–4% decrease of polarization and purity occurred followed by proportional invert content growth. For better result it is necessary to restrict the area where concentrate is in a contact with oxygen, for instance by proper barrel or tank shape. RDS less than 56% was not sufficient for long-term storage (Fig. 4)

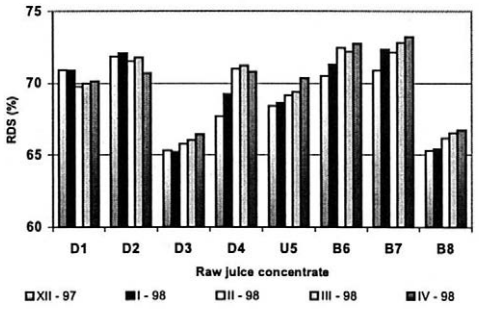

Figure 1. Refractometric dry solid content – RDS (campaigne 97/98)

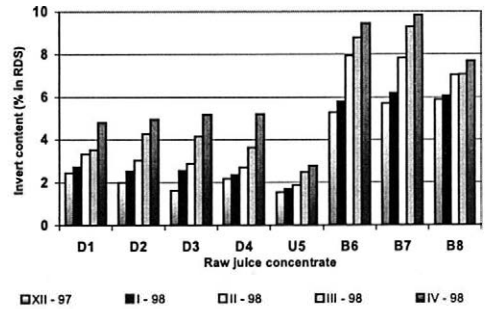

Figure 2. Invert content (campaigne 97/98)

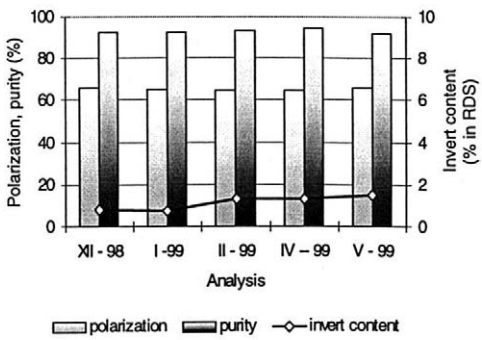

Figure 3. Composition of concentrates with high (71%) initial RDS

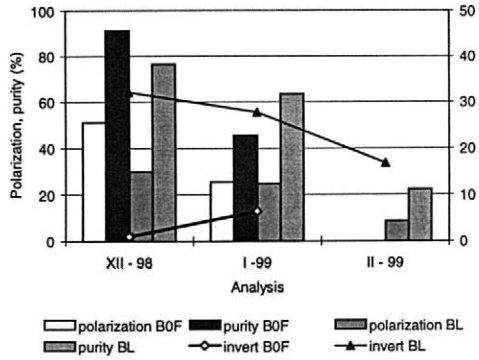

Figure 4. Composition of concentrates with low initial RDS (59% (B0F) and 39% (BL)

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Properties of Ionomer Packaging Films with Linked Bacteriocins

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Abstract

The active components of two commercial bacteriocin products Perlac 1901 (Laboratorios Amerex, Spain) and Chrisin (Chr. Hansen, Denmark) were fixed on the surface of ionomer film (Surlyn 1605 and 8140, DuPont, USA) using the Ugi's reaction. The antimicrobial agents can gradually release from the film into the contact liquid medium. The activity of the bacteriocins on film was proved by inhibition of *Lactobacillus delbrqeckii* (subsp. *Lactis LTI 30*) growth during incubation in liquid MRS medium under 37°C. The total amount of active components released from 1 dm^2 of prepared films corresponded to 250–310 μm of Perlac 1901 and 240–270 μm of Chrisin (240–270 IU). The immobilization of bacteriocins influenced significantly in all tested modifications the transparency of packaging film. The important changes were not determined for the tensile strength, sealability, gas and humidity permeability and coefficient of friction.

Keywords: bacteriocin; nisin; active packaging; food packaging

INTRODUCTION

Active packaging with antimicrobial activity represents an alternative approach to treatments of foods with antimicrobials traditionally used in food processing. The weak linking of bacteriocins on packaging material provide the promising active packaging system, several types of which have been already investigated (1–4). The aim of our work was to study the possibility of immobilization of two types of bacteriocin on the surface of a ionomer packaging film.

EXPERIMENTAL

Packaging film. Ionomer films (50 μm thickness) made of Surlyn 1605 and Surlyn 8140 (Du Pont, USA) were used.

Bacteriocins: Two types of bacteriocins were tested, (i) Perlac 1901 (Amerex, Czech Republic) and (ii) Chrisin (Chr.Hansen, Denmark).

Bacteriocin immobilization. The bacteriocins were bound on the ionomer film surface using Ugi's reaction in the presence of cyclohexyl isocyanide and glutaraldehyde. The efficiency of immobilization was checked by FT-IR spectrometry.

Activity testing. The activity of the bacteriocin on film was determined by incubation with of *Lactobacillus delbrqeckii* (subsp. Lactis LTI 30) in liquid MRS medium under 37°C. The rate of testing microorganism growth was evaluated by spectrophotometry at 615 nm.

RESULTS AND DISCUSSION

The efficiency of immobilization of bacteriocins was proved by FT-IR spectra. The presence of nisin on the surface of prepared films was indicated by absorption maxima at 1648 and 1526 cm^{-1} ($-NH_2$ groups).

The activity of bacteriocins linked on the surface of ionomer film was tested by incubation with of *Lactobacillus delbrqeckii* (subsp. Lactis LTI 30) in liquid MRS medium at 37°C. The MRS medium was in contact with the tested films before inoculation for 0–2 days at 4°C. The significant delay of indicator microorganism growth was determined in all tested cases compared with control. The results for the both Surlyn films with immobilized Chrisin are given in Fig. 1 and Table 1. For the films with bound Perlac 1901 the obtained data were very similar.

The results confirm that bacteriocins can be fixed rather firmly on a iomomer film using Ugi's reaction without the loss of its activity. It was proved, that antimicrobial effect was caused by bacteriocins and not by the other components using during immobilization. As the activity against indicator microorganism was also determined in medium extracts after removing of film with immobilized bacteriocins, it is obvious, that bacteriocins gradually releases from the film surface. It is also obvious from data in Fig. 1 and Table 1, as inhibition of *Lactobacillus* growth was stronger after longer extraction into cultivation medium. The activity of bacteriocins released from 1 dm^2 of

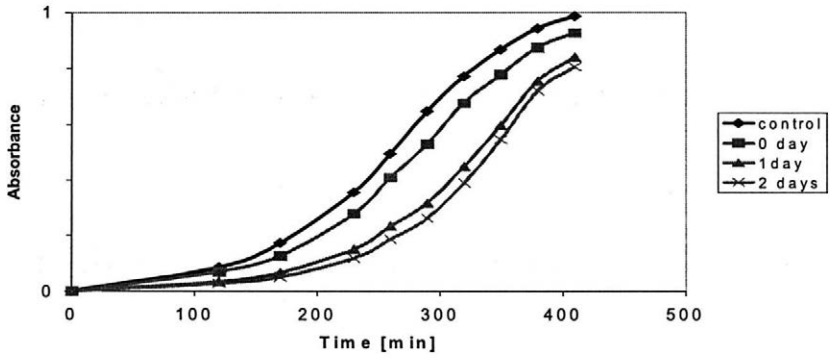

Figure 1. Growth of indicator microorganism *L. delbrqeckii* in medium in which Surlyn 8140 with linked Chrisin was extracted for 0–2 days (series 4)

Table 1. Influence of Surlyn 1605 and Surlyn 8140 with immobilized Chrisin on the lag time of growth of *Lactobacillus delbrqeckii* (subsp. Lactis LTI 30) during six series of tests

Time of film extraction before inoculation (days)	Lag time [min]					
	series					
	1	2	3	4	5	6
	Surlyn 1605					
Control	200	259	205	352	190	368
0	220	262	220	365	195	370
1	230	268	232	380	215	375
2	235	273	258	380	259	382
	Surlyn 8140					
Control	275	300	300	180	320	198
0	285	310	305	200	327	202
1	285	315	312	245	335	210
2	298	322	325	258	348	240

prepared films corresponded to 250–310 μm of Perlac 1901 and 240–270 μm of Chrisin (240–270 IU).

For the practical purposes the effect of the preservation agent immobilization on functional properties of polymer film must be known. It was found that the linking of bacteriocins influenced significantly the transparency of packaging film in all tested modifications. The important changes were not determined for the tensile strength, sealability, gas and humidity permeability and coefficient of friction.

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Antimicrobial Effect of Saccharide Esters with Lauric Acid

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Abstract

Antimicrobial properties of 6-*O*-lauroylsucrose and 6-*O*-lauroylglucose against *Bacillus subtillis* LCC 666, *Escherichia coli* DBM 3104, *Penicillium expansum* DBM 4061, *Aspergillus* sp. DMF 0501, and *Fusarium culmorum* DMF 0103 were studied. It was found out that 6-*O*-lauroylsucrose ester and 6-*O*-lauroylglucose ester inhibited the growth of *Bacillus subtilis* LCC 666 more than that of *Escherichia coli* DMB 3104. The antifungal activity of 6-*O*-lauroylsucrose and 6-*O*-lauroylglucose was found and a concentration dependence below 0.6 mmol/l and 1.1 mmol/l , respectively was observed in the mould colony growth. As our results indicated the maximal concentration effect was achieved by the concentration corresponding to the critical micellar concentration of the tested substances.

Keywords: antimicrobial; antimicrobial; fungi; fatty acid esters

INTRODUCTION

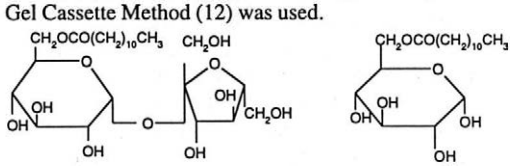
To find a new natural substances which inhibit commonly occurring food-borne microorganisms, some lauric acid derivatives have been tested in past. Sugar esters of lauric acid have been found to show the best antimicrobial properties (1, 2).

Antimicrobial activity has been found to be greatly dependent on the degree of substitution of substance (2). Monoesters showed the best level of both antifungal and antibacterial activity. The sucrose esters posses the antimicrobial effects against mould genera (e.g. *Alternaria, Aspergillus, Penicillium, Candida*) (3) and against some bacteria (e.g. *Listeria monocytogenes, Staphylococcus aureus*) (3–5) as well. No inhibition effect has been shown against lactic acid bacteria (2). The inhibition of gramnegative bacteria has been found at the presence of EDTA (6).

The chemical structure of sucrose ester with fatty acids seemed to be advantageous for the use in food production. Sucrose esters are good nonionic surfactants with a good detergent properties and high biodegradability. Therefore, they are commonly used as emulsifiers in food or cosmetic products (7).

