ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

POTRAVINÁŘSKÉ VĚDY

FOOD SCIENCES

3

ROČNÍK 11
PRAHA 1993
CS ISSN 0332-0568

ČESKÁ AKADEMIE ZEMĚDĚLSKÝCH VĚD
SLOVENSKÁ AKADÉMIA PÔDOHOSPODÁRSKYCH VIED

POTRAVINÁŘSKÉ VĚDY
FOOD SCIENCES

This journal is covered by Agrindex of FAO (AGRIS database), Food Science and Technology Abstracts, Dairy Science Abstracts, Chemical Abstracts, WLAS, TOXILINE PLUS and Czech Agricultural Bibliography.

Contact address: Slezská 7, 120 56 Prague 2, Czech Republic, tel. 251 098

Potr. Vědy, *11*, 1993 (3) : 161-256

Potr. Vědy, *11*, 1993 (3) : 161-162

FOOD QUALITY AND ITS ASSESSMENT IN THE EDUCATION OF FOOD SCIENTISTS

The quality is defined as the degree, in which the product or the service fulfils the consumers' requirements. Food quality becomes more and more important in the developed industrial societies. The requirements are rapidly growing with the increasing life standard and cultural level.

In food products, the quality is often reduced to the food safety which is, however, only a very small part of the quality. The food quality includes, naturally, the nutritional value, which is the main reason, why food is purchased and consumed. Other, practical aspects of quality are very important as well, such as the degree of finalization, packaging, labeling, availability or price.

Psychological, social or philosophical aspects of food quality are often neglected by food scientists. Among the psychological characteristics, the sensory value is, without doubt, the most obvious one as all consumers may easily assess the sensory quality, which is then often the most critical factor in their decision on future purchase. In addition of laboratory methods of sensory analysis, consumer studies are usually necessary. Other psychological factors should be considered as well, mostly of emotional character. Ecological foods (bioproducts) belong to this group more than to the aspects of safety.

Among the social factors, the social status of food, the servicing, environment, tradition, examples of socially active citizens, education, advertisement and similar factors influence the consumers' requirements and thus the quality.

Personal philosophy of the consumer (or a group of consumers), ethical and esthetical opinions, taboo, etc., influence his or her requirements and the quality sometimes more than other factors.

The food quality has thus a very complex character, and therefore, the quality assessment has a complex, multidisciplinary character as well. The determination of food safety includes various biological, particularly microbiological methods, and several instrumental methods. The physiological and histological examination is often necessary. The determination of rheological and other physical properties of food products is generally desirable.

The determination of the nutritional value does not mean only the content of nutrients, but also their availability and interactions. In many cases, experiments with laboratory animals or epidemiological studies cannot be avoided. Very complex procedures are necessary for the control of the origin of raw materials, processing and storage conditions, but they are necessary to control the proper labeling.

Psychological methods are rapidly developing, especially methods of the sensory analysis, but other psychological characteristics should be studied as well. Sociological methods have to be included in the food quality assessment, often based on consumer studies.

The quality assessment is very expensive and time consuming, therefore, chemometrics should be applied as much as possible, especially in the sampling. Statistical methods may help to reduce cost and to save time on one hand, and to better utilize the information obtained by the analytical operations on the other hand.

In the education of food scientists, the above considerations should be taken into account. The education should not include the training in instrumental and microbiological methods only. Other biological, enzymic, and physiological methods are necessary, and psychological and sociological methods are important, and have to be included in the curriculum. Statistics and chemometrics are useful sciences for proper education of specialists in quality assessment.

Proper education for food quality assessment is thus multidisciplinary, very complex and difficult, and long training is necessary. Therefore, it is preferable to prepare specialized curricula for the quality assessors, and not to combine their education with that of food technologists, food engineers, veterinary scientists or agriculturists, who have difficult curricula themselves. The best procedure for a food quality assessor is to work in a team with food technologists, agriculturists and veterinary scientists, sometimes even with psychologists and sociologists, in order to achieve their task in the most satisfactory way.

Jan Pokorný

Potr. Vědy, *11*, 1993 (4) : 163-170

DETERMINATION OF CYANOGENIC GLYCOSIDES OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ) AND CASSAVA PRODUCTS

Marek DOLEŽAL, Sakiru A. FATAI, Michal VOLDŘICH[1], Jan VELÍŠEK

Institute of Chemical Technology – Department of Food Chemistry and Analysis and [1]Department of Food Preservation and Meat Technology, Technická 5, CS-166 28 Praha 6, Czech Republic

Hydrogen cyanide, both glycoside bound and non-glycosidic cyanide, in fresh cassava tissues and in processed cassava products employing a spectrophotometric (pyridine/barbituric acid reagent) and electrochemical method which uses a cyanide selective electrode has been determined. Results obtained by these two methods were essentially similar. Statistically significant higher values for fresh cassava samples and non-glycosidically bound cyanide were obtained by the electrochemical method. The accuracy of the methods differed only in the case of one fresh cassava sample. Both methods have shown that they are relatively simple, rapid, precise and capable of determining cyanide levels down to a level of about 1 mg per kg in either fresh cassava or processed cassava products.

cassava or manioc (*Manihot esculenta* Crantz); gari; cyanogenic glycosides; cyanogens; linamarin; hydrogen cyanide; analysis

Cyanogens occurring as secondary metabolites in plants consumed by humans and domestic animals are usually β-glycosides of 2-hydroxynitriles, called cyanogenic glycosides (C o n n, 1980; T e w e, I y a y i, 1989). More than 100 plant families have been shown to contain these substances and more than 20 cyanogenic glycosides have been reported in economically important higher plants in Rosaceae (primarily fruit kernels of the genus *Prunus, Cydonia, Sorbus* and *Malus*), Viciaceae (*Leguminosae; Phaseolus*), Euphorbiaceae (*Manihot*), Caprifoliaceae (*Sambucus*), Linaceae (*Linum*) and Poaceae (*Graminae; Sorghum*).

Cyanogenic glycosides readily undergo enzymatic hydrolysis by means of specific β-glucosidases (β-D-glucoside glucohydrolases, EC 3.2.1.21), which split off the sugar moiety of the glycoside. The resulting cyanohydrin dissociates by the action of hydroxynitrile lyase (EC 4.1.2.11) or spontaneously to the parent carbonyl compound and hydrogen cyanide.

The cassava or manioc plant (*Manihot esculenta* Crantz) is widely distributed in the tropics where its tubers have achieved considerable importance as a source of industrial and dietary saccharides for some 200 - 300 million people. Generally, sweet and bitter varieties of the plant are distinguished on the basis of a low and high level of cyanogens concentrated especially in the peel. The amount of cyanogens (expressed as hydrogen cyanide) generally ranges from 30 to 100 mg per kg while that of cassava peel often exceeds 2,500 mg per kg (W o o d, 1966). The popular cassava product gari contained hydrogen cyanide at a level of 13 - 62 mg per kg (I k e d i o b i et al., 1980). The minimum lethal dose of hydrogen cyanide taken orally by man is of the order of 0.5 - 3.5 mg per kg body weight. Occassionally, sufficient quantities of cyanogenic foods are consumed to cause fatal poisoning in humans. Chronic intoxications resulting in tropical ataxic neuropathy and enlargement of the thyroid gland (endemic goitre) due to the formation of thiocyanate (rhodanide) ions from cyanides by thiosulphate: cyanide sulphurtransferase, (rhodanase, EC 2.8.1.1) are very common. Precautions must be therefore taken to limit the levels of hydrogen cyanide in cassava and cassava products such as gari (a traditional food made of peeled cassava tubers which are grated, fermented, lightly fried and sometimes with the addition of a little palm oil), fuku and fufu in Africa, penjeum in Indonesia, farinha de mandioca in South America, etc.

The measurement of cyanide levels is of great importance to the plant breeders interested in breeding low cyanide cultivars, food technologists developing processing methods producing edible cassava products and toxicologists assessing the extent of cassava and cassava product toxicity.

A number of indirect and direct methods, varying in specificity, sensitivity and other attributes, are available for the determination of cyanogenic glycosides in plant tissues and some of these methods have been used for the analysis of cassava and cassava products. Most are based on the estimation of cyanide liberated by acid hydrolysis (B r a d b u r y et al., 1991) or using endogenous (native) β-glucosidase linamarase in fresh cassava or exogenous enzyme in processed cassava products (bitter almond β-glucosidase is inactive). The liberated cyanide is separated by steam distillation or aspiration and determined spectrophotometrically (W o o d, 1965; E s q u i v e l, M a r a v a l h a s, 1973; C o o k e, 1978; I k e d i o b i et al., 1980; N a m b i s a n, S u n d a r e s a n, 1984) or electrochemically using ion-selective electrodes or enzyme electrodes (B l a e d e l et al., 1971; V o l d ř i c h et al., 1990; R a s h e d et al., 1991). Gas-liquid chromatography (N a r t e y, 1968) and especially high-performance liquid chromatography using reversed-phase technique and amperometric detection of cyanides liberated with immobilized linamarase takes advantages of

the selectivity provided by liquid chromatography and sensitivity provided by an amperometric measurement (D a l g a a r d, B r i m e r, 1984; B r i m e r, D a l g a a r d, 1984).

The present paper deals with the development of two simple, rapid and sensitive methods, i.e. a spectrophotometric method using pyridine/barbituric acid reagent and an electrochemical method employing a cyanide-selective electrode, which would be suitable for the indirect evaluation of both glycosidic and non-glycosidic cyanide in cassava as well as in cassava products. The aim of this work was to simplify the already available methods and to compare the obtained results statistically.