EXPERIMENTAL

6-*O*-lauroylsucrose (α-D-glucopyranosid-b-D-fructofuranosyl monododecanoate) (Fig. 1) and 6-*O*-lauroylglucose (α-D-glucopyranosid monododecanoate) (Fig. 1) were prepared by reaction of 3-lauroylthiazolidin-2-thion with corresponding sugar (8, 9). The purity of substances was 95% w/w tested by use NMR. The critical micelar concentration (CMC) was determined by surface tension measuring (tensiometer C LAUDA) of different concentrations of tested substances in water at 20°C. HLB values were calculated according the method of Davies (10). HLB values of 6-*O*-lauroylsucrose and 6-*O*-lauroylglucose were calculated to be 15.7, 10.2, respectively and the values of CMC were determined to be 0.2 0.3 mmol/ (Fig. 2). Agar Spot Diffusion Method was used to test the antimicrobial activity (11), for the testing the antifungal activity the IFR Gel Cassette Method (12) was used.



6-*O*-lauroylsucrose 6-*O*-lauroylglucose

Figure 1. The structure of tested substances

RESULTS AND DISCUSION

The antibacterial effect of 7 different concentrations of 6-*O*-lauroylsucrose and 8 different concentrations of 6-*O*-lauroylglucose were tested against *Eschericha coli* DBM 3104 and *Bacillus subtilis* LCC 666 (Table l).

The lowest concentration which caused the inhibition zone of *Bacillus subtilis* LCC 666 was 0.3 mmol/l. No inhibition effect against *Escherichia coli* DBM 3104 was found. The lowest concentrations necessary for making the inhi-

Table 1. Effect of various concentrations of 6–*O*–lauroylsucrose and 6–*O*–lauroylglucose as tested by agar difussion method

Concentrations		*Escherichia coli* DBM 3104	*Bacillus subtilis* LCC 666	*Penicillium expansum* DBM 4061
[mg.ml^{-1}]	[mmol.l^{-1}]	z [mm]	z [mm]	z [mm]
			6–*O*–lauroylsucrose	
15.0	28.59	NT	NT	NT
10.0	19.06	NT	NT	3
5.0	9.53	–[1]	9	1
1.0	1.91	–[1]	9	–[1]
0.5	0.95	–[1]	8	–[1]
0.4	0.76	NT	8	NT
0.3	0.57	NT	8	NT
			6–*O*–lauroylglucose	
15.00	41.38	–[1]	NT	9
10.00	27.59	–[1]	NT	7
5.00	13.79	–[1]	13	7
1.00	2.76	–[1]	11	NT
0.50	1.38	–[1]	11	NT
0.40	1.10	–[1]	11	NT
0.30	0.82	–[1]	10	NT
0.20	0.55	–[1]	8	NT

z the diameter of inhibition zone of tested sample
NT non tested
[1]was not possible to determine the diference between the diameter of inhibition zon of control sample and tested sample

bition zone of *Penicillium expansum* DBM 4061 were 9.5 mmol/l of 6-*O*-lauroylsucrose and <13.8 mmol/l of 6-*O*-lauroylglucose.

The antifungal effect of 6-*O*-lauroylsucrose and 6-*O*-lauroylglucose as tested by gelatine cassettes is presented at Figs 3 and 4. The concentration scale from 0.1 mmol/l to 2.0 mmol/l of 6-*O*-lauroylsucrose and 6-*O*-lauroylglucose were tested against *Aspergillus* sp.

DMF 0501, *Penicillium expansum* DBM 4061 and *Fusarium culmorum* DMF 0103. It was found out that the concentrations higher that 0.6 mmol/l of 6-*O*-lauroylsucrose inhibited the growth of colony *Aspergillus* sp. DMF 0501 almost completely and the increasing concentration of 6-*O*-lauroylsucrose did not increase the inhibition of colony growth. The concentration of 6-*O*-lauroylglucose lower than 0.6 mmol/l did not show any concentration effect on the colony growth. It was found out that the concentration higher that 0.6 mmol/l inhibited the growth of colony *Aspergillus* sp. DMF 0501 almost completely and the increasing concentration of 6-*O*-lauroylsucrose did not increase inhibition of colony growth. With concentration below 0.6 mmol/l a concentration effect was observed in the colony growth. The same effect was found with *Penicillium expansum* DBM 4061 and *Fusarium culmorum* DMF 0103.

As it is presented in Figs 2 and 3, the change in colony growth rate of tested mould took place when the concentration of tested substances run over CMC. At the concentration higher than CMC, the colony growth rate

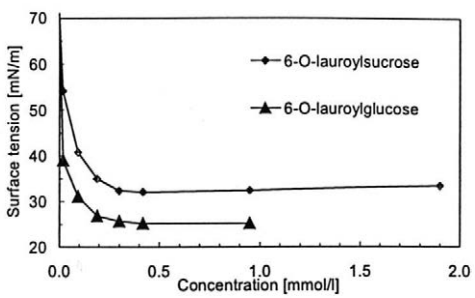


Figure 2. The dependence of surfase tension on different concentrations of active substance in water

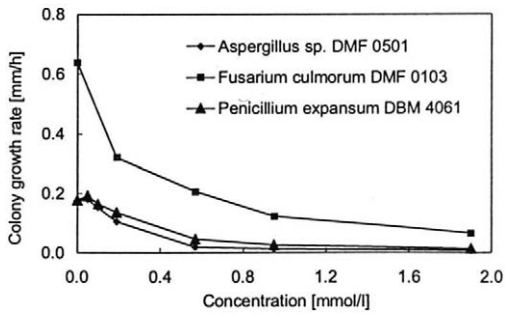


Figure 3. The dependence of the colony growth rate on 6-O-lauroylsucrose concentrations

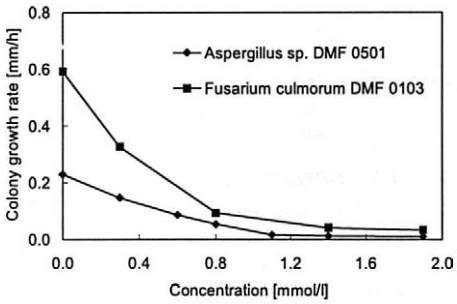


Figure 4. The dependence of the colony growth rate on 6-O-lauroylglucose concentrations

decreased as the surface tension decreased to the lowest values. The difference between the concentration of 6-*O*-lauroylsucrose and 6-*O*-lauroylglucose at which the growth rate change accomplished could be explained by the different polarity of these substances which deals with HLB values.

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Immunoassay of Organochlorine Pesticides

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Abstract

Enzyme immunoassays to DDT and endosulfan with both photometric and luminescent detection were developed by using monoclonal antibodies, which can be classified as class-specific or pesticide specific. Because the organochlorine pesticides are poorly solubilized in water solutions, the effect of organic solvents on antibody affinity have been tested. Developed ELISAs were used to analyze samples of different origin (soil, lettuce, fish). The recovery for both organochlorine pesticides was usually around 100%. The results of ELISAs were evaluated by GC-ECD.

Keywords: pesticide; DDT; endosulfan; immunoassay; fish

INTRODUCTION

The main problem of food safety is its contamination with biologically active compounds like pesticides, hormones and drugs. Therefore, the monitoring of food samples is of great importance. Unfortunately, the nowadays methods, most often chromatography and electrophoresis, are very complicated and include several cleaning steps before analysis. Simplifying and minimising the analysis are the key problems. The initial screening test methods should be technically simple, inexpensive and useful for the routine analysis of a large number of samples. These requirements can be met by enzyme immunoassay (ELISA] with photometric and/or chemiluminescent detection. The aim of this study was to develop indirect ELISA for pesticides from both DDT (dichlorodiphenyltrichloroethane) and endosulfan families.

The goal of our work was to obtain high-affinity monoclonal antibodies (MAbs) to the main families of chlorinated insecticides, i.e. the DDT family and the chlorinated cyclodiene family. Since hapten design is a key step in the development of immunoassays for small molecules, the synthesis of several haptens with different spacer arms attached through different molecular sites, and their use to produce MAbs to both chlorinated insecticide families, was undertaken. MAbs were subsequently used as the primary immunoreagents to develop ELISAs as an alternative to chromatographic techniques for the screening of large number of food and environmental samples.

EXPERIMENTAL

Five haptens resembling as much as possible the general chemical structure of the DDT were synthesised. Similarly, four haptens resembling cyclodiene family were synthesised. All the haptens were covalently attached to BSA using the modified active ester method. Hapten-to-protein molar ratios were estimated to be between 14 and 21. All the haptens were also covalently attached to ovalbumin using the mixed-anhydride method, having molar ratios between 5 and 11. Female mice (BALB/c) were immunised with BSA – hapten conjugates using standard procedures. Mouse spleen lymphocytes were fused with murine myeloma cells (P3-X63-Ag 8.653) at a 5:1 ratio using polyethylene glycol 1,500. Indirect format was used for ELISA. Plate was coated with ovalbumin-hapten conjugate. Then antibody and standard or sample were added and incubated for 2 h. Next, plate was incubated for 1 h with peroxidase-labelled rabbit anti-mouse immunoglobulins. Finally, peroxidase activity was determined either by standard o-phenylendiamine procedure, or by chemiluminescent assay by using several substrates, among them SuperSignal substrate (SS, Pierce) was the best (e.g. see Scheme 1 for DDT immunoassay).

Scheme 1

Indirect competitive ELISA-DDT

Coating of microplate (100 µl/well) with ovalbumin-hapten (**OVA-DDT 5,** stored at –18°C) solution in 0.05 M carbonate/bicarbonate buffer, pH 9.6

↓

Incubation overnight at room temperature

↓

Washing of microplate (4 times) with PBS – 0.05% Tween 20 solution, pH 7.4 (200 µl/well)

↓

Addition of 50 µl per well of antigen **p,p'-DDT** (stored at refrigerator) diluted with PBS in 10% methanol

↓

Addition of 50 µl per well of monoclonal antibody solution (**LIB – DDT 5.25 or LIB – DDT 5.52**) diluted in PBS – 0.1% BSA

↓

Incubation for two hours at room temperature

↓

Washing of microplate (4 times) with solution PBS – 0.05% Tw (200 µl/well)

↓

Addition of 100 ml per well of **peroxidase conjugate** (**SwAM – Px**) diluted 1/1000 in PBS – 0.05%Tween 20 1000 times, [15 µl of SwAM-Px in 15 ml of PBS – 0,05% Tween 20]

↓

Incubation for one hour at room temperature

↓

Washing of microplate (4 times) with PBS – 0.05% Tween 20 solution, pH 7.4 (**200** µl/well)

↓

Addition of 100 µl per well of **freshly** prepared substrate solution [15 ml of citrate-phosphate buffer pH 5.0 , 15 ml of H_2O_2, 7.5 mg of OPD]

↓

Incubation for 10 min at room temperature

↓

Stop the reaction with **2 M** H_2SO_4 (50 µl/well)

↓

Absorbance measurement at 492 nm

RESULTS AND DISCUSSION

Enzyme immunoassays (with peroxidase [HRP] and/or alkaline phosphatase [ALP] as the label) to DDT and endosulfan with photometric and luminescent detection were developed. Preferably HRP was used, because of simpler and more faster detection. The advantages of luminescent assay in comparison with photometric detection are a wider working range for pesticide analysis and a lower consumption of immunoreagents. Because the organochlorine pesticides are poorly solubilized in water solutions, the effects of organic solvents and Tween 20 on immunoglobulins affinity have also been tested. Limited solubility of organochlorine pesticides resulted in adsorption effects on plastic materials (like tips, polystyrene microplates, etc). To avoid adsorption effects several experiments using Tween 20 and organic solvents were carried out. The best results were obtained with methanol in final concentration 10–20%. These concentrations decrease adsorption to the minimum and have also minimum effect to nativity of immunoglobulins (no denaturation effects were observed). The developed immunoassay format using organochlorine pesticides solubilized in methanol is therefore recommended for use.

Developed immunoassay formats have been applied to analyze real samples. The main problem encountered in this area was to remove fat from analyzed sample. Organochlorine pesticides are preferably solubilized in hydrophobic compounds, i.e. mainly in fat, but fat is an interfering compound for all analytical techniques, as well as for immunoassays. We tried to use first of all dilution of samples to be analyzed, as it is typically used in immunoassays. In case of spiked milk samples, to high extent it is possible to use this methodology and to distinguish milk samples contaminated over the detection limit. For meat samples, fat must be removed before analysis, e.g. by using gel chromatography, because it has fatal interfering effects.

Developed ELISAs were used to analyze samples of different origin, and the results were compared to those obtained by GC-ECD as reference method (Table 1).

Table 1. Determination of DDT family (mg/kg) by both GC-ECD and ELISA

Method	Standard	Soil (only DDE)	Lettuce	River fish fat
GC-ECD	3.36±0.14	1.06±0.05	1.17±0.09	4.50±0.22
GC recalculated*	3.96±0.15	1.06±0.05	0.64±0.05	5.54±0.24
ELISA	4.02±0.21	0.97±0.09	0.56±0.12	5.01±0.28

*results recalculated with respect to cross reactivities of DDE and DDD derivatives

Therefore, the developed indirect ELISAs with both photometric and chemiluminescent detection can be used as screening methods for large number of food and environmental samples, or even as an alternative to chromatographic techniques.

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Perspectives of *Listeria Monocytogenes* Immunoassay Detection

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Abstract

The overall objective is to develop rapid, specific test methods for the immunochemical detection of listeria in foods, with the aim of introducing appropriate quality control into food production processes. The novel polyclonal antibodies to the ten peptide sequences (MAP 1–10) representing *Listeria monocytogenes* pathogenicity proteins were raised and characterised. Immunoglobulin G fractions against MAP 1, 2, 5 and 6 specifically bound these peptides in indirect enzyme immunoassay. AntiMAP 1 and 9 interact also with listeria cells.

Keywords: food-borne pathogens; *Listeria monocytogenes*; virulence proteins; immunoassay; peptide synthetic antigen

INTRODUCTION

Listeria monocytogenes has a multi-level mechanism of pathogenesis involving a number of proteins. The target proteins in this study are the internalins and the actin-assembly protein. We considered several criteria when selecting peptides to represent the virulence of these proteins:

i) uniqueness of primary sequence of the peptide – must be present only in *L. monocytogenes*, and not in other bacteria, in particular other *Listeria* species
ii) surface exposure of the peptide – if the sequence is not on the protein surface, then it is unlikely that antibodies will bind to the whole protein
iii) secondary structure of the peptide – peptides containing, for example, turns or coils, are more likely to stimulate an immune response than straight chain structures (provided, of course, that the synthetic peptide in isolation adopts the same conformation as in the parent protein)
iv) antigenicity of the peptide – this is linked to iii), but must include consideration of other factors like the hydrophilicity of the constituent amino acids

The characteristics of the chosen peptides are shown in Table 1.

EXPERIMENTAL

As the chosen peptides are not immunogenic themselves, multiple antigenic peptides (MAPs) based on a lysine

Table 1. Characteristics of peptide sequences used for antibody production

Target protein	Sequence	Predicted 2^0 structure (Stultz *et al.*)	Hopp & Woods max. (hydrophilicity index)	Jameson-Wolf max. (antigenic index)	MAP number
Actin-assembly protein:					
	^{41}DEWEEEKTEEQPS^{53}	helix 0.4	2.0	1.7	10
	^{72}IKELEKSNKV^{81}	helix 0.5–0.7	1.6	1.1	2
	^{143}AEIKKRRKAI^{152}	helix 0.7	1.7	0.9	7
	^{225}WVRDKIDENPEV^{236}	loop 0.6 (>230)	1.8	1.3	6
	^{265}FPPPPTDEEL^{274}	loop 0.8	1.1	1.3	8
Internalins:					
inlA	^{78}DITPLANLTNL^{88}	loop 0.4–0.9	–0.3	–0.15	4
	^{447}HVDGKETTKE^{456}	loop 0.4	1.4	0.9	5
inlB	^{139}KDLKKLKSLS^{148}	helix 0.7 (>145)	1.3	1.3	9
	^{276}GDYEKPNVKW^{285}	loop 0.7	1.5	1.7	1
inlC + i-inlC	^{88}QSLAGMQFFT^{97}	helix 0.5	0.3	1.7	3

core were prepared. Eight-branch MAPs with the chosen oligopeptides have molecular weights in excess of 10,000 Daltons and are thus immunogenic without further coupling. For single peptides, as well as for MAPs, solid phase peptide synthesis was used. After synthesis, the MAPs (No. 1–10) were lyophilised (yields were in the region of 5–15 mg) and used for rabbit polyclonal antibody production. Novel antibodies to peptide sequences representing *Listeria monocytogenes* pathogenicity proteins were raised. Antisera obtained were purified by affinity chromatography. Resulting immunoglobulin (IgG) fractions were lyophilised with lactose in ratio 1:1 (w:w) and used in all experiments.

RESULTS AND DISCUSSION

The rabbit antisera were used for isolation of immunoglobulin (IgG) fractions. IgG were isolated and purified on protein A – Prosep A (glass carrier) column by means of affinity chromatography. All solutions, as well as antisera, were sterilized by membrane filtration. IgG fractions were then lyophilised with lactose, as a carrier, in ratio 1:1 (w:w).

IgG fractions against MAP 1, 2, 4, 5, 6, and 9 specifically bound these peptides in indirect competitive enzyme immunoassay (ELISA). Antibodies against MAP 2, 4 and 6 have showed the best affinities to their corresponding antigens (Table 2).

Cross reactivities of all polyclonal antibodies with MAPs were tested. The lowest cross reactivity was found for antiMAP 4, only 7% with MAP 7. AntiMAP 2 cross reactes with MAP 4 (19%) and antiMAP 6 with MAP 2 (87%) and MAP 7 (18%). The highest cross reactivity possesses antiMAP 1, which interacts very well with MAP 9 (154%) and MAP 2 (114%).

The interactions of prepared antibodies with different listeria species were followed in indirect competitive enzyme immunoassay (ELISA) format. Surprisingly, only anti-MAP 1 and MAP 9 recognize *Listeria monocytogenes*, while all other antibodies do not bind microbial cells.

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The Analysis of Heterocyclic Aromatic Amines in Various Types of Fried Meat

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Abstract

Heterocyclic aromatic amines (HAAs) are mutagenic compounds that are formed during heating of meat and fish. These substances are products of the reaction of creatine with amino acids and carbohydrates. The substances that are mainly found in cooked meat and fish products are 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoline (MeIQ), 2-amino-3,4,8-trimethyl-imidazo[4,5-f]quinoxaline (4,8-DiMeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Various kinds of meat (beef, pork, lamb, poultry) were fried at various cooking conditions (temperature, time of frying). The highest concentrations of the aromatic amines were as follows: MeIQ 4.1 μg/kg, IQ 23 μg/kg, 4,8-DiMeIQx 24 μg/kg, PhIP 27 μg/kg, MeAαC was not detected.

Keywords: heterocyclic amines; fried meat; mutagens; carcinogens; food analysis; HPLC

INTRODUCTION

The International Agency on Cancer Research (IARC) has classified several of the HAAs as possible human carcinogen and has recommended a reduction in the exposure to these compounds (1). In order to estimate and minimise exposure to HAAs it is necessary to analyse their quantities in foods.

HAAs are normally found in heated meat and meat products. The concentration of HAAs depends on the time and temperature regime used during cooking (2, 3). An increase in the heating temperature and the heating time normally results in an increased mutagenic activity.

The samples were extracted according to the method of Rivera and Galceran (4, 5) with some modifications.

All samples were analysed for their concentrations of IQ, MeIQ, 4,8-DiMeIQx, PhIP and MeAαC (2-Amino-3-methyl-9H-pyrido[2,3]indole).

EXPERIMENTAL

The samples were fried on both sides simultaneously using a teflon-coated heating device. The fried meat was homogenised with a mixer. 8 g of the homogenised samples were saponified with 25 mL 0.1 N NaOH for 6 h at room temperature, mixed with 26 g Extrelut and extracted with 100 mL dichloromethane (DCM). The DCM fraction which contained the HAAs was divided into two equal parts.

For the purification of PhIP and MeAαC an Oasis MCX Cartridge was used. PhIP and MeAαC were eluted from the Oasis column with 2 ml 5% ammonium hydroxide with 95% methanol. The solvent of the eluate was evaporated and the residue was dissolved in methanol (500 μL). PhIP and MeAαC_were then qualitatively and quantitatively determined by HPLC with fluorescence detection.

For the purification of IQ, MeIQ and 4,8-DiMeIQx a propylsulfonic column (PRS) coupled to a silica column (C_{18}) was used. After loading the PRS column with the DCM fraction the PRS column was washed with 2 mL DCM and then coupled to the C_{18} column. This tandem was first eluted with 20 mL 0.5 M ammonium acetate at pH 8.0 to pass the HAAs onto the C_{18} column. After washing the C_{18} column was eluted with 0.8 ml MeOH/NH_3 (9:1). The eluate was evaporated and the residue was dissolved in methanol (100 μL). Chromatographic analysis was performed using liquid chromatography-mass spectrometry (LC-MS).

RESULTS AND DISCUSSION

Poultry. HAAs were determined in fried poultry meat at different frying temperatures.

Relatively low amounts of HAAs could be found in fried poultry meat. MeAαC was not detected in poultry meat.