MATERIAL and METHODS

Material and chemicals — Fresh cassava tubers (obtained one week after harvest) and cassava flour gari were bought at a local market in Lagos, Nigeria and kindly supplied by the Nigerian Embassy in Prague, Czech Republic. Linamarin (Sigma Chemical Co., USA), pyridine (Loba Chemie, Austria), barbituric acid, potassium cyanide and all the other chemicals (Lachema, Czech Republic) were commercial products.

Enzyme preparation — Partially purified linamarase was prepared from the peel of cassava tuber according to the method described by C o o k e et al. (1978) as simplified by N a m b i s a n and S u n d a r e s a n (1984). The obtained solution was stored at 4 °C in a refrigerator. Its activity was determined by adding different amounts (0.05 - 0.4 ml) of the enzyme preparation to 1 ml of phosphate buffers of different pH value (ranging from pH 5.5 to 6.5). The mixture was incubated for 15 min at 25 °C, 30 °C or 35 °C, the reaction was stopped by the addition of 1 ml sodium hydroxide solution (c = 0.2 mole/dm^3) and neutralized with hydrochloric acid (c = 0.2 mole/dm^3). The released hydrogen cyanide was determined spectrophotometrically. The minimum amount of enzyme required for complete recovery of linamarin as cyanide was 0.1 ml (0.4 ml of this preparation were used in all experiments), incubation time of 15 min at 30 °C was sufficient for complete reaction.

Apparatus — Spectrophotometric measurements were performed on a Unicam SP 1800 spectrophotometer (Pye Unicam, Great Britain), electrochemical measurements were performed using a selective cyanide electrode Selectrode F1042CN and a pH meter TTT2 (Radiometer, Denmark).

Extraction of cyanogenic glycosides from samples — Cassava root or gari samples (10 g) were homogenized in 30 ml of boiling 80% (v/v) ethanol, the resulting suspension was centrifuged, the supernatant transfered to a 100ml

volumetric flask and the solid residue extracted twice more with ethanol. The combined extracts were cooled to a room temperature and the flask was filled up using the same solvent. Aliquots of 0.5 to 2 ml were evaporated to dryness under reduced pressure. The residue was dissolved in 1 ml phosphate buffer (pH 6) and used for the determination of cyanogenic glycosides.

Spectrophotometric determination of cyanides — For the determination of the total cyanide content, 1 ml of a linamarin standard solution, or 1 ml of extracted cassava (gari) glycosides dissolved in phosphate buffer of pH 6 (see above), or 1 ml of these glycosides fortified with linamarin in a 25 ml volumetric flask was incubated with 0.4 ml of linamarase preparation for 15 min at 30 °C. At the end of the incubation period, 1 ml of sodium hydroxide (c = 0.2 mole/dm^3) was added followed by 1 ml hydrochloric acid (c = 0.2 mole/dm^3). After the addition of 1 ml of chloramine B solution (1%, w/w) and standing for 1 min, 3 ml of the pyridine/barbituric acid reagent (3 g of barbituric acid were dissolved in 30 ml of pyridine and 3 ml of concentrated hydrochloric acid and adjusted to 100 ml) and water up to 25 ml were added. The absorbancy of the solution was measured after 10 min of standing at 570 nm against water. The same procedure (the same amount of water was used instead of the enzyme preparation) was analyzed for the determination of non-glycosidic cyanide.

The calibration curve was constructed using buffered solutions of potassium cyanide prepared by dilution of a standard potassium cyanide solution (c = 0.1 mole/dm^3).

Electrochemical determination of cyanides — Solutions of linamarin, extracts of cassava and gari or fortified extracts in phosphate buffer of pH 6 were prepared according to the already described procedures. After the addition of sodium hydroxide and hydrochloric acid (see above) at the end of the incubation period, 30 ml of sodium hydroxide and 10 ml of phosphate buffer were added, the volume was adjusted to 50 ml and the content of cyanides was measured using cyanide selective electrode.

The calibration curve was constructed using buffered solutions of potassium cyanide prepared by dilution of a standard potassium cyanide solution.

RESULTS and DISCUSSION

Spectrophotometric and electrochemical methods which have been employed for the evaluation of cyanogens in plant tissues by other authors differ individually in many aspects such as the means of the endogenous β-glycosidase inactivation, hydrolysis of cyanogenic glycosides, isolation of the released cyanide, use of different reagents for the spectrophotometric determination of hydrogen cyanide,

etc. They further differ in their speed, reproducibility and sensitivity. We have modified and simplified these procedures using partially purified linamarase (with much quicker preparation of the enzyme and relatively quick determination of glycosidic cyanide in comparison with measurements using purified enzyme; C o o k e et al., 1978). The use of boiling ethanol inactivating the endogenous linamarase enabled the simultaneous determination of non-glycosidic cyanide. The extract was also suitable for both the spectrophotometric and the electrochemical determination and both the total (bound) and the free cyanide. Both these methods proved to be sensitive enough to allow the determination of cyanogens (cyanide) in cassava as well as in cassava products such as gari, i.e. in samples which differed in their cyanide content about ten times.

I. Cyanide content of peeled cassava tuber

Analysis No.	Spectrophotometric method			Electrochemical method		
	A	B	C	A	B	C
1	49.8	28.9	24.8	52.4	29.8	26.6
2	50.5	29.2	24.8	50.3	28.7	25.5
3	49.8	28.6	25.4	50.3	29.2	26.6
4	50.5	28.6	24.8	52.4	29.2	26.6
5	50.5	28.9	25.4	52.4	28.7	25.5
Average [mg.kg^{-1}]	50.2	28.8	25.0	51.5	29.1	26.1
Standard deviation [mg.kg^{-1}]	0.26	0.27	0.25	0.88	0.50	0.44
Coefficient of variance [%]	0.51	0.90	0.99	1.70	1.71	1.68

A = outer layer (0-5 mm), B = central layer (5-10 mm), C = inner layer (15 mm)

The results obtained analyzing cyanogens in three parallel series of cassava samples (five samples each) are presented in Table I. The non-glycosidic cyanide of cassava root represented less than 1 % of the total cyanide. The amount of gari cyanide both glycoside bound and non-glycosidic cyanide is summarized in Table II.

The absorbance of the characteristic reddish-violet colour produced with the pyridine/barbituric acid reagent was reasonably stable and could be read with no loss of accuracy even after several hours of storage at room temperature. The results

II. Cyanide content of gari

Analysis No.	Spectrophotometric method		Electrochemical method	
	Glycoside-bound	Non-glycosidic	Glycoside-bound	Non-glycosidic
1	1.95	1.36	1.86	1.36
2	1.98	1.36	1.89	1.48
3	1.96	1.45	1.88	1.42
4	1.94	1.42	1.88	1.36
5	1.96	1.39	1.82	1.42
Average [mg.kg^{-1}]	1.96	1.40	1.87	1.41
Standard deviation [mg.kg^{-1}]	0.017	0.038	0.028	0.051
Coefficient of variance [%]	0.868	2.720	1.500	3.660

of sensitivity tests indicated that this spectrophotometric method was capable of determining cyanide down to a concentration of about 0.01 mg/ml, which corresponds to the cyanide level of cassava or cassava products down to about 1 mg/kg. Approximately the same sensitivity was observed in the electrochemical method employing cyanide selective electrode. The advantage of the latter method was a relatively quicker measurement of cyanide level.

In cassava samples A and C (the cyanide content) and in gari (the glycoside bound cyanide) the electrochemical method produced statistically significantly higher values ($P = 95$ %) compared with the values obtained for the materials using the spectrophotometric method. On the other hand, there was a good agreement between the results obtained for fresh cassava sample B and non-glycosidically bound cyanide of gari between the two methods (t -test).

The data in Tables I and II demonstrate that the two methods have essentially the same precision as indicated by the coefficient of variance. Comparison of these two methods on the probability level of $P = 95$ % showed that their accuracies differed only in the case of cassava A sample analysis.

The limitation of these two comparable methods lies however in the fact that they both measure the total cyanogens level, the sum of linamarin and lotaustralin content.

References

BLAEDEL, W. - EASTY, D. - ANDERSON, L. - FARRELL, T.: Potentiometric determination of cyanide with an ion selective electrode. Application to cyanogenic glycosides in Sudan grasses. Anal. Chem., *43*, 1971 : 890-894.

BRADBURY, J. H. - EGAN, S. V. - LYNCH, M. J.: Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glucosides. J. Sci. Food Agric., *55*, 1991 : 277-290.

BRIMER, L. - DALGAARD, L.: Cyanogenic glycosides and cyanohydrins in plant tissues. Qualitative and quantitative determination by enzymatic post-column cleavage and electrochemical detection after separation by high-performance liquid chromatography. J. Chromatogr., *303*, 1984 : 77-88.

CONN, E. E.: In: BELL, E. A. - CHARLAMBOUS, B. V.: Cyanogenic Glycosides. Berlin, Heidelberg, New York, Springer Verlag 1980 : 461-492.

COOKE, R. D.: An enzymatic assay for the total cyanide content of cassava (*Manihot esculenta* Crantz). J. Sci. Food Agric., *29*, 1978 : 345-352.

COOKE, R. D. - BLAKE, G. G. - BATTERSHILL, J. M.: Purification of cassava linamarase. Phytochemistry, *17*, 1978 : 381-383.

DALGAARD, L. - BRIMER, L.: Electrochemical detection of cyanogenic glycosides after enzymatic post-column cleavage. J. Chromatogr., *303*, 1984 : 67-76.

ESQUIVEL, T. F. - MARAVALHAS, N.: Rapid field method for evaluating hydrocyanic toxicity of cassava root tubers. J. Agric. Food Chem., *21*, 1973 : 321-322.

IKEDIOBI, C. O. - ONYIA, G. O. C. - ELUWAH, C. E.: A rapid and inexpensive enzymatic assay for total cyanide in cassava (*Manihot esculenta* Crantz) and cassava products. Agric. Biol. Chem., *44*, 1980 : 2803-2809.