Beef. HAAs were determined in beef at different frying temperatures and frying time.

The highest amounts of HAAs could be found in beef. With increasing frying time and frying temperatures the detected amounts also increased. MeAαC could not be found in any of the samples. The concentration of MeIQ is lower than the concentration of IQ, 4,8-DiMeIQx and PhIP.

Table 1. HAAs in fried poultry meat and in beef

	Surface temperature (°C)	Frying time (min)	IQ (μg/kg)	MeIQ (μg/kg)	4,8-DiMeIQx (μg/kg)	PhIP (μg/kg)	MeAαC (μg/kg)
Fried poltry	140	20	1.1 ± 0.4	0.9 ± 0.3	0.4 ± 0.3	3.8 ± 1.8	n.d.
	160	20	0.75 ± 0.25	0.4 ± 0.2	0.4 ± 0.2	4.2 ± 1.8	n.d.
	180	20	1.5 ± 0.5	1.0 ± 0.5	0.5 ± 0.3	7.8 ± 1.0	n.d.
Beef	120	15	2.6	0.6	0.5	2.4	n.d.
	160	25	5.1	1.1	6.2	19	n.d.
	220	35	23	4.1	24	27	n.d.

Table 2. HAAs in pork and in lamb

	Temperature (°C)	Frying time (min)	IQ (μg/kg)	MeIQ (μg/kg)	4,8-DiMeIQx (μg/kg)	PhIP (μg/kg)	MeAαC (μg/kg)
Pork	Level 4	20	n.d.	n.d.	1.0	0.3	n.d.
	Level 4	25	n.d.	n.d.	2.1	5.6	n.d.
	Level 4	30	n.d.	n.d.	10.1	26.8	n.d.
Lamb	Level 4	15	n.d.	n.d.	n.d.	0.1	n.d.
	Level 4	20	n.d.	n.d.	2.8	1.7	n.d.
	Level 4	25	n.d.	n.d.	8.2	9.1	n.d.

Pork. HAAs were determined in pork that was fried for different times at a specific temperature.

Relatively high amounts could be found for 4,8-DiMeIQx. The amounts that could be found for PhIP were as high as in beef. With increasing frying time the amounts also increased. IQ, MeIQ and MeAαCcould not be detected.

Lamb. HAAs were determined in lamb that was fried for different times at a specific temperature.

Relatively high amounts could be found for PhIP and 4,8-DiMeIQx. IQ, MeIQ and MeAαC could not be detected.

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Impact of Bioacidification on Overall Beer Quality

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Abstract

The project of biocidification was focused on strain selection, development of bioacidification strategy and evaluation of lowered pH impact on yeast performance and beer quality. *Lactobacillus plantarum L10* strain has shown satisfactory parameters for use in the acidification of unhopped wort. The acidification was carried out in a fed-batch system and the final acidified wort was used for blending with hopped wort. These blends were inoculated with lager type yeast and fermented in EBC columns at 12°C. The positive effect of lower wort initial pH values ranging from 5.15 to 4.0 was found on vicinal diketones uptake as well as organoleptic markers of "young beer". Wort attenuation was retarded at initial pH values lower than 4.8.

Key words: beer; bioacidification; fed-batch; *Lactobacillus plantarum*; volatile compounds; diacetyl

INTRODUCTION

Mash or wort bioacidification has become a popular method for beer quality improving which brings higher brewhouse productivity and consequent economical savings. German brewmasters working under regulations of Beer Purity Law (Reinheitsgebot) have only possibility to acidify mash and wort (or produce acid malt, respectively) by introduction of lactic acid bacteria to the brewing process (1). Another aspect encouraging brewers to use bioacidification strategy is impact on beer organoleptic properties positively contributing to flavour characteristics particularly in production of non-alcoholic or low-alcoholic beer (2)

Shift in pH level of mash benefits higher yield of extract by improved action of crucial enzymes involved in mashing process and therefore, more rapid conversion of starch to utilisable sugars (β amylase activity) and higher content of soluble nitrogen (proteinase activity) (3). Decrease in mash viscosity resulting from higher β-glucanase activity favourably affects filtration process in lauter tun (4). For wort production, several important technological parameters can be improved by bioacidification. Lower pH value allows easier separation of precipited proteins due to approaching their pI point of which is about 5.0–5.2 and therefore, avoid beer from nonbiological haze formation. Facilitated removal of tannins together with enhanced extraction of other polyphenolic compounds balance character of beer bitterness. Moreover, pH level of final beer under 4.5 prevents the product from bacterial contamination causing biological hazes (3).

Lactic acid bacteria are considered to be strongly inhibited by hop resins so that most of applications have been carried out on sweet worts and mashes. Selection of the suitable strain is based on criteria respecting properties of fermented substrate (sugar profile of mash and wort, inhibition effect of hop substances) and process efficiency (high lactic acid productivity) (1, 3).

Among many different developed strategies of bioacidification continuous system with immobilized bacterial cells has been shown as most efficient. This type of process has been developed in pilot scale by Pittner and Back. Wort is acidified in bioreactor loaded by immobilized *Lactobacillus amylovorus* TL3 cells. This method has already been patented in relation to the technology of alcohol free production in Bavaria brewery (5).

EXPERIMENTAL

Microorganismes. LAB strains (LCC Prague, CCM Brno, DMF ICT, Prague); *Lactococcus lactis* ssp. *lactis* 416, 670, 685, 686, 702/68, 731T, *Lactobacillus plantarum* L10, *Lactobacillus casei* ssp. *rhamnosus* 1828, *Streptococcus thermophilus* 786; *Saccharomyces cerevisiae* – industrial lager yeast strain.

Material. Bioreactor of 6L working volume, standard equipment, controlled by computer programme; EBC columns of 2L.

Analytical methods. biomass by OD measurement, determination of sugars and lactic acid by HPLC, determination of volatile compounds by GC head-space, attenuation by digital densimeter, determination of L(+) lactic acid content by enzymatic test.

Cultivation strategy. Screening experiments included testing of growth on hopped and unhopped wort, lactate formation and testing of resistency to iso-α-bitter acids.

Fed-batch cultivation of *Lactobacillus plantarum* L10 at 30°C, working volume from 1.5 L to 6 L, feeding by peristaltic pump according to simulation programme.

Acidified unhopped wort blended with the hopped wort of common gravity ⇨different initial pH values: **I** A: 5.15, B 5.0, C 4.8; **II** A 4.7, B 4.5, C 4.35, D 4.2, E 4.0.

First fermentation in EBC columns (2 L) at 12°C, inoculation with lager type yeast.

RESULTS AND DISCUSSION

Screening experiments including different homofermentative strains of *Lactococcus*, *Lactobacillus* and *Streptococcus* genera were carried out to prove strains capability of growth on brewing semifinished products. No one of selected strains was able to growth sufficiently on hopped wort. Unhopped wort appeared to be suitable substrate for acidification by *Lactobacillus plantarum* L10 (Fig. 1) while clear inhibitory effect has further been shown in growth tests of this strain in the presence of decreasing level of iso-a-bitter acids (ranging from 20 ppm to 2 ppm). Lactic acid production by *Lactobacillus plantarum* L10 on different type of media with addition of iso-a-bitter acids is described in Table I. indicating that bacteriostatic action of iso-a-bitter acids was diminished in case of cultivation of this strain on MRS medium.

Lactobacillus plantarum L10 met important selection criteria satisfactorily and it was singled out of tested LAB strains for further bioacidification. Fed-batch cultivation gives advantage of reduced level of inhibition caused by lactic acid accumulation in fermented wort. Final acidity after 4 day fermentation reached pH value 3.25. Such acidified wort was used for dilution of original wort to achieve worts of different initial pH values and these blends were inoculated with lager type of industrial brewing yeast. Wort attenuation, pH level and volatile compounds evolution were followed in the course of first fermentation in each of the EBC columns. Furthermore, filtered young beer was evaluated in basic organoleptic markers.

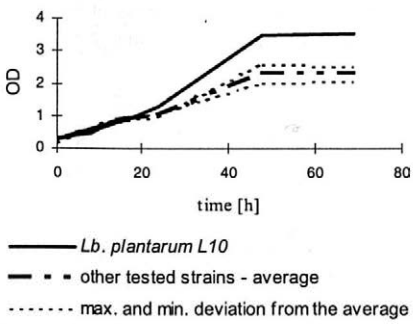

Figure 1. Growth of *Lb. plantarum* L10 strain on unhopped wort in comparison to other tested strains

Table 1. *Lb. plantarum* L10: Lactic acid production on different media with addition of iso-α-acids

Medium	LA (g/l)
MRS	14.9
MRS+4ppm	9.9
MRS+12ppm	7.6
GLU	6.5
GLU+4ppm	4.5
GLU+12ppm	3.2
UHW	2.9
UHW+4ppm	0.1
UHW+12ppm	0.0

The course of attenuation, a major process of first fermentation step when the wort original gravity falls, was retarded at pH values lower than 4.8. Long lag phase extended duration of fermentation about three days comparing to experiments accomplished at pH level ranging from 5.15 to 4.8. No significant differences were observed in corresponding attenuation curves of experiment set I and II.

Removal of certain volatile compounds during beer ageing is essential to obtain product free of off-flavour. Besides acetaldehyde and sulfuric compounds, very olfactive vicinal diketones are responsible for undesirable beer aroma, namely diacetyl resembling rancid butter, even though their concentrations in beer are very low. Typical course of vicinal diketons evolution is wide peak shaped curve. Diacetyl and 2,3-pentanedione uptake came out faster in columns with lower initial pH values. For example, diacetyl level in column AII at 9th day of fermentation is twofold higher comparing to column EII. Concerning diacetyl content, achieved results suggest that decrease in pH about 0.8 units can shorten maturation time for about six days. Nevertheless, both diacetyl and 2,3-pentanedione reached only slightly different final values.

Integral part of beer appraisal was the sensory analysis of main organoleptic markers. Colour, taste and foam formation were evaluated respecting typical attributes of young beer. Following trends were traced with decreasing pH level: colour was improved to more intensive gold-brown; taste was found well balanced and finer in bitterness; more stable foam was observed when beer was poured into the glass.

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Changes of Agaritine during Household Processing of Champignons

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Abstract

Champignons, the most widely cultivated edible mushrooms (*Agaricus* sp.) contain natural toxin agaritine and other derivatives of phenylhydrazines. Levels of agaritine in *Agaricus* sp. samples obtained from retail market were measured by reversed phase high performance liquid chromatography. Changes of agaritine content during household processing of mushrooms (boiling in water, frying in oil, microwave heating, drying, dry baking, freezing and thawing of frozen product) were investigated. Household processing efficiently reduces levels of the suspected toxins in the mushrooms. The decrease of agaritine levels during these processes ranged between 23% and 88%.