NAMBISAN, B. - SUNDARESAN, S.: Spectrophotometric determination of cyanoglucosides in cassava. J. Assoc. Off. Anal. Chem., *67*, 1984 : 641-643.

NARTEY, F.: Studies on cassava, *Manihot ultissima* Pohl - I. Cyanogenesis: the biosynthesis of linamarin and lotaustralin in etiolated seedlings. Phytochemistry, *7*, 1968 : 1307-1312.

RASHED, S. M. - VOLDŘICH, M. - VRBOVÁ, E. - MAREK, M. - JARÝ, J.: Enzyme electrodes for the determination of cyanogenic glycosides. Sbor. VŠCHT v Praze, *E52*, 1991 : 19-30.

TEWE, O. O. - IYAYI, E. A.: Cyanogenic Glycosides. In: CHEEKE, P.R.: The Toxicants of Plant Origin. Boca Raton, Florida, CRC Press 1989 : 44-60.

VOLDŘICH, M. - RASHED, S. M. - VRBOVÁ, F. - MAREK, M.: Enzymová potenciometrická elektroda pro stanovení kyanogenních glykosidů peckového ovoce. Potrav. Vědy, *8*, 1990 : 253-260.

WOOD, T.: The cyanogenic glucoside content of cassava and cassava products. J. Sci. Food Agric., *16*, 1965 : 300-305.

WOOD, T.: The isolation, properties, and enzymic breakdown of linamarin from cassava. J. Sci. Food Agric., *17*, 1966 : 85-90.

Received for publication April 6, 1993

Potr. Vědy, *11*, 1993 (3) : 163-170

M. Doležal, Sakiru A. Fatai, M. Voldřich, J. Velíšek (Vysoká škola chemicko-technologická – Ústav chemie a analýzy potravin a Ústav konzervace potravin a technologie masa, Praha)

Stanovení kyanogenních glykosidů kassavy (*Manihot esculenta* Crantz) a výrobků z kassavy

Pro stanovení kyanovodíku vázaného v glykosidech a kyanovodíku přítomného v jiných formách v kassavě a ve výrobcích z kassavy byla vypracována a ověřena spektrofotometrická metoda (činidlo pyridin/barbiturová kyselina) a elektrochemická metoda (selektivní kyanidová elektroda). Výsledky získané oběma metodami byly velmi podobné. Statisticky významně vyšší hodnoty pro kyanidy u čerstvých hlíz kassavy a pro neglykosidové kyanidy byly získány elektrochemickou metodou. Přesnost obou metod se lišila pouze v jednom případě analýzy čerstvého vzorku kassavy. Obě metody se ukázaly být relativně jednoduché, rychlé, přesné a umožňovaly stanovení kyanidů přítomných v množství asi 1 mg/kg jak v enzymově aktivních čerstvýh vzorcích, tak i v enzymově inaktivních výrobcích z kassavy.

kassava; maniok (*Manihot esculenta* Crantz); gari; kyanogenní glykosidy; kyanogeny; linamarin; kyanovodík; analýza

Potr. Vědy, *11*, 1993 (3) : 171-180

GEOMETRICAL ISOMERIZATION OF DOUBLE BONDS OF FATTY ACID METHYLESTERS

Vladimír FILIP

Institute of Chemical Technology – Department of Milk and Fat Technology, Technická 5, CS-166 28 Praha 6, Czech Republic[*]

The geometrical isomerization of double bonds of methyloleate and methyllinoleate was realized with p-toluenesulphinic acid as a catalyst to the conversion grade 78-81 %. The presence of volatile products of fatty acid methylesters which inhibit an isomerization catalyst played a substantial role. The positional isomerization did not take place. Trans-isomers of fatty acid methylesters were isolated by a crystallization from acetone from the reaction mixture: concentration of methylesters 5-7 % w/w, trans-9-octadecenoate at -30 °C, the mixture 9-trans-12-trans isomer and 9-trans-12-cis-octadecadieonate at -60 °C, fractions containing especially cis-9,trans-12-octadecadienate at -72 °C. Sulphur compounds were not proved in methylelaidate after the double fractional crystallization and rectification. Whereas isomers of 9,12-octadecadienoic acid methylesters contained sulphur at the level 60-1400 ppm. It was not possible to exclude the interaction of substrate-catalyst at the application of p-toluenesulphinic acid.

geometrical isomerization; linoleic acid; octadecadienoic acid isomers; octadecenoic acid isomers; oleic acid; positional isomerisation; p-toluenesulphinic acid

Configuration of double bonds in fatty acids occurring in nature is usually cis, exceptions are represented partly by fatty acids of animal (W o o d, 1983) or microbiological (O k u y a m a et al., 1991) origin when hydrogenated reactions are realized in a macro- and/or microorganism. Isomeric reactions accompanying hydrogen addition upon the double bond in configuration cis at partial catalytic

* The work was carried out at the Department of Technical Chemistry and Petrolchemistry at RWTH Aachen

hydrogenation of oils and fats (P a t t e r s o n, 1983) represent the most frequent case of the origin of fatty acid double bonds with configuration trans. It is always parallelly realized addition, geometrical and positional isomerization of double bonds (v a n d e r P l a n k, 1971). Methylstearate and the mixture of cis and trans isomers of octadecenoic acid methylesters in positions 7, 8, 9, 10, 11 as a minimum, which can be characterized by a certain distribution, is a result of partial catalytic hydrogenation e.g. methyloleate, i.e. 9-cis octadecenoic acid methylester.

The cis-trans isomerization of double bonds is a reversible process, in which the double bond in position cis is energetically richer. The equilibrium of cis-trans isomerization of double bonds is shifted to the benefit of the trans-isomer 75 trans : : 25 cis (L i c h t f i e l d et al., 1963), up to date 80 : 20 (S n y d e r, S c h o l - f i e l d, 1982) and is stated as an equilibrium value. Geometrical and simultaneously also positional isomerization undergoes catalysis with strong acids (an ionic mechanism), a catalytic effect of free radicals. Geometrical isomerization is preferred at the catalytic effect of free radicals (J o h n s o n, P r y d e, 1983). Selenium powder (L i c h t f i e l d et al., 1963) and nitrous acid (L i c h t - f i e l d et al., 1965; G r a n d g i r a r d et al., 1987) have been used for cis-trans isomerization from the formerly suggested and still used catalyst up to the present day. A great disadvantage of these catalysts consists in their partial interaction with substrate, the product of isomerization cannot be used e.g. for the studying of hydrogenation, and partial positional isomerization takes place (selenium). Mercaptans or sulphides (K i r c h e r, 1963) belong to further isomeric catalysts. In this case thiyl radical is an active agent. p-Toluenesulphinic acid suggested for isomerization of olefins (G i b s o n, S t r a s s b u r g e r, 1976) and also used for isomerization of unsaturated fatty acid esters (S n y d e r, S c h o l f i e l d, 1982) represents a modern catalyst of this type. Due to its instability it is prepared from the sodium salt and it is expected to decompose itself consequently into the p-toluenesulphonic acid and the aromatic thiolsulphonate, which apparently represents the catalyst itself:

$$3\ ArSO_2H \longrightarrow ArSO_3H + ArSO_2SAr + H_2O$$

The authors say that the catalyst is suitable especially for the preparation of isomers of polyunsaturated fatty acids because these isomers do not contain any products of interaction of fatty acids with catalyst, and positional isomerization is not realized. The published data do not state, however, quantification of the substrate-catalyst reactivity and of the purity of isomerization products.

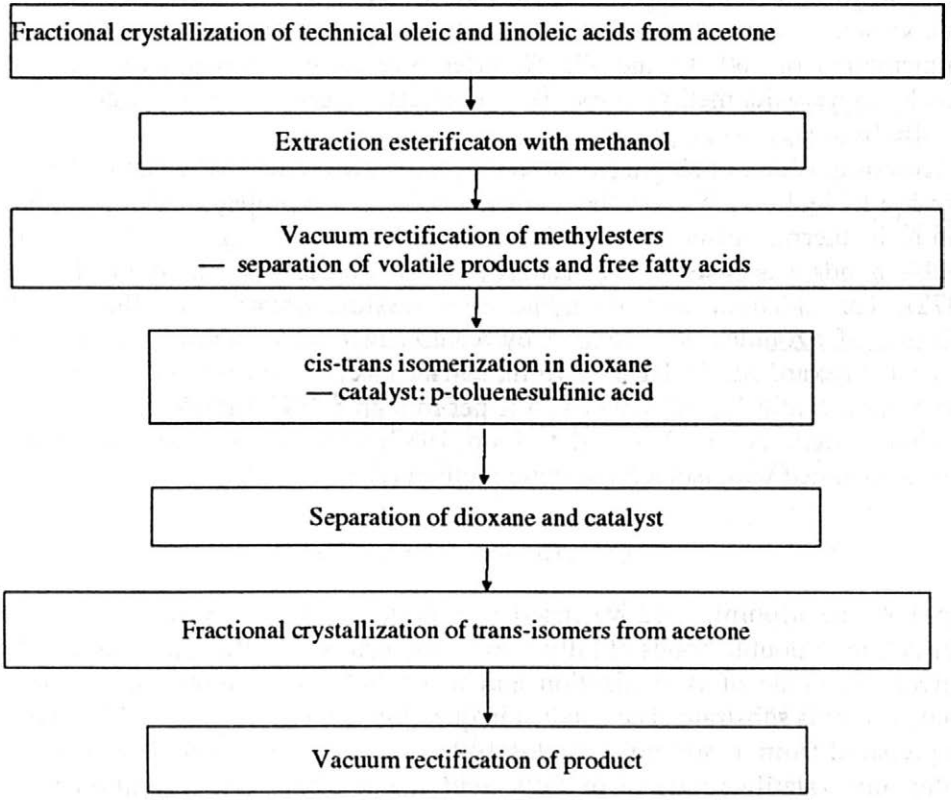
The second approach to the preparation of fatty acid isomers is represented by selective addition of halogen with its consequent elimination when the double bond

in a trans position originates selectively. It is possible to presume the presence of the reactant in the product in these cases.

In this paper the following is described: the preparation of trans isomers of cis-9-octadecenoic acid and cis-9,cis-12-octadecadienic acid of a high purity grade with the aim of further use of the isomers prepared in this way for study of their catalytic hydrogenation, and/or analytical purposes. The problem of substrate--catalyst interaction is discussed.

MATERIAL and METHODS

Sodium salt of p-toluenesulphinic acid, 97 % (Fluka Chemie AG, Buchs). Initial raw material: technical oleic acid (78.8 %) and technical linoleic acid (63.7 %) underwent fractional crystallization from acetone (Fig.1) (concentration about 7 % w/w). In both cases saturated fatty acids were separated at the temperature of -20 °C



1. The preparation of geometrical isomers of fatty acid methylesters

to -25 °C. Oleic acid was obtained at the temperature of -40 °C and recrystallized at the temperature of -50 °C (purity 97.79 %) Linoleic acid was obtained by crystallization at the temperature of -60 °C after separation of saturated fatty acids and oleic acid (B r o w n, K o l b, 1955) - purity 83.05 %. Fatty acid were transferred to methylesters by extraction esterification and vacuum rectified to remove volatile products.

Isomerization of methylesters (S n y d e r, 1982) was carried out in dioxane (3 ml solvent per 1 g ester). The catalyst – p-toluenesulphinic acid – was prepared from its sodium salt closely before isomerization, catalyst amount 0.11 mole per mole of methyloleate; isomerization time at the boiling point of dioxane 30 minutes. A double amount of the catalyst was used for isomerization of methyllinoleate. The solvent was distilled off; the catalyst was repeatedly extracted with a water solution $NaHCO_3$.

Methylelaidate was separated from methyloleate by the repeated crystallization from acetone at the temperature of -30 °C, isomers of octadecadienoic acid methylesters were obtained in two fractions by acetone crystallization at the temperatures of -60 °C and -72 °C after preceding separation of isomers of octadecenoic acid methylesters. The products of crystallization were vacuum rectified.

The composition of single isomer methylesters was analysed by a GLC method (S c h o l f i e l d, 1981) on the capillary column Chrompack CP-Sil-88, 50 m, 355 K isotherm, carrier gas He 0.5 ml/min, FID detector (Fig. 2). The position of double bonds was observed by means of ester ozonolysis (v a n d e r P l a n k, 1972). The aldehyde and aldehydic ester mixture obtained by the reduction splitting of ozonides was divided by a GLC method on the capillary column Hewlett-Packard SE 54-HP-FS, 50 m, carrier gas N_2 1 ml per min, temperature programme 5 min 325 K isotherm, 5 K per min up 525 K, FID detector. The total sulphur content (A b r a h a m, d e M a n, 1987) after the conversion to sulphates was determined with isotachophoretic method (Z e l e n s k ý et al., 1984).

RESULTS and DISCUSSION

p-Toluenesulphinic acid was used as a modern catalyst for the cis-trans isomerization of double bonds of fatty acid methylesters with the aim to reach a high conversion grade of isomerization and to exclude the potential interaction of catalyst versus substrate. The catalyst in question is unstable as an acid and has to be prepared from a sodium salt closely before the application. The absence of water and volatile products of fatty acid methylesters is a condition for the attainment of an equilibrium state. These volatile products originating in the course

of preparation were not quantified but results describing the influence of their separation to the obtained conversion grade are given (Table I). Volatile products were separated by rectification under Ar atmosphere as the first distillate of yellowish shade.

I. Influence of the substrate purity to the obtained conversion grade of cis-trans isomerization (C)

Substrate	Way of substrate purification	Water concentration [%]	C [%]
Methyl oleate	distillation	0.07	68.5
	distillation	0.02	76.4
	rectification	0.02	80.6
Methyl linoleate	distillation	0.02	44.5 - 63.0
	rectification	0.02	75.0 - 78.5

The conversion grade over 80 % was reached at the isomerization of methyloleate, hence it is more than the references say for the catalysts of the Se or nitrous acid type (L i c h t f i e l d et al., 1963, 1965) and the conversion grade is comparable as it is published for the same catalyst (S n y d e r et al., 1982;

II. Content (% w/w) and distribution of double bonds (%) of 9-octadecenoic acid methylesters (FAME) before (substrate) and after (product) isomerization

FAME	Substrate	Product
cis-$C_{18:1}$	97.79	0.44
trans-$C_{18:1}$	0.29	95.50
$C_{18:2}$	-	-
n_C		
6	-	-
7	-	-
8	0.45	-
9	99.17	99.90
10	0.07	-
11	0.24	0.10
12	0.07	-

G i b s o n et al., 1976). The reached conversion grade, related to what has been stated of the influence of volatile products, cannot be considered as a datum describing the equilibrium state of isomerization. It is in discrepancy with the formely published data on the trans-cis equilibrium 75 : 25 (L i c h t f i e l d et al., 1963, 1965). The real value is rather near to the ratio 80 : 20. Within the experimental error both substrates were proved not to undergo positional isomerization (Table II). Methylelaidate obtained by fractional crystallization from acetone and then purified by rectification was a colourless liquid. The total sulphur concentration before and after isomerization is the same (Table III). The methylelaidate obtained in a described way does not apparently contain further sulphur compounds.

III. The total sulphur content in methylesters before and after cis-trans isomerization

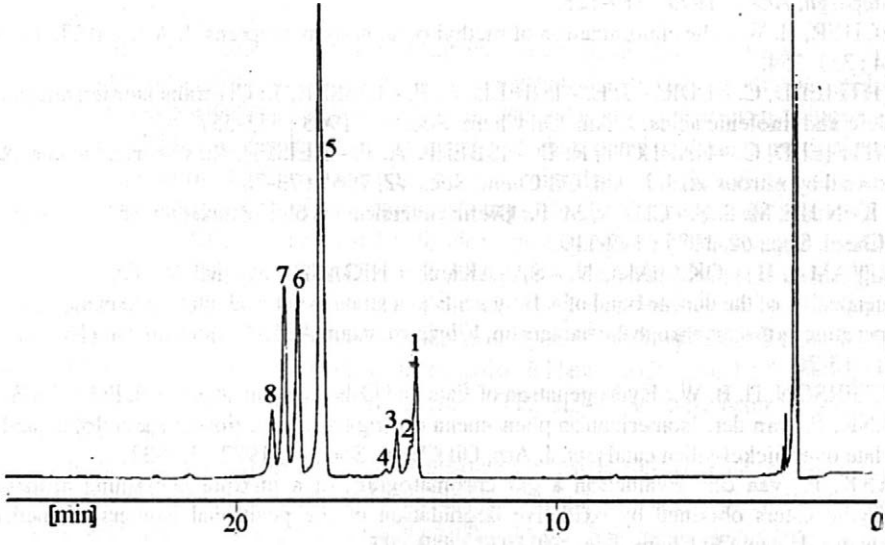
Ester		S [ppm]
Methyl oleate		33.9
Methyl elaidate		33.9
Methyl linoleate		20.8
Product of isomerization	1. fraction	58.6
	2. fraction	1400

With regard to the lower purity of the original methyllinoleate (83.0 %) the product of isomerization also contained methylelaidate, which was separated by fractional crystallization (Table IV). Isomers of 9,12-octadecadienoic acid methylesters were separated in two fractions. The mutual relationship of trans9,trans12-; trans9,cis12- and cis9,trans12-isomers 3.4 : 1 : 1 results from the composition of reaction mixture. The mutual proportion of individual isomers in the first and second fractions points to very close solubility of trans9,trans12- and trans9,cis12-isomers. It is not possible to separate both isomers either by the stated technique, or by consequent crystallization and to obtain total trans-isomer of a higher purity in this way. On the other hand, cis9,trans12-isomer shows features similar to the original isomer in total cis configuration in view of solubility. The content of sulphur in isomers of octadecenoic acid methylesters is 3-70 times higher compared with the original substrate in contrast to methylelaidate (Table III). This fact is also displayed in the colour of these isomers. The second fraction is already strikingly yellowish and stinks after sulphur compounds. The mutual interaction catalyst - isomer fatty acid methylesters takes place also in the case of

IV. Content (% w/w) of isomers of 9-octadecenoic and 9,12-octadecadienoic acid methylesters (FAME) before (substrate) and after (product) isomerization and purification (1. and 2. fraction)

FAME	Substrate	Product	1. fraction	2. fraction
trans-$C_{18:1}$	0.05	11.85	10.19	1.93
cis-$C_{18:1}$	12.98	3.00	0.35	12.50
$\Sigma C_{18:1}$	13.03	14.85	10.54	14.43
trans9,trans12-$C_{18:2}$	-	51.59	72.78	13.83
cis9,trans12-$C_{18:2}$	0.61	13.84	0.35	41.29
trans9,cis12-$C_{18:2}$	0.50	14.64	15.82	20.60
cis9,cis12-$C_{18:2}$	83.05	4.14	0.31	8.90
Conjugated $C_{18:2}$	0.98	0.48	0.15	0.87
$\Sigma C_{18:2}$	85.14	84.69	89.41	85.49

isomerization of fatty acid esters with p-toluenesulphinic acid. It is however possible to separate quantitatively these reaction products of polar character by fractional crystallization from acetone at the temperature to -30 °C in the case of



2. GLC chromatogram of isomers of linoleic acid methylesters - product of isomerization: octadecenoic acid methylesters: trans9 (1); trans11 (2); cis9 (3); cis11 (4); octadecadienoic acid methylesters: trans9,trans12 (5); cis9,trans12 (6); trans9,cis12 (7); cis9,cis12 (8)

methylelaidate, whereas in crystallization at the temperature of -60 °C crystallization in common with octadecenoic acid methylesters takes place. It is not possible to separate quantitatively the products of the stated interaction from fatty acid methylesters even by consequent rectification. The probability of the origin of fatty acid methylesters containing sulphur increases with the rising number of double bonds.