Keywords: agaritine; champignons; HPLC; household processing; decrease

INTRODUCTION

Their special, delicious flavor has made some kinds of mushrooms afterthought ingredients in a variety of meals throughout ages. The popularity of mushrooms as dietary constituents is, besides their attractive sensory properties, based on their low energy content: they have a typically low amount of lipids and, in addition, they are a good source of dietary fiber and minerals (1). World annual estimated production of champignons is 1.7 mil. tons (2).

Derivatives of phenyl hydrazine represented mainly by agaritine are natural toxicants occurring in champignons (*Agaricus* sp.) – the most widely cultivated edible mushrooms. Levels of agaritine (Fig. 1) (N-(g-L(+)-glutamyl)-4-hydroxymethylphenyl hydrazine) reported in literature range between 100 and 1700 mg/kg fresh weight, concentrations of other phenyl hydrazines potentially present in these mushrooms are commonly significantly lower (3).

Data obtained from toxicological studies on experimental animals demonstrated adverse health effects after their chronic oral exposure to mushrooms products. For instance, life-time feeding of Swiss albino mice with raw, dry baked and/or freeze dried *Agaricus bisporus* induced tumours in these animals (4). Not surprising, when published, such studies gave rise to serious concern as to a possible human health risk from consumption of the cultivated mushroom. Although it has not been established whether consumption of biological relevant amounts of these mushrooms constitutes a tolerable risk or not, it is undoubtedly advisable to abstain from exaggerated dietary intake to phenyl hydrazine derivatives.

Although some people have the habit of consuming fresh cultivated mushrooms raw in salads, most typically, mushrooms are processed in the kitchen by cooking, dry baking or frying.

In the presented study, the influence of various ways of household processing on the agaritine content in ready-

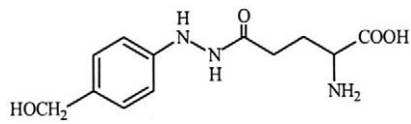

Figure 1. Structure of agaritine (β-N-[γ-L(+)-glutamyl]-4-hydroxymethylphenylhydrazine)

to-consume mushroom-based products were investigated with the aim to generate more data relevant for estimation of dietary intake of agaritine (it should be noted that consumption information has to be available for this purpose).

EXPERIMENTAL

Materials. Fresh cultivated mushrooms *Agaricus bitorquis* (species cultivated in the Czech Republic) were purchased from grower. The agaritine standard was synthesized by Dr Henrik Frandsen, Danish Veterinary and Food Administration according to (5, 6).

Methods. A fresh stock solution of agaritine in methanol (0.02 mg/ml) was prepared weekly and stored in the refrigerator. Twenty grams of fresh mushrooms were ho-

mogenized in 100 ml methanol for 10 minutes. The homogenate was shaken for 30 minutes and solids were then removed by filtration. The volume of the filtrate was adjusted to 200 ml with methanol, 10 ml of this crude extract were evaporated to dryness and the residue dissolved in 2 ml Milli Q water. 20ml aliquot of solution filtered through a microfilter into a vial was injected onto the HPLC column.

Identification and Quantification. HPLC analysis were performed using a Hewlett-Packard HP 1100 liquid chromatograph equipped with DAD or HP 1050 with UV. Separation was carried out on column (250 × 4 mm), LiChrospher 100 RP-18 (5 μm), with precolumn (4 × 4 mm), LiChrospher 100 RP-18 (5 μm). The mobile phase was methanol:water, 10% methanol 0–5 min, then 80% methanol to 15 min at a flow rate 1 ml/min. Retention time of agaritine was about 3.3 min. The agaritine was detected at 237 nm (maximum of absorption spectrum). Under experimental conditions described for sample preparation the limit of detection was 0.2 mg/kg of mushrooms and the repeatability of measurement as the relative standard deviation was 4.2%.

RESULTS AND DISCUSSION

Generally, all the procedures that were investigated (boiling in water, frying in oil, microwave heating, drying, dry baking, freezing and thawing of frozen product) resulted in a significant decrease of parent toxin (Table 1).

Table 1. The influence of storage and household processing on the agaritine content

Process	Conditions	Time	Decrease of agaritine*
Drying	22°C	28 hours	35%
		6 hours	30%
Drying	40°C	24 hours	33%
		6 hours	29%
Drying	70°C	6 hours	86%
Freezing without thawing	−18°C	30 days	41%
Freezing with thawing	−18°C	30 days	77%
Cooking	boiling water	5 min	66%
Cooking	boiling water	60 min	88%
Dry baking	200°C	10 min	23%
Frying	sunflower oil 180°C	10 min	57%
Microwave heating	1000 W 2450 MHz	1 min	65%

*) 100% = content in fresh mushrooms before processing

With exception of dry baking which only reduced the phenylhydrazines in the mushroom to around 75% of the original content, household processing of fresh cultivated mushroom commonly reduced the agaritine level by as much as 75–55% of the original content.

Taken together, these data show that the consumer of household processed cultivated mushroom might be still exposed to substantial quantities of agaritine. Regarding mushroom-based products from retail market, the lowest exposure to agaritine occurs when canned products are consumed. Typically less than 10% of original agaritine content in fresh mushrooms (200–400 mg/kg), i.e. 10–30 mg/kg, can be determined in such products (3, 7). A comparison of concentrations in mushrooms and brine indicates a near-equilibrium situation between solid and liquid phases achieved during can storage. Also storage of freshly harvested fruit bodies in the refrigerator resulted in a reduction in agaritine content.

Considering all the available data, it can be assumed that sporadic eating of *Agaricus* sp. as a part of a varied diet carries a minimal risk and is of little toxicological concern. On the other hand it would be highly undesirable if the mushrooms are taken as a substantial part of a special diet, for example a slimming diet; such excessive consumption should be avoided.

Acknowledgment: This study was carried out within the project No. MSM 223300004 supported by the Ministry of Education, Youth and Sports of the Czech Republic.

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Chlorinated Propanols and Levulinic Acid in Soy Sauces

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Keywords: chlorinated propanols, 3-chloro-1,2-propanediol, 1,3-dichloropropanol, levulinic acid, hydrolysed protein

INTRODUCTION

Soy sauce is made by the fermentation of a mixture of soyabean and wheat flour. The growing market demand for soy sauce has led to attempts to speed up the fermentation by using acid hydrolysis of the soya protein, but this gives an inferior product. As a compromise several soy sauces are prepared as a blend of traditionally fermented and acid hydrolysed products (1).

Traditionally fermented sauces, not containing acid hydrolysed protein, may be regarded as a premium product and command higher prices. There is therefore a need for tests for the authenticity of such sauces. The measurement of levels of levulinic acid, produced by the action of acid on the sugars present, has been examined and proposed for this purpose. The Chinese National Standard regulates a levulinic acid level in naturally fermented sauces of less than 1.0 mg/ml (2).

Acid hydrolysis also leads to the formation of toxic chlorinated propanols from the reaction of hydrochloric acid with glyceride residues in the vegetable material (3). The major chloropropanol, 3-monochloropropanediol (3-MCPD) has been declared a genotoxic carcinogen by the European Union's Scientific Committee for Food (4). Another significant chloropropanol, 1,3-dichloropropanol has been found to be carcinogenic (5). Both of these chloropropanols have been found in acid hydrolysed vegetable protein (HVP), however industry practices have led to a major reduction in levels of chloropropanols in HVP in recent years.

In 1999 European Union Rapid Alert System for Foodstuffs reported that soy sauces in Denmark, Germany, Sweden and the Netherlands had been found to contain 3-MCPD at levels up to 124 mg/kg (6). A survey carried out in the UK this year reported levels of 3-MCPD in soy sauces of up to 30 mg/kg (7).

We have analysed a limited number of soy sauces containing a range of 3-MCPD concentrations in order to compare the levels of 3-MCPD, 1,3-DCP and levulinic acid.

METHODS

3-MCPD was determined by the Association of Official Analytical Chemist's Official First Action method (8).

1,3-DCP was determined using a new automated headspace gas chromatographic technique coupled with mass spectrometric detection. The method used direct injection onto a J&W 30M × 0.25 mm 0.25 μm DBWax column and incorportaed a deuterium labelled internal standard. It has been fully tested and shown to have a limit of detection of better than 0.005 mg/kg. Full details of the method and its validation will be published later.

Levulinic acid was determined using the method of Wang *et al.* (2).

RESULTS

The quantitative results are given in the Table 1.

Table 1. Levels of 3-MCPD, 1,3-DCP and levulinic acid in soy sauces.

Sample	3-MCPD [mg/kg]	1,3-DCP [mg/kg]	Levulinic acid [mg/ml]	Sample description
A	< 0.01	< 0.005	1.5	
B	< 0.01	< 0.005	2.0	
C	< 0.01	< 0.005	2.00	Naturally fermented
D	0.01	< 0.005	7.5	
E	0.01	< 0.005	2.1	Naturally fermented
F	0.2	< 0.005	1.4	
G	0.5	< 0.005	0.7	
H	1.6	< 0.005	2.5	
I	4.5	< 0.005	5.4	
J	15	0.01	5.1	With hydrolysed soya
K	42	2.62	4.4	Naturally fermented
L	46	0.59	5.5	
M	78	4.13	2.9	
N	100	4.28	4.1	

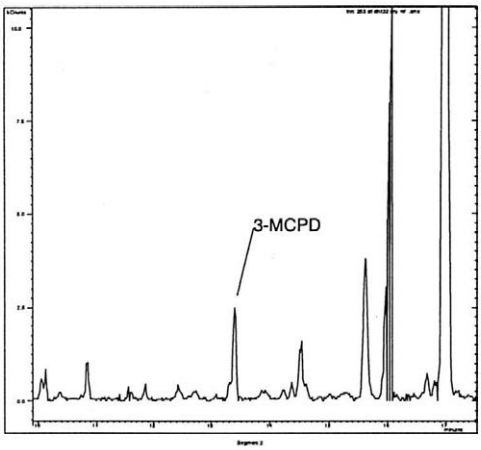

Figure 1. Reconstructed ion chromatogram at m/z 253 showing 3-MCPD in sample K

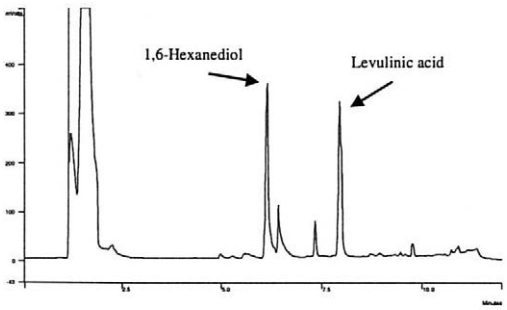

Figure 3. Levulinic acid in sample K

Figure 1 shows the reconstructed ion chromatogram at m/z 253 for sample K containing 42 mg/kg 3-MCPD.