The positional isomerization does not take place at the application of the stated catalyst, it is not however possible to exclude the interaction of the catalyst and reactant.

References

ABRAHAM, V. - deMAN, J. M.: Determination of total sulphur in canola oil. J. Am. Oil. Chem. Soc., *64*, 1987 : 384-387.

BROWN, J. N. - KOLB, D. K.: In: Progress in the Chemistry of Fats and other Lipids. London, Pergamon Press 1955 : 58-76.

GIBSON, T. W. - STRASSBURGER P.: Sulfinic acid catalyzed isomerization of olefins. J. Org. Chem., *41*, 1976 : 791-793.

GRANDGIRARD, A. - JULLIARD, F. - PREVOST, J. - SEBEDIO, J. L.: Preparation of geometrical isomers of linolenic acid. J. Am. Oil Chem. Soc., *64*, 1987 : 1434-1440.

JOHNSON, R. W. - PRYDE, E. H.: Isomerization, Conjugation and Cyclization in Fatty Acids. Champaign, AOCS 1979 : 319-325.

KIRCHER, H. W.: The elaidinization of methyl oleat with mercaptans. J. Am. Oil Chem. Soc., *41*, 1964 : 351-354.

LICHTFIELD, C. - LORD, J. E. - ISBELL, A. F. - REISER, J.: Cis-trans isomerization of oleic, linoleic and linolenic acids. J. Am. Oil Chem. Soc., *40*, 1963 : 553-557.

LICHTFIELD, C. - HARLOW, R. D. - ISBELL, A. F. - REISER, R.: Cis-trans isomerization of oleic acid by nitrous acid. J. Am. Oil Chem. Soc., *42*, 1965 : 73-78.

LIE KEN JIE, M. S. F. - CHAN, M. F.: Olefin inversion involving unsaturated fatty esters. J. Am. Oil Chem. Soc., *62*, 1985 : 109-110.

OKUYAMA, H. - OKAJIMA, N. - SASAKI, S. - HIGASHI, S. - MURATA, N.: The cis/trans isomerization of the double bond of a fatty acids as a strategy for a adaptation to changes in ambient temperature in the psychrophilic bacterium, *Vibrio* sp. strain ABE-1. Biochim. Biophys. Acta, *1084*, 1991 : 13-20.

PATTERSON, H. B. W.: Hydrogenation of Fats and Oils. London, Appl. Sci. Publ. 1983 : 7-10.

PLANK, P., van der: Isomerization phenomena during hydrogenation of methyloleat and methyl elaidate over nickel-silica catalysts. J. Am. Oil Chem. Soc., *49*, 1972 : 327-332.

PLANK, P., van der: Evaluation a gas chromatogram of a mixture containing aldehydes and aldehydic esters obtained by oxidative degradation of the positional isomers of methyl octadecanoate. J. Am. Oil Chem. Soc., *49*, 1972 : 489-493.

SCHOELFIELD, C. R.: GC equivalent chain lengths of fatty acid methylesters on a Silar 10C glass capillary column. J. Am. Oil Chem. Soc., *58*, 1981 : 662-663.

SNYDER, J. M. - SCHOLFIELD, C. R.: Cis-trans isomerization of unsaturated fatty acids with p-toluenesulfinic acid. J. Am. Oil Chem. Soc., *59*, 1982 : 469-470.
WOOD, R.: Geometrical and Positional Monoene Isomers in Beef and Several Processed Meats. AOCS-Monography No.10, 1983 : 341-358.
ZELENSKÝ, I. - ZELENSKÁ, V. - KANIANSKY, D. - HAVAŠI, P. - LEDNÁROVÁ, V.: Determination of inorganic anions in river water by column-coupling capillary isotachophoreris. J.Chromatogr., *294*, 1984 : 317-322.

Received for publication February 2, 1993

V. Filip (Vysoká škola chemicko-technologická – Ústav technologie mléka a tuků, Praha)

Geometrická isomerizace dvojné vazby methylesterů mastných kyselin

Moderní katalyzátor pro geometrickou isomeraci dvojných vazeb je kyselina p-toluensulfinová, místo tradičních katalyzátorů – práškového Se a kyseliny dusité (L i c h t f i e l d et al., 1963; 1965), navržená poprvé pro izomeraci olefinů (G i b s o n, S t r a s s b u r g e r, 1976) a použitá i pro isomerizaci esterů nenasycených mastných kyselin (S n y d e r, S c h o l f i e ld, 1982). Tato nestálá látka se rozkládá na kyselinu p-toluensulfonovou a aromatický thiolsulfát, který zřejmě představuje vlastní katalyzátor. Uvádí se, že nedochází k polohové isomeraci, údaje o interakci katalyzátor - substrát nejsou k dispozici.

Výchozí suroviny – technická kyselina olejová a technická kyselina linolová - byly podrobeny frakční krystalizaci z acetonu, získané mastné kyseliny o čistotě 97,79, resp. 83,05 % byly po převedení na metylestery isomerizovány kyselinou p-toluensulfinovou v dioxanu. Methylester kyseliny trans-9-oktadecenové a směsi methylesterů trans9,trans12- společně s trans9,cis12- a dále cis9,trans12- kyseliny oktadekadienové byly získány krystalizací z acetonu při teplotě -30 °C, resp.-60 °C a -72 °C a následně vakuově rektifikovány (obr.1). Složení methylesterů jednotlivých isomerů (S c h o e l f i l d, 1981) bylo zjišťováno metodou plynové chromatografie na kapilární koloně Chrompack CP-Sil-88 (obr. 2). Poloha dvojných vazeb byla zjišťována redukčním štěpením ozonidů a získaná směs aldehydů a aldehydesterů byla dělena metodou plynové chromatografie na kapilární koloně Hewlett-Packard SE 54-HP-FS. Ve výchozích látkách a v produktech izomerace (tab. III) byla stanovena veškerá síra (A b r a h a m, d e M a n, 1987).

Těkavé produkty substrátu inaktivující katalyzátor, musí být ze směsi separovány (tab. I). Při isomeraci methyloleátu bylo dosaženo stupně konverze přes 80 %, tedy více jako uvádí literatura pro katalyzátory typu Se nebo kyseliny dusité (L i c h t f i e l d et al.1963, 1965) a srovnatelně jako je publikováno pro použitý katalyzátor (S n y d e r et al., 1982; G i b s o n et al., 1976). V případě isomerace methyllinoleátu byl dosažený stupeň konverze o cca 3 % nižší. Dosažený stupeň konverze není možné považovat za údaj popisující rovnovážný stav isomerace. Jeho

reálná hodnota se blíží poměru 80 : 20. Pro oba substráty bylo prokázáno, že v rámci experimentální chyby, nedochází k polohové isomeraci (tab. I a IV). Methylelaidát získaný frakční krystalizací z acetonu a purifikovaný následně rektifikací byla bezbarvá kapalina, obsahující stejné množství sirných sloučenin jako výchozí substrát (cca 35 ppm S).

Isomery methylesterů kyseliny 9,12-oktadekadienové byly separovány ve dvou frakcích. Ze složení reakční směsi vyplývá vzájemný poměr isomerů trans9,trans12-, trans9,cis12- a cis9,trans12- 3,4 : 1 : 1. Výsledek vzájemného zastoupení jednotlivých isomerů ve frakci 1. a 2. (tab. IV) ukazuje na velmi blízkou rozpustnost isomerů s konfigurací dvojné vazby v poloze trans-9. Uvednou technikou není proto možné ani následnou krystalizací uvedené isomery oddělit a získat tak úplný trans-isomer o větší čistotě. Isomer cis9,trans12 vykazuje naproti tomu, z hlediska rozpustnosti, vlastnosti blízké původnímu isomeru v úplné cis konfiguraci. Na rozdíl od methylelaidátu, obsahují isomerované a přečištěné methylestery isomerů kyseliny oktadekadienové 3 až 70krát více látek obsahujících síru nežli výchozí substrát (tab. III). I v případě isomerace esterů mastných kyselin kyselinou p-toluensulfinovou, podobně jako při použití Se nebo kyseliny dusité, tedy dochází ke vzájemné interakci katalyzátoru s methylestery mastných kyselin. Tyto reakční produkty polárního charakteru je však možné v případě methylelaidátu kvantitativně odstranit frakční krystalizací z acetonu při teplotách do -30 °C, zatímco při krystalizaci při teplotách pod -60 °C již dochází ke společné krystalizaci s methylestery kyseliny oktadecenové. Ani následnou rektifikací není možné produkty uvedené interakce kvantitativně separovat od metylesterů mastných kyselin. Původní literatura údaje o interakci katalyzátoru s methylestery mastných kyselin neuvádí (Gibson, Strassburger, 1976; Snyder, Scholfield, 1982).

Při aplikaci uvedeného katalyzátoru nedochází k polohové izomeraci, nelze však vyloučit interakci katalyzátoru a reaktantu.

geometrická isomerizace; kyselina linolová; isomery kyseliny oktadecenové; isomery kyseliny oktadekadienové; kyselina olejová; polohová isomerace; p-toluesulfinová kyselina

Potrav. Vědy, *11*, 1993 (3) : 181-187

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF SACCHAROSE AND LACTOSE IN MILK CHOCOLATE

Jana ČOPÍKOVÁ, Martina NOVEKOVÁ, Ladislava SVOBODOVÁ

Institute of Chemical Technology – Department of Carbohydrate Chemistry and Technology, Technická 5, CS-166 28 Praha 6, Czech Republic

The described HPLC system allows simultaneous determination of saccharose and lactose in milk chocolate products. The carbohydrates are extracted with water from defatted samples and chromatographed on ion exchange resin in Pb^{2+} form with deionised water as a mobile phase. The method is statistically evaluated and compared with the official method which is based on copper reduction. The estimated HPLC method is an environmentally non-polluting procedure.

. chrcmatography; milk chocolate; saccharose; lactose

Common methods of saccharose and lactose determination in chocolate is based on copper reduction and polarimetry (ČSN, 1985; Official Methods of Analysis, 1990). These methods are reasonably accurate, but they need extensive sample preparation, time and skilled analyst. H u r s t and M a r t i n (1977, 1980) developed a new efficient chromatographic method which was later adopted by the Association of Official Analytical Chemists (Official Methods of Analysis, 1990). The new chromatographic method allows determination of fructose, glucose, saccharose and lactose. This classical high performance liquid chromatography (HPLC) method dictates the use of reversed phase column run with acetonitril (Č o p í k o v á et al., 1983). The great disadvantage of common methods and the chromatographic method mentioned above is the fact that they are not environmentally non-polluting. The chromatography on ion exchange resins (B u l l, 1990) enables to determinate a wide mixture of carbohydrates involving the complicated mechanisms of separation. As distilled water or diluted solutions are the mobile phase, this type of chromatography is a very useful technique without disadvantages connected with common methods or chromatography with acetonitril.

MATERIAL and METHODS

A high pressure liquid chromatograph was equipped with HPP 5001 pump, refractive index detector RIDK 101, HPLC injection valve LCI 30 with 20 µl loop (all CTM Praha), integrator Spectra Physics 4100 (USA), and stainless steel column 250 x 8 mm filled with ion exchange resin Ostion LG KS 0800 in Pb^{2+} form (dp = 6.3 µm, Spolek pro chemickou a hutní výrobu, Ústí n. L.)) working at 85 °C. The mobile phase was degassed deionised water with flow rate 0.5 ml/min.

The content of saccharose and lactose in three representative samples of milk chocolate was determined by the official method ČSN (1985) and by means of chromatography. The official ČSN method is based on copper reduction before and after chocolate sample hydrolysis.

The following procedure was chosen for sample preparation before chromatography: Grated milk chocolate (8 g) was defatted with petroleum ether (b. p. 40 - 65 °C, 2 x 80 ml) at ambient temperature, the solid was filtered off and dried. Pulverized sample was then dissolved in water (50 ml) at 40 °C, placed into 100 ml volumetric flask and solutions of Carrez I and Carrez II (0.5 ml of each) were added. After mixing, 1,2,3-propanetriol (4.0 g) as an internal standard was added and the volume was made up to the mark with water at 20 °C. The mixed content of flask was filtered, an aliquot of clear filtrate (25 ml) pipetted into 100ml volumetric flask and a mix-bed ion exchange resin (1 g, Amberlite MB-3) was added. After making up to the mark, the solution was passed through membrane filter (0.45 µm, Synpor) and a clear filtrate was directly used for HPLC.

The chromatograms were evaluated by the method of internal standard. The concentration of saccharose and lactose in standard solutions can be seen from Table I.

RESULTS and DISCUSSION

The results of chromatographic resolution are presented in Table I. The concentrations of standard solutions were chosen according to the expected content of saccharose and lactose in chocolate samples. The use of as an internal standard 1,2,3-propanetriol improved the accuracy of chromatographic determination as resolution of saccharose and lactose (R_s = 1.27) was not excellent, nevertheless sufficient. The column efficiency corresponded to practice.

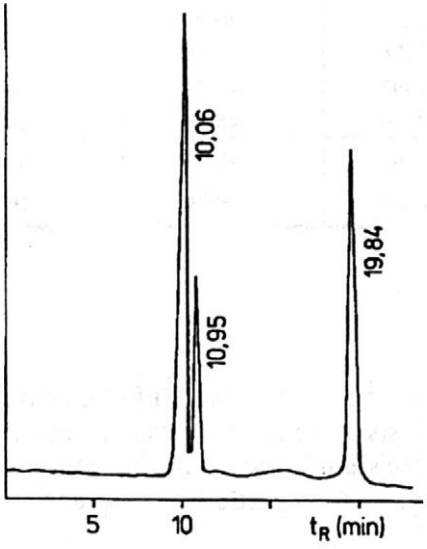
The results of carbohydrate determination by the official method and HPLC method are presented in Table II and statistical evaluation of obtained data is in Tables III and IV. The statistical evaluation (F e l i x, B l á h a, 1962; B r o o k e s et al., 1966) was based on the comparison of standard errors of means (S.E.), means

(c_{mean}) and significance of constants (a) and coefficients (b) in linear regression equations :

$$c_{HPLC} = a + b \cdot c_{CSN} \qquad [1]$$

I. Chromatographic resolution data

Substances	t_R [min]	R_S (saccharose/lactose) [1]			n_{sach} [m^{-1}]
Saccharose	10.06	1.27			13,300
Lactose	10.95				13,400
1,2,3-propanetriol	19.84				31,100
	c_1	c_2	c_3	c_4	c_5
	[g per 100 ml]				
Saccharose	1.00	1.00	1.00	1.00	1.00
Lactose	0.50	0.25	0.20	0.10	0.05
1,2,3-propanetriol	1.00	1.00	1.00	1.00	1.00
	R_f				
Saccharose	1.65	1.64	1.65	1.63	1.60
Lactose	1.77	1.75	1.75	1.75	1.72



1. HPLC chromatogram of sample No. 2

II. Saccharose and lactose determination in three milk chocolate samples, using ČSN and HPLC methods

Sample No.	ČSN		HPLC	
	saccharose [%]	lactose [%]	saccharose [%]	lactose [%]
1	21.72	27.30	21.40	26.84
	21.78	27.43	21.20	26.34
	21.65	27.30	19.45	26.70
	21.55	26.93	21.30	27.90
	21.89	27.30	20.54	26.06
	21.61	26.86	21.90	28.15
c_{mean}	21.70	27.19	20.97	27.00
S.E.	0.11	0.21	0.79	0.77
2	31.21	14.41	31.47	15.25
	31.70	14.41	30.39	14.76
	30.98	14.18	30.62	15.00
	31.89	14.61	31.76	14.45
	31.72	14.39	32.10	14.50
	31.50	14.50	31.41	15.19
c_{mean}	31.50	14.42	31.29	14.86
S.E.	0.31	0.13	0.60	0.31
3	48.31	2.32	48.30	1.93
	48.29	2.28	46.96	1.87
	48.72	2.27	48.38	1.91
	48.37	2.60	49.39	2.05
	48.30	2.33	48.67	2.04
c_{mean}	48.40	2.36	48.34	1.96
S.E.	0.16	0.12	0.79	0.07

For the purposes of exact comparison of the official and HPLC method *F*-test and *t*-test were assessed. These statistical tests of significance were calculated with probability equal to 95 % (and/or 5 % level of significance) with degrees of freedom corresponding to the numbers of the analysis of the particular sample.

III. Significance tests

Sample No.	n	v	F-test			t-test		
			$F_{0,05}$	saccharose	lactose	$t_{0,05}$	saccharose	lactose
1	6	5	5.05	48.82	13.20	2.57	2.00	0.10
2	5	4	6.39	3.67	5.82	2.78	0.14	0.52
3	6	5	5.05	23.41	2.78	2.57	0.56	0.48

It can be seen from the values of S.E. in Table II and from results of F-test (Table III) that the accuracy of both methods was not the same. The official method is more accurate in comparison with the HPLC method, as most of the values of S.E. in case of HPLC method is higher than the S.E. values of the official method. The half of the calculated F-test values is higher than the values quoted from tables.

A comparison of the mean value of both methods has proved (Table II, t-test) that there was not a real difference between the two means as the calculated t-test values are lower than the values quoted from tables.

Statistical evaluation of the linear regression equations (1) for saccharose and lactose tested the null hypothesis of constants (a) ($a = 0$) and hypotheses that the coefficients (b) are equal to one ($b = 1$). The adoption of these hypothesis would confirm that the official and HPLC methods give values for saccharose and lactose without any significant difference in the whole estimated range of concentrations.

IV. Regression analysis

	t - test				
	$a = 0$	$b = 1$	n	v	$t_{0,05}$
Saccharose $c_{HPLC} = -1.137 + 1.024 \,.\, c_{CSN}$	1.85	1.35	17	15	2.13
Lactose $c_{HPLC} = -0.113 + 1.005 \,.\, c_{CSN}$	0.62	0.28	17	15	2.13

The calculated values of t-test in Table IV were the lower than the values quoted from tables. The null hypothesis of constants (a) and hypothesis of coefficients $b = 1$ was confirmed for both carbohydrates in milk chocolate.

The HPLC column with ion exchange resin in Pb^{2+} form provides commonly excellent resolution of saccharose and lactose in food products containing these carbohydrates. This study shows that ion exchange resin Ostion LG KS 0800 in Pb^{2+} form allows the simultaneous determination of saccharose and lactose in milk chocolate in 20 min. At the same time the described HPLC method gives results without any significant difference from results obtained by the official method.