Figure 2 shows the reconstructed ion chromatogram at m/z 79 for sample K containing 2.6 mg/kg 1,3-DCP.

Figure 3 shows levulinic acid at 4.4 mg/ml in sample K, and the internal standard (1,6-hexanediol)

DISCUSSION

Three samples (A to C) contained no detectable chloropropanols. Six samples (D to I) contained 3-MCPD but no detectable 1,3-DCP. Five samples contained both 3-MCPD and 1,3-DCP.

A single sample (J) contained a high level of 3-MCPD (15 mg/kg) but a low level of 1,3-DCP (0.01 mg/kg). This sample was labeled as containing hydrolysed soya protein. This could have been added as an HVP preparation. We have found that HVP generally contains less than 0.05 mg/kg 1,3-DCP.

Samples K to N contained high levels of both 3-MCPD (42 to 101 mg/kg) and 1,3-DCP (0.6 to 4.3 mg/kg). We therefore assumed that they contain a significant proportion of acid hydrolysed soyabean and/or wheat protein.

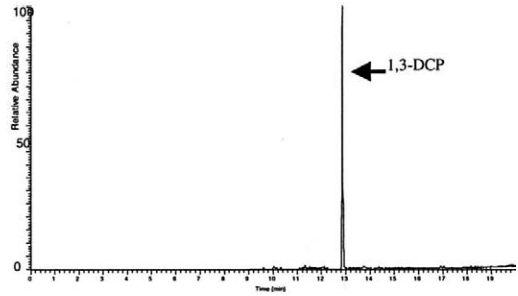

Figure 2. Reconstructed ion chromatogram at m/z 79 showing 1,3-DCP in sample K

The levulinic acid levels are in agreement with those previously reported for soy sauces (2). Only a single sample (G) contained less than the CNS regulatory limit of 1 mg/ml levulinic acid. This sample contained 0.5 mg/kg 3-MCPD which was likely to be due to the addition of acid HVP as no 1,3-DCP was detected.

Three samples (C, E and K) were labeled as being naturally brewed or fermented. Each of these contained levulinic acid in excess of the CNS limit although C contained no chloropropanols and E only 0.01 mg/kg 3-MCPD. Sample K contained high levels of both 3-MCPD (42 mg/kg) and 1,3-DCP (2.6 mg/kg) and also had a higher level of levulinic acid (4.4 mg/kg). This sample clearly contained acid hydrolysed material.

The results suggest that detection of chloropropanols might be a better indicator of acid hydrolysis of soy sauces than levulinic acid determination. It has also been shown that some sauces contain unacceptable levels of the carcinogen 1,3-DCP in addition to the previously reported 3-MCPD.

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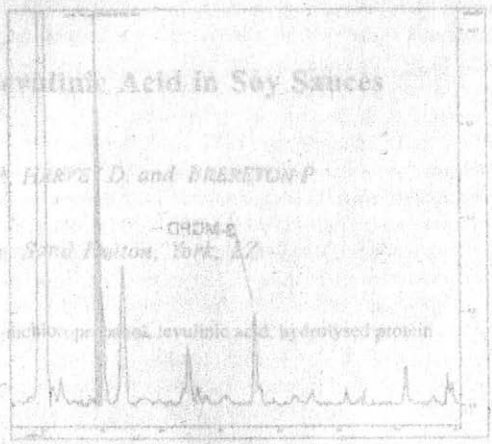

Last Minute

Optimisation of the Synthesis of Cross-Linked Amino Acids and their Nuclear Magnetic Resonance Characterisation

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The thermal treatments necessary for the industrial production and the microbiological stabilisation of foods, may modify the structure of the side chains of food proteins. Very often these processes are due to the Maillard reaction. However, also without participation of sugars, some amino acids can undergo leaving group elimination to give reactive unsaturated intermediates which can undergo nucleophile addition to give the so called cross-linked amino acids (1, 2). Thus a dehydroalanine residue may be formed through elimination of a leaving group from serine, *O*-phosphorylserine, *O*-glycosylserine, and cystine and may undergo Michael addition by another nucleophilic amino acid residue. For example when lysine is in a suitable position, its e-amino group reacts to give a secondary amine which is normally indicated with the trivial name of lysinoalanine (LAL). The principal characteristic of this compound and its analogues is that they are stable in the conditions of acidic protein hydrolysis, and are relatively easy to analyse in respect to other compounds, such as isopeptides.

The only cross-linked amino acid that has been studied extensively in foods is LAL, but analogous reactions can involve ornithine to give ornithinoalanine (OAL), cysteine to give lanthionine (LAN), and histidine to produce histidinoalanine (HAL). Moreover also threonine can eliminate to produce dehydroaminobutyric acid (i.e. methyldehydroalanine) that reacts with the same aminoacids to give methyl-lysinoalanine (Me-LAL), methyl-ornithinoalanine (Me-OAL), methyl-lanthionine (Me-LAN), and methyl-histidinoalanine (Me-HAL), respectively.

The lack of commercial standards has prevented the development of systematic studies on the formation of the other cross-linked aminoacids in foods and on their impact on protein digestibility. We have therefore developed an improved method for the synthesis of these cross-linked amino acids.

All these compounds are formed in foods as diastereomeric mixtures because the enatiomeric pure nucleophile can attack dehydroalanine on both sides. Thus, for example, LAL is a mixture of two diastereoisomers: *S*-lysino-*S*-alanine (*S,S*-LAL) and *S*-lysino-*R*-alanine (*S,R*-LAL), and OAL is a mixture of *S*-ornithino-*S*-alanine (*S,S*-OAL) and *S*-ornithino-*R*-alanine (*S,R*-OAL) being the configuration of lysine and ornithine, respectively, preserved. We have developed a method for the determination of the diastereoisomeric ratio based on ^{13}C-NMR (3). This method is particularly reliable because it does not require any derivatisation. The aim of the work was to know exactly the diastereoisomeric ratio of the standards to be used for the quantification in foods. This has a nutritional relevance because it has been demonstrated that the two diastereoisomers of LAL have different affinity to copper (II) and cobalt (II). The greater nephrotoxicity of *S,R*-LAL have been related to its higher Cu(II) affinity (4).

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Lysinoalanine in Solutions for Enteral Nutrition and Infant Formulas

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Most industrial foods are thermally treated to assure their microbiological stabilisation and to obtain specific sensory properties. During these processes the amino acids can undergo modification in their side chains. For example, elimination of a leaving group from *O*-phosphoryl-serine, *O*-glycosylserine, and cystine generates dehydroalanine residues, which may undergo Michael addition by another nucleophilic amino acid residue. Thus, when lysine is in a suitable position, its e-amino group reacts to give a secondary amine that is normally indicated with the trivial name of lysinoalanine (LAL). This compound is relatively easy to analyse in respect to others, such as isopeptides, because it does not decompose in the acidic conditions of protein hydrolysis.

These reactions do not involve the participation of sugars and are particularly extensive when the proteins are submitted to aqueous alkali treatments, such as those used in preparing soy protein concentrates or while recovering proteins from cereal grains and milling by-products, oilseeds, such as cottonseeds, peanuts, safflower seeds and flaxseeds, and dairy products, such as sodium caseinate. Other alkali procedures are used for destroying microrganisms, for preparing peeled fruits, and to induce fiber-forming properties in textured soybean foods (vegetable soy proteins used, for example, as meat analogues).

LAL reduces the digestibility of proteins treated with alkali (1), has some nutritional consequences (2, 3), and was demonstrated to promote lesions in rat kidney cells causing nephrocitomegaly (4).

Great attention has been paid in the past on the presence of LAL in infant formulas, this has induced industry to look for better techniques for thermal stabilisation of liquid formulas (5), while spray dried ones are less critical.

A careful check of the literature has shown that the solutions for enteral nutrition had never been submitted to analysis of LAL residues. We have, therefore, collected some commercial samples and submitted them to analysis, which were performed with a HPLC method (6) proposed very recently to determine the addition of casein or caseinates in natural Mozzarella cheese. This method is based on derivatisation with 9-fluorenylmethylchloroformate (FMOC-Cl), solid phase extraction, reverse phase chromatography and fluorescence detection.

The values encountered were in the range 150-800 μg/g protein, much higher than those found in infant formulas. The few samples that were labelled as treated with a UHT methodology had relatively low values. Taking into account the reduced digestibility and essential amino acids availability, it would be very useful to try to decrease the thermal treatments of these products that are consumed by patients in severe physical conditions.

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Kinetic Modeling
A Tool to Understand Maillard Reaction Mechanisms

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Quite often food scientists have tried to fit a zero-, first- and second order reaction or even a model with a fractional order, to their data. However, the order of a reaction is a parameter that gives a mathematical description of time- or concentration-dependence, it does not necessarily give information on the reaction mechanism. If we attempt to unravel and explain a particular reaction in more detail, we should propose a reaction mechanism. This is another approach to kinetic modelling (1). The following steps should be taken into account:

- Differentiate between primary and secondary reaction routes
- Determine critical reaction conditions (pH, temperature etc.)
- Determine the effect of reactant concentrations and calculate the mass balance
- Propose a reaction mechanism
- Build a model by setting up differential equations based on the network of the proposed mechanism. The coupled differential equations can be solved by numerical integration using a suitable algorithm.

Various factors influence the Maillard reaction and they can be considered as product and processing variables. By manipulating these variables, the balance of the various chemical pathways making up the Maillard reaction changes. As an example, the Maillard reaction between glucose and glycine may demonstrate this point in more detail. Based on the assumption that Amadori product is a main intermediate of the Maillard reaction (2 and on experimental data, an initial kinetic model was proposed (Fig.1). The brown colour measured in terms of absorbance of the reaction mixture, was related to the advanced Maillard products also known as melanoidins [M], by the molar extinction coefficient E, Abs = E[M], considering 1 cm measuring cells (3). The model fits the trend in the data, however the fit is certainly not satisfactorily, especially in what colour is concerned (Fig. 3a). Moreover, a considerable pH drop was observed.

Tressl (4) stated that under acidic conditions the major pathway in browning is the 3-deoxyosulose route. In the browning reaction of glucose, the key intermediate would therefore be the 3-deoxyhexosulose (DH), which is formed, from the 1,2-enaminol precursor of Amadori compound and not the Amadori compound itself. As a result an extra pathway, characterised by k_8 was included and a new kinetic model was proposed (Fig.2). A major improvement on the fit was obtained (Fig. 3b).

Kinetic modeling appears to be a powerful tool to model complicated consecutive and parallel reactions in foods. It is both helpful for deriving relevant kinetic parameters as well as for obtaining insight into reaction mechanisms. Here a major guidance in understanding the reaction mechanism was given.