List of symbols

a	constant in regression equation
b	coefficient in regression equation
c_i	concentration of saccharose, lactose or 1,2,3-propanetriol in standard solutions [g per 100 ml]
c_{mean}	average content of carbohydrate in sample [%]
c_{HPLC}	carbohydrate content in sample determinated by HPLC [%]
c_{CSN}	carbohydrate content in sample determinated by ČSN [%]
t_R	retention time [min]
R_s	resolution of two substances [1] (S n y d e r, K i r k l a n d, 1973)
R_f	response factor [1] (S n y d e r, K i r k l a n d, 1973)
n_{sach}	efficiency of column [m^{-1}] (S n y d e r, K i r l a n d, 1973)
n	number of samples
v	degree of freedom
t-test, F-test	statistical tests [1] (F e l i x, B l á h a, 1962; B r o o k e s, et al., 1966)
sacch	saccharose
lact	lactose

References

BULL, G. F. M.: The application of HPLC to the determination of low molecular weight sugar and polyhydric alcohols in food: A review. Food Chem., *35*, 1990 : 117-152.

BROOKES, C. J. - BETTELEY, I. G. - LOXSTON, S. M. : Mathematics and Statistics for Chemists. New York, J. Wiley and Sons 1966 : 284-316.

ČOPÍKOVÁ, J. - HANZLOVÁ, H. - VOZKA, S.: Vysokoúčinná kapalinová chromatografie cukrů. Prům. Potr., *34*, 1983 : 243-248.

FELIX, M. - BLÁHA, K.: Matematickostatistické metody v chemickém průmyslu. Praha, SNTL 1962.

HURST, W. J. - MARTIN, R. A. Jr.: Rapid high pressure liquid chromatographic determination of carbohydrates in milk chocolate products. J. A.O.A.C., *60*, 1977 : 1180-1184.

HURST, W. J. - MARTIN, R. A. Jr.: High perfomance liquid chromatographic determination of carbohydrates in chocolate: Collaborative study. J. A.O.A.C., *63*, 1980 : 595-599.

SNYDER, L. R. - KIRKLAND, J. J.: Introduction to Modern Liquid Chromatography. New York, John Wiley and Sons 1973.
ČSN 56 0146. Metody zkoušení výrobků cukrovinkářského průmyslu a trvanlivého pečiva. 5. část Stanovení obsahu sacharidů. 1986.
OFFICIAL METHODS of ANALYSIS A.O.A.C.: 15th Edition 1990 : 773.

Received for publication February 17, 1993

J. Čopíková, M. Noveková, L. Svobodová (Vysoká škola chemicko-technologická – Ústav chemie a technologie sacharidů, Praha)

Chromatografické stanovení sacharosy a laktosy v čokoládě

Ke stanovení obsahu sacharosy a laktosy v mléčné čokoládě se v současné době běžně používá polarimetrická nebo oxido-redukční metoda (ČSN, 1985), přičemž obě dvě metody jsou časově náročné. V literatuře je popsáno chromatografické stanovení cukrů v mléčné čokoládě systémem, při němž se jako mobilní fáze používá acetonitril (H u r s t, M a r t i n, 1977, 1980; Č o p í k o v á et al., 1983). Acetonitril je jedovatý a ekologicky škodlivý, což je velká nevýhoda při rutinním využívání této techniky. V předkládaném sdělení je ověřeno stanovení obsahu sacharosy a laktosy v mléčné čokoládě chromatografickým dělením na koloně naplněné ionexem Ostion LG KS 0800 v Pb^{2+} formě. V tomto případě se jako mobilní fáze používá deionizovaná voda. Naměřené výsledky byly statisticky porovnány s výsledky, které poskytuje metoda daná normou ČSN 56 0146. Ze statistického hodnocení vyplynulo, že obě metody poskytují shodné výsledky, které jsou u chromatografické metody zatíženy větší chybou. Skutečnost, že chromatografické metody jsou zatížené větší chybou než metody tradiční, je doposud téměř běžnou analytickou praxí. Zároveň bylo pomocí testování konstanty a koeficientu v regresní rovnici $c_{HPLC} = a + b \,.\, c_{CSN}$ pro sacharosu i laktosu ověřeno, že metody nejsou vzájemně zatíženy systematickou chybou a poskytují shodné výsledky v měřeném koncentračním rozsahu.

chromatografie; mléčná čokoláda; sacharosa; laktosa

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION
Slezská 7, CS-120 56 Praha 2, Czech Republic
FAX.: (00422) 257 090

In this institute scientific journals dealing with the problems of agriculture and related sciences are published on behalf of the Czech Academy of Agricultural Sciences and Slovac Academy of Agricultural Sciences. The periodicals are published in the Czech or Slovak languages with summaries in English or in English with summaries in Czech or Slovak.

Subscription to these journals be sent to the above-mentioned address.

Periodical	Number of issues per year	pages
Rostlinná výroba (Plant Production)	12	96
Veterinární medicína (Veterinary Medicine - Czech)	12	64
Živočišná výroba (Animal Production)	12	96
Zemědělská ekonomika (Agricultural Economics)	12	96
Lesnictví (Forestry)	12	96
Zemědělská technika (Agricultural Engineering)	4	80
Ochrana rostlin (Plant Protection)	4	80
Genetika a šlechtění (Genetics and Plant Breeding)	4	80
Zahradnictví (Horticultural Science)	4	80
Potravinářské vědy (Food Sciences)	6	80

Potr. Vědy, *11*, 1993 (3) : 189-196

MINOR LIPOPHILIC COMPONENTS IN CRUDE RAPESEED OIL

Jan POKORNÝ, Jan VELÍŠEK, Jiří PÁNEK, Jarmila KÁŇOVÁ, Helena PAŘÍZKOVÁ [1], Marie HOLASOVÁ [1], Richard KOPLÍK, Jiří ČMOLÍK[2]

Institut of Chemical Technology – Department of Food Chemistry and Analysis, Technická 5, CS-166 28 Praha 6;
[1]Research Institute of Food Industry, Radiová 7, CS-102 31 Praha 10;
[2]SETUZA, a. s., CS-401 29 Ústí nad Labem, Czech Republic

The contents of chlorophyll and carotenoid pigments, tocopherols, glucosinolate degradation products, and iron were determined in 47 samples of crude rapeseed oil, obtained in the following way: (A) by expeller pressing and filtration, (B) by expeller pressing and centrifuging, (C) by solvent extraction, (D) by mixing expeller pressed and solvent extracted oils (2 : 1 v/v). Solvent extracted oils were more stable against oxidation than expeller pressed oils. Positive correlation between the stability and the content of chlorophyll pigments was observed in all oils. The correlation between the tocopherol content and the stability was negative in filtered pressed oils and positive in mixed crude oils. Iron had no negative effect on the oxidative stability.

rapeseed oil; autoxidation; stability; minor lipids

Rapeseed – main Czechoslovak oilseed, is processed by subsequent expeller pressing and solvent extraction. Crude oils are purified by filtration and centrifuging, and degumming. Both crude oils are then mixed, and refined in the mixture.

Refinig losses, the quality of refined oil, and its stability against the oxidative rancidity depend on the content of various minor nonlipidic substances present in crude oil, such as free fatty acids, chlorophyll or carotenoid pigments, tocopherols, trace metals, and glucosinolate degradation products (N i e w i a d o m s k i, 1983). Oxidation products produced by lipoxygenase oxidation in seeds (M e s h e h d a n i et al., 1990) are important as well as they initiate the autoxidation.

Czechoslovak rape is the low-erucic and either high or medium glucosinolate winter (*Brassica napus* L.) cultivar. Usually, seeds are rich in chlorophyll and carotenoid pigments (H o l a s o v á et al., 1989) so that the stability of oil against oxidative rancidity may be, generally, considered as low (C h o, D e M a n, 1989), compared to oils produced from the Canadian summer rape (*Brassica rapa* L.) cultivars.

In this paper, we report our results on the composition of various minor substances in crude rapeseed oils, and their relations to the oxidative stability.

MATERIAL and METHODS

Czechoslovak zero-erucic winter rapeseeds grown in the years 1987-1991, and stored from two to ten months, were used for the plant-scale processing. Both mixtures of high glucosinolate cultivars (Belinda, Germany, Silesia and Solida - - Czechoslovakia) and low glucosinolate cultivars (Darmor, France, Ceres, Germany, Sonata - Czechoslovakia) were studied. The water content was up to 12 % at the time of cropping, and between 7 - 9 % before the processing. Processing conditions were reported in another paper (Čmolík et al., 1983). About 2/3 of oil was obtained by expeller pressing. This oil was purified from coarse particles by filtration, and then purified by centrifuging to remove the finer particles. Cakes from the expeller pressing were solvent extracted (Extraktionstechnik, Hamburg), the extracted oil was degummed by hydration with water, centrifuged, and mixed with expressed oil for the further refining after a temporary storage in tanks.

Chlorophyll and carotenoid pigments were determined by spectrophotometry and spectrofluorimetry (Pokorný et al., 1989; Holasová et al., 1990). Tocopherols were determined by HPLC (Pocklington, Dieffenbacher, 1988), the content of volatile glucosinolate degradation products by capillary GLC (Velíšek et al., 1990), and the ionic iron content spectrophotometrically after dry ashing (Čoupková et al., 1986). The stability against oxidative rancidity by the Schaal oven test at 60 °C, the induction period being the time necessary to attain the weight increase of 500 mg and 200 mg, respectively (Čmolík et al., 1991).

RESULT and DISCUSSION

In total of 47 crude rapeseed oils were analyzed: A – crude expeller pressed and filtered oils (10 samples), B – crude expeller pressed and centrifuged oils (14 samples), C – crude solvent extracted oils (14 samples), and D – mixtures of crude expeller pressed and solvent extracted oils mixed in the ratio of 2 : 1 v/v (9 samples). Ranges of experimental values are given in Table I which shows relatively high contents of chlorophyll pigments, in agreement with our earlier results (Holasová et al., 1992), and relatively high content of iron (not widely different from results of other laboratories). Among tocopherols, the content of γ-tocopherol was generally slightly higher than that of α-tocopherol, and the content of δ-tocopherol was only very low. The content of carotenoids was of the

I. Ranges of properties of crude rapeseed oils

Component analyzed [mg . kg^{-1}]	Expressed filtered oil (A)	Expressed centrifuged oil (B)	Extracted oils (C)	Mixed crude oils (D)
Chlorophyll a	1.0 - 5.0	0.9 - 4.6	1.7 - 9.0	1.4 - 4.7
Chlorophyll b	0.2 - 3.8	0.1 - 3.5	0.5 - 3.5	0.1 - 2.2
Total chlorophylls	1.8 - 8.1	1.3 - 8.2	2.5 - 11.5	1.9 - 6.1
Pheophytin a	2..2 - 49.6	22.4 - 46.3	26.3 - 44.8	24.1 - 44.6
Pheophytin b	1.4 - 3.6	1.2 - 4.8	26.3 - 44.8	24.1 - 44.6
Total pheophytins	24.6 - 53.2	23.9 - 49.2	0.5 - 3.7	1.7 - 4.0
Chlorophyll pigments	27.7 - 56.5	26.0 - 53.0	28.0 - 48.6	25.8 - 47.4
α-Tocopherol	179 - 317	177 - 395	30.7 - 55.8	28.3 - 50.2
γ-Tocopherol	306 - 406	312 - 481	252 - 427	213 - 320
δ-Tocopherol	6 - 32	11 - 36	372 - 482	263 - 428
Total tocopherols	497 - 715	602 - 900	10 - 35	17 - 33
Carotenoid pigments	39.2 - 61.5	39.5 - 60.6	635 - 927	579 - 802
Iron	8.4 - 22.6	1.6 - 8.1	41.2 - 69.6	36.8 - 64.2
Cyanobutene	0.6 - 16.3	4.0 - 15.8	1.6 - 5.6	0.2 - 5.0
Cyanopentene	0.8 - 6.1	1.4 - 6.0	3.3 - 40.3	0.1 - 15.5
Total nitriles	1.4 - 24.4	5.4 - 21.6	3.2 - 46.1	0.03 - 12.9
Butenyl ITC	0.1 - 2.3	0.1 - 5.7	6.5 - 81.2	0.1 - 28.2
Pentenyl ITC	0.01 - 0.4	0.02 - 1.4	2.9 - 14.3	1.3 - 8.8
Total ITC	0.1 - 2.7	0.1 - 7.1	3.5 - 17.2	1.8 - 10.8

same order as that of pheophytins but there was no correlation between the content of carotenoid pigments and that of pheophytins. On the contrary, significant relations were found between carotenoids and chlorophylls which accompany pheophytins in small amounts.

The content of glucosinolate degradation products was rather high, and in no close correlation with the content of glucosinolates and their degradation products in seeds. Nitriles prevailed over isothiocyanates, in agreement with our previous results (V e l í š e k et al., 1991), and there was no significant correlation between the level of nitriles and isothiocyanates in expeller pressed oil, but the two groups of degradation products were interrelated in extracted oils.

Results of the stability determination are given in Table II. The stability of oils against oxidative rancidity was determined with use of the Schaal oven test at 60 °C which gives results better correlating with those of a storage test than the AOM value in crude oils. The end of the induction period is not distinctly evident in rapeseed oil so that we used the absorption of 200 mg or 500 mg oxygen by 1 kg oil as the end of the induction period. Correlations between the two methods were fairly good in all oils tested, pressed centrifuged oils excepted but the correlation coefficients (A – $r = 0.8737$, B - $r = 0.2064$, C – $r = 0.8725$, D – $r = 0.9252$) were not high enoughto allow the application of one method exclusively.

II. Stabilities against autoxidation after Schaal at 60 °C

Group of oils	Stability [days]	
	Schaal 500	Schaal 200
Expressed and filtered	17.0 - 36.2	8.3 - 26.0
Expressed centrifuged	17.2 - 28.0	8.8 - 18.8
Solvent extracted	21.2 - 52.8	14.4 - 41.2
Mixed	15.8 - 31.6	8.0 - 20.1

The effect of various minor substances on the stability of crude oils against autoxidation is evident from the examples in Table III. Surprisingly, the stability was correlated with the content of chlorophylls and pheophytins in the positive way both in expeller pressed and in extracted oils even when chlorophylls are well known photosensibilizers promoting the autoxidation (E n d o et al., 1984). Contrary to pheophytins, chlorophylls are present only in small amounts (Table I) but their content seems to be more important (Table III) than that of pheophytins, even when the latter are active photosensibilizers as well. In the dark, however, pheophytins act as moderate inhibitors of oxidation (H o l a s o v á et al., 1989), and relatively high amount of carotenoids present in crude oils may quench the photooxidizing activity as well (L e e, M i n, 1990). Anyway, the oxidative stability in crude oils is probably not due to chlorophylls but they are indicators of the state of oil only (insufficient ripeness).

The stability against autoxidation depended on the content of tocopherols in mixed oils (group D) even when the correlation was not statistically significant. Tocopherols are well known natural inhibitors of oxidation. The relations were significant in expeller pressed and filtered oils (group A) but, contrary to expectations, the correlation coefficients exhibited negative values (Table III) which means that oils containing higher amounts of tocopherols were less stable

III. Linear correlations between minor components of crude reapeseed oil and the stability against autoxidation by Schaal oven test

Group of crude rapeseed oils	Component tested	Correlation coefficients	
		Schall 500	Schaal 200
Expressed and filtered ($N = 10$)	chlorophyll a	0.581	0.663*
	pheophytin a	0.572	0.433
	pheophytin b	0.893*	-0.150
	α-tocopherol	-0.664*	-0.768*
	γ-tocopherol	-0.769*	-0.638*
	δ-tocopherol	-0.716*	-0.824*
	total tocopherols	-0.810*	-0.830*
	carotenoids	0.495	0.537
Expressed and centrifuged ($N = 14$)	total tocopherols	-0.308	-0.018
Solvent extracted ($N = 14$)	chlorophyll a	0.596*	0.805*
	chlorophyll b	0.583*	0.823*
	total chlorophylls	0.627*	0.861*
	pheophytin b	-0.250	-0.446
	α-tocopherol	-0.249	-0.246
	δ-tocopherol	-0.610*	-0.749*
Mixes oils ($N = 9$)	chlorophyll b	0.574	0.551
	pheophytin b	0.656*	0.706*
	γ-tocopherol	0.467	0.580
	δ-tocopherol	0.618	0.632
	iron	0.551	0.244

* statistically significant ($P = 95$ %)

against oxidation. It is true that tocopherols may act as prooxidants at high concentrations, corresponding to those in crude rapeseed oil (H e i m a n n, P e z o l d, 1957), but nearly the same amount of tocopherols in mixed oils (D) had positive effect, and no correlation was observed in solvent extracted oils (group C). Therefore, it seems that tocopherols in crude pressed and filtered oils (A) have no real prooxidant effect but act only as indicators of the state of oil. The controversial correlation of oxidative stability of various types of crude rapeseed oil on the tocopherol content in obviously due to the presence of different

concentrations of phospholipids and related polar substances in oils A, B. C, and D, respectively. The synergistic effect of various types of phospholipids on the antioxidative effect of tocopherols was observed in our recent model experiments which are now being prepared for publication.

Heavy metals with transient valency states are active prooxidants (B e n j e l l o u n et al., 1991) in vegetable oils. The concentration of iron was very high, especially in crude expressed and filtered oils (A), nevertheless, no negative effect of iron was observed. On the contrary, its concentration was in a rather positive correlation with the oxidative stability (Table III). Iron is probably found in inactive complexes, perhaps with phospholipids. It was found (P o k o r n ý et al., 1986) that iron salts of phosphatidic acids had no prooxidative effect in rapeseed oil, even when iron salts may catalyze the oxidation of phospholipids in emulsions (K a w a k a t s u et al., 1984).

No relation between the oxidative stability and the content of isothiocyanates was detected, even when sulphur compounds, such as thioethers, act as antioxidants (K a r a h a d i a n, L i n d s a y, 1988). The relation of oxidative stability and the content of nitriles were found insignificant too.

References

BENJELLOUN, B. - TALOU, T. - DELMAS, M. - GASET, A.: Oxidation of rapeseed oil: Effect of metal traces. J. amer. Oil Chem. Soc., *68*, 1991 : 210-211.

ČMOLÍK, J. - DAVÍDEK, J. - POKORNÝ, J.: Experience with processing zero-erucic rapeseed by combination of pressing and extraction. In: Fat Science. London, Elsevier 1983 : 687-694.

ČMOLÍK, J. - POKORNÝ, J. - PÁNEK, J. - KÁŇOVÁ, J. - VELÍŠEK, J. - PAŘÍZKOVÁ, J. - HOLASOVÁ, M. - KOPLÍK, R.: Effect of minor constituents of crude rapeseed oil on the stability of refined oil against the oxidative deterioration. In: GCIRC VIIIth int. Rapeseed Congr., Saskatoon, C 1991, paper 42.

ČOUPKOVÁ, M. - WINTEROVÁ, L. - MACH, I.: Modifikovaná metoda ke stanovení obsahu železa v rafinovaných olejích a neemulgovaných ztužených tucích. Zprav. tuk. Prům., *2*, 1986 : 94-98.

CHO-AN-YING, F. - DEMAN, J. M.: Sulphur and chlorophyll content of Ontario canola oil. Canad. Inst. Food Sci. Technol. J., *22*, 1989 : 222-226.

ENDO, Y. - USUKI, R. - KANEDA, T.: Prooxidant activities of chlorophylls and their decomposition products on the photooxidation of methyl linoleate. J. amer. Oil Chem. Soc., *61*, 1984 : 781-784.

HEIMANN, W