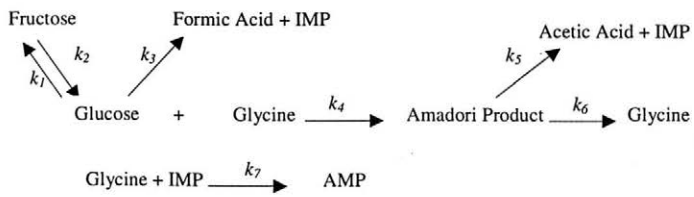

Figure 1. Initial Kinetic model of glucose/glycine Maillard reaction (IMP – Intermediate Maillard Products; AMP – Advanced Maillard Products)

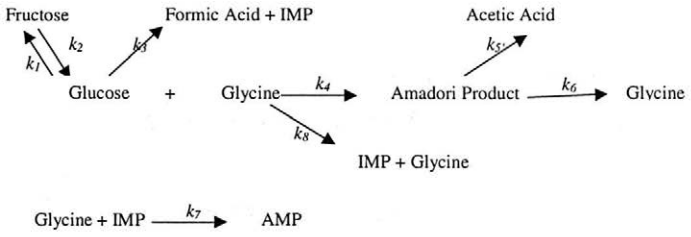

Figure 2. Improved Kinetic model of glucose/glycine Maillard reaction (IMP – Intermediate Maillard Products; AMP – Advanced Maillard Products

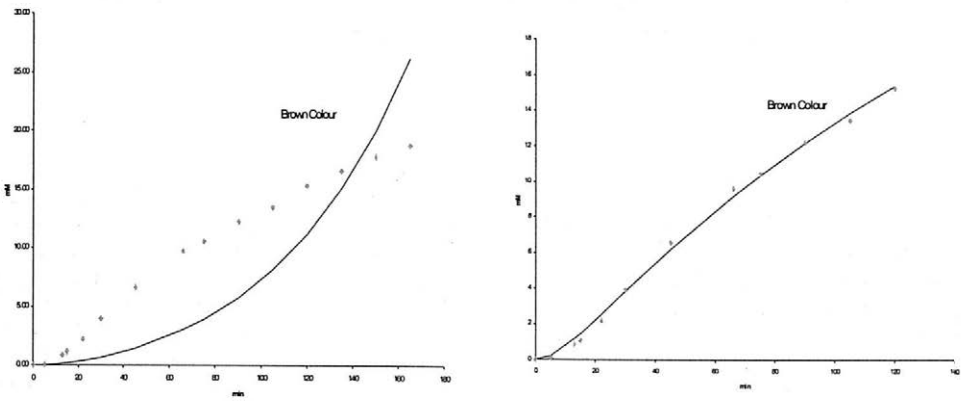

Figure 3. Comparison of the fits, of brown colour, a) before and b) after adding the step k_8

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Influence of Ascorbic Acid Infusion on Lipid Oxidation in Frozen Asparagus

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Abstract

The present study examined the effect of ascorbic acid infusion and/or blanching on hexanal formation in frozen asparagus, as well as their effect on the flavour of asparagus. Ascorbic acid was introduced into the asparagus by vacuum-infusion prior to freezing. Ascorbic acid contents and hexanal contents were determined in the fresh asparagus spears and in the spears after 5 and 7 weeks of storage at –18°C. Acceptability of flavour and appearance was determined after 7 weeks of frozen storage. Infusion increased ascorbic acid contents considerably, but ascorbic acid levels decreased during storage for all treatments. Lipid oxidation rates varied among the treatments, showing lowest rates for the combination treatment of infusion and blanching and highest for the non-treated asparagus spears. High flavour scores related to low hexanal contents and high ascorbic acid contents. Ascorbic acid infusion and blanching reduced lipid oxidation rates considerably, resulting in lower concentrations of volatile secondary lipid oxidation products and improved flavour and appearance characteristics.

Keywords: flavour; lipid oxidation; asparagus; infusion; ascorbic acid

INTRODUCTION

The vegetable asparagus is an attractive vegetable, but also one of the most perishable products shipped commercially (1). Even during refrigerated storage, asparagus has a high rate of respiration which results in a maximum fresh storage life up to four weeks (2). Frozen storage would be an interesting alternative for refrigeration, and would allow more time between harvesting and processing or consumption. However, it is known that asparagus is susceptible to lipid oxidation and consequently off-flavours develop very rapidly in frozen asparagus. Ascorbic acid is a natural antioxidant, which acts as an oxygen scavenger and exerts chelating action (3). Introduction of ascorbic acid into asparagus is thought to be useful as it might reduce lipid oxidation rates and enhance the flavour of frozen asparagus. In the present study the effect of infusion of ascorbic acid and blanching on lipid oxidation rates and sensory characteristics of asparagus was examined.

EXPERIMENTAL

Materials and treatments. Green asparagus spears were obtained from a local producer in Nebraska (USA) immediately after harvesting and were divided in four groups. A control (non-treated) sample was frozen (–18°C) without any further treatment (Control). The three other groups were infused with a 1% ascorbic acid solution (Inf), or blanched for 4 minutes (Blanch), or both infused and blanched prior to frozen storage (Inf-blanch). For infusion 500 mL 1% ascorbic acid solution was placed in a tray. The asparagus spears were placed in the solution and vacuumised at 100 mbar at room temperature for 3 minutes. Subsequently, the spears were left submerged in the solution to allow infusion. Each treatment group (Control, Inf, Blanch, Inf-blanch) was divided in three parts. The first part was immediately analysed for ascorbic acid and hexanal content. The two other groups were stored at –18°C for 5 and 7 weeks and subsequently analysed.

Ascorbic acid analysis. Asparagus tips (cut 5 cm from the tip; 25 g) were blended with 100 mL oxalic acid solution (0.5%; Aldrich, Milwaukee, USA). The extract was centrifuged at 2000 rpm for 5 minutes. The supernatant was titrated with an indophenol solution (50 mg 2,6 dichloro-indophenol Na salt and 42 mg Na_2CO_3 in 300 mL water) until a rose-pink colour persisted for at least 5 seconds. Six ascorbic acid solutions varying in concentration (Ascorbic acid: Aldrich, Milwaukee, USA) were used for calibration. The ascorbic acid content of each stored sample was determined in two replicate measurements.

Hexanal determination. Asparagus tips (40 g) were blended with 150 mL 0.5 % NaCl solution for 2 minutes.

Hundred mLs of the mixture were transferred to headspace vials (125 mL), which were placed in a 40°C water bath for 45 min to allow equilibration. For static headspace analysis 2 mL of the headspace was injected onto the OV-101 packed column (10%, 80/100 mesh; Supelco, USA), 3.0 m length, 0.32 mm i.d. of a Hewlett Packard gas chromatograph (Model 18835B; Kansas City, USA). The GC was equipped with a flame ionisation detector at 350°C. The GC oven temperature was kept at 140°C throughout the analysis. Six hexanal in 0.5% NaCl solutions varying in concentration were used for calibration. Two replicate measurements of each stored sample were carried out.

Acceptability. Tips of asparagus spears were evaluated by 10 assessors of an experienced panel. They rated levels of perceived acceptability for both flavour and appearance using a 9-point hedonic scale (1: dislike extremely; 9: like extremely).

RESULTS AND DISCUSSION

The effect of infusion of ascorbic acid and blanching on lipid oxidation rates and sensory characteristics was examined directly after treatment, and after 5 and 7 weeks of frozen storage. Infusion increased the ascorbic acid content of the fresh asparagus considerably (Fig. 1). Infusion resulted in an 8 % weight increase as well. Blanching reduced both the originally present and the infused ascorbic acid, as expected. During storage ascorbic acid contents decreased for all treatments and was nearly depleted in the control sample after 7 weeks of storage. Maximum ascorbic acid retention after storage was observed for the combined infusion and blanching treatment.

The changes in hexanal content of the asparagus spears during storage are presented in Fig. 2. Before storage hexanal concentrations are extremely low. Hexanal formation in the infused spears hardly differed from the formation in the control sample, which indicates that lipid oxidation rates were rather similar. Infusion as single treatment affected lipid oxidation only slightly. Blanching reduced lipid oxidation rates considerably, obviously enzymatic formation of volatiles is an important factor. However, hexanal content still increased in some degree during storage. Therefore, autoxidation seems to play a role as well. The combination treatment (infusion and blanching) gave best results, hexanal could not be detected in the asparagus spears throughout the storage period.

Evaluation of the flavour and appearance characteristics of the asparagus after 7 weeks of storage revealed considerable differences between the treatments (Table 1). The combination of infusion and blanching resulted in highest flavour scores (5.0). The non-treated asparagus spears were rated lowest (0.8). Appearance showed similarity with flavour. Flavour scores and hexanal content were significantly (negatively) correlated (Spearman's ranked correlation test, $p<0.05$), showing that hexanal is a reasonable indicator for off-flavour formation. In addition,

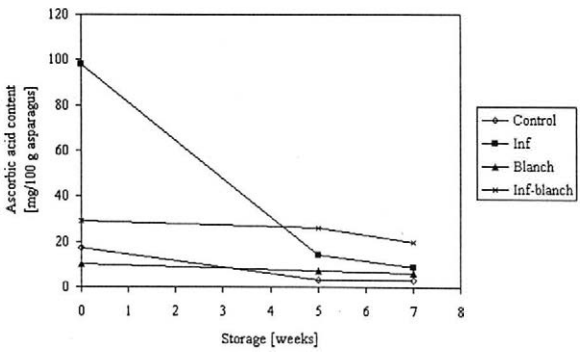

Figure 1. Effect of infusion (Inf), blanching (Blanch) and a combination of both treatments (Inf-blanch) on the ascorbic acid content of asparagus tips after frozen storage

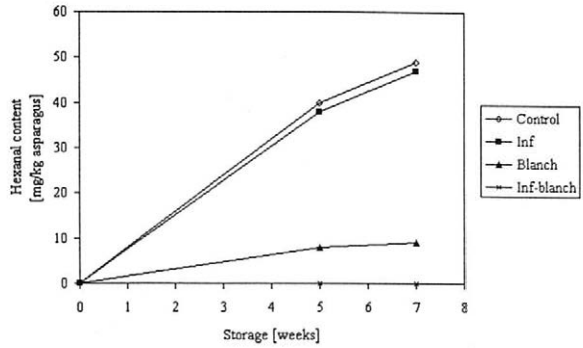

Figure 2. Effect of infusion (Inf), blanching (Blanch) and a combination of both treatments (Inf-blanch) on the hexanal content of asparagus tips after frozen storage

Table 1. Effect of infusion (Inf), blanching (Blanch), and a combination of both treatments (Inf-blanch) on the acceptability of flavour and appearance of asparagus tips after frozen storage (1: dislike extremely; 9: like extremely)

	Flavour	Appearance
Control	0.8	3.9
Inf	1.4	5.0
Blanch	4.0	4.4
Inf-Blanch	5.0	5.9

hexanal content and ascorbic acid content showed a significant negative correlation (Spearman's ranked correlation test, $p<0.05$).

In conclusion, ascorbic acid infusion and blanching reduced lipid oxidation rates, resulting in lower concentrations of volatile secondary lipid oxidation products and improved flavour and appearance characteristics.

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Kinetic Modelling of the Maillard Reaction

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INTRODUCTION

The Maillard reaction is a complex reaction starting with the condensation of reducing sugars with free amino groups of amino acids or proteins. In order to be able to control complex chemical reactions they need to be studied in a quantitative way. With knowledge of kinetics, it becomes possible to describe the changes quantitatively and to predict changes from certain time-temperature combinations. Kinetics is also a tool for understanding reaction mechanisms.

In the present study, a kinetic model was developed for the reactions occurring in monosaccharide-casein systems and extensively tested for different heating temperatures.

EXPERIMENTAL

A reducing sugar (150 mM of glucose or fructose) and sodium caseinate (3% w/w) were dissolved in a phosphate buffer (0.1 M, pH 6.7). These model systems were heated in closed glass tubes in an oil bath at 100, 110 or 120°C.

The concentration of melanoidins was calculated from the absorbance (measured at 420 nm) using the extinction coefficient of 1000 $mol^{-1}l\,cm^{-1}$.

Multiresponse modelling was performed by setting up differential equations for the various reaction steps, numerically solving them and fitting them to the data. The parameters of the model were estimated by non-linear regression using the determinant criterion.

RESULTS AND DISCUSSION

The main reaction products and reaction routes, which were identified in the heated sugar-casein systems, are summarised in the model of Fig. 1. Besides their taking part in the Maillard reaction, glucose and fructose are also subject to isomerisation and degradation reactions. The kinetic model was fitted to the experimental data of the glucose-casein and fructose-casein systems heated at 120°C. In general, the fits were very good. Only a minor lack of fit was observed for the formation of formic acid in the fructose-casein system. This fit could be improved by the assumption that formic acid formation was catalysed by the protein.

The heating temperature will have an effect on the rate of the various reactions. In order to be able to predict the reactions at various temperatures, the temperature dependence has to be determined. The relationship between the rate constant (k) and temperature (T) is frequently indicated by the well-known Arrhenius equation:

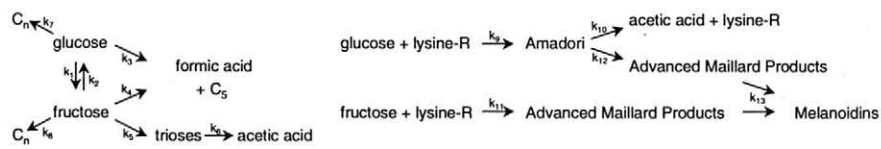
$$k = k_0 \exp(-E_a/RT)$$

where: k_0 – the so-called pre-exponential factor
R – the gas constant (8.31 $J.mol^{-1}K^{-1}$)
E_a – the activation energy

In stead of the rate constants, the activation energies were estimated when fitting the model to the data of the systems heated at different temperatures.

The model fitted the sugar-casein reactions at different heating temperatures very well. The only exception was the loss of available lysine when the fructose-casein system was heated at 100°C: the lysine residues did not decrease until 60 minutes, while the model predicts lysine to decrease immediately.

Therefore a new model was proposed (see Fig. 2) in which unidentified reaction compounds (C_n and C_5) react with lysine residues and form AMP. This adapted model fitted the lysine loss in the fructose-casein systems heated at 100°C much better, while no significant difference was



Figue 1. Reaction products and reaction routes identified in the heated sugar-casien systems

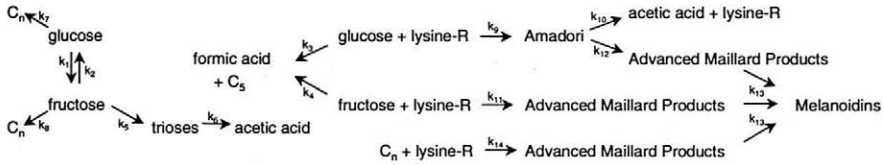


Figure 2. New model of the sugar-casein reactions

observed at higher temperatures. The activation energies, which were calculated for every reaction step, were around 120 kJ/mol (as expected for chemical reactions). It was observed that the reaction between casein and fructose is much more temperature dependent (E_a is 175 kJ/mol), and therefore less important at lower temperatures, than the reaction between intermediate reaction compounds and the protein.

ORAL PRESENTATION – SUPPLEMENT

Monitoring Aroma Generation by the Maillard Reaction in Real Time

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Abstract

Atmospheric Pressure Chemical Ionisation-Mass Spectrometry (APCI-MS) monitored the volatiles produced by the Maillard reaction in skimmed milk powder (SMP), in real time. The effect of different reaction conditions on the amount and rate of aroma generation was measured.

Keywords: Maillard, continuous; aroma; skimmed milk powder

INTRODUCTION

The Maillard reaction is a complex set of reactions leading to the production of colour and flavour in food products. Formation of products is affected by reaction conditions (e.g., time, temperature and moisture content). Understanding the effect of these conditions on flavour production allows control of the balance of flavour products and minimises undesirable flavours.

The aim of this work was to develop a method to monitor volatile production with time, in a heated solid food system. Kinetic data could be obtained by monitoring many products simultaneously.

In the past, the Maillard reaction has been monitored by measuring a single indicator, such as depletion of starting materials (1), water production (2) or colour development (1, 3). These factors may not necessarily be related to the production of specific flavour compounds, which may be identified and quantified by extraction and GC-MS analysis (4, 5). However, these methods are not well-suited for kinetic investigations. An on-line method for following the Maillard reaction in a liquid system has been developed previously (6).

This paper describes the use of direct APCI-MS to monitor volatiles in the headspace above heated SMP. There is no chromatography, so real-time quantification of products in the headspace above a sample is achieved. The method was employed to investigate reaction at different heating temperatures and moisture contents.

EXPERIMENTAL

Sample preparation. Water content of SMP samples was varied by storage of the dry material above saturated salt solutions and measured by gain in mass.

APCI-MS. APCI-MS was operated in positive ion full scan mode for the mass range 30–180 (scan time 10 s; inter-scan delay 0.02 s). Data was collected using MassLynx software. Headspace (5 ml/min) was drawn continuously for 60min into the APCI ionisation source from a glass bottle containing the SMP sample. Samples were heated between 70 and 120°C, with water contents between 0 and 8.3g water/100g dry solids.

RESULTS AND DISCUSSION

The APCI-MS method gave a time-intensity profile from the headspace above each heated SMP sample (Fig. 1). It was possible to simultaneously follow the generation of many reaction products, then distinguish them on the basis of both the amount and profile of production. The total ion chromatogram (TIC, Fig. 1A) represents all volatiles in the headspace and this remained fairly constant. Individual compounds were identified, including acetone, acetic acid, diacetyl, acetoin, furfural and furfuryl alcohol. These showed differences with time. For example, acetone reached a maximum early on in the heating period, before declining in intensity (Fig. 1B). In contrast, furfural only started to increase towards the end of an hour (Fig. 1C).

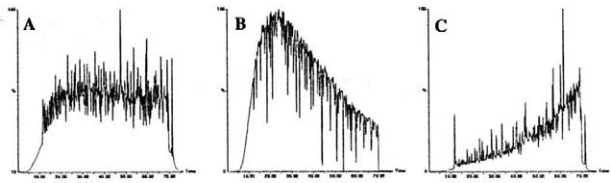

Figure 1. APCI time-intensity profiles for SMP with moisture content 4.6 g/100g dry solids, heated at 90°C. A – TIC; B – acetone; C – furfural

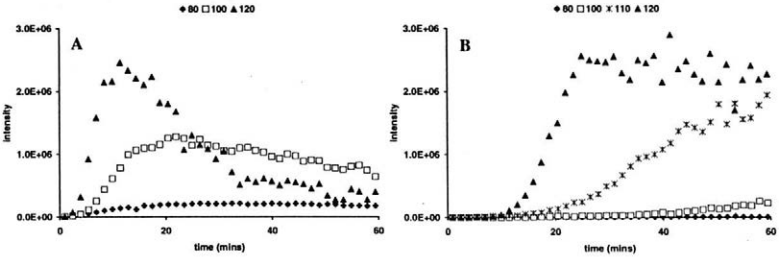

Figure 2. Time-intensity plots showing production of acetone at three temperatures (A) and furfural at four temperatures (B)

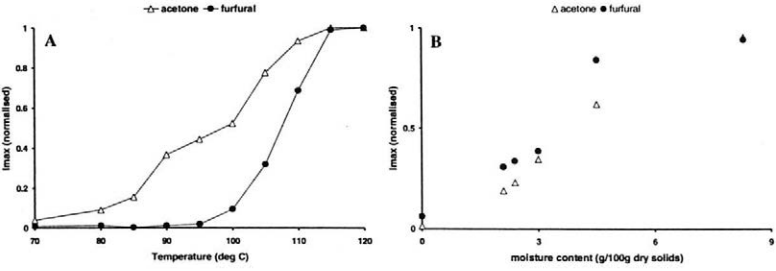

Figure 3. Increase in Imax (normalised to highest value for each compound) with temperature (A) and moisture content (B) for acetone and furfural

The APCI measurements also showed the effect of reaction conditions on the amount of volatiles produced. Fig. 2 shows the effect of temperature on acetone and furfural production. The data were used to calculate the maximum intensity (I_{max}) reached for each compound at each temperature, which was then normalized for easy comparison (Fig. 3A). Imax increased with temperature for each compound, but at different rates. The amount of acetone increased over the whole temperature range, whilst furfural started to increase only above 95°C. These differences may reflect different activation energies for the different compounds, and/or the stage of the Maillard reaction at which they are produced. The effect of initial moisture content on the production of acetone and furfural (at 90°C) is shown in Fig. 3B. The amounts of both compounds increase over the moisture range studied.

The APCI-MS technique is limited to smaller, more volatile Maillard reaction products and therefore does not represent the full flavour profile of the heated SMP. Due to the lack of chromatography, compounds with the same ion mass cannot be monitored separately. However, the method is powerful in that it can provide real time profiles of volatile production during heating a solid system. It enables investigation of the effect of reaction conditions on the rate and amount of aroma generation and hence has the scope for more detailed kinetic analysis. Further experiments are underway with other systems based on SMP.

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INSTRUCTIONS FOR AUTHORS

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New knowledge on chemical reactions during processing and storage of foods

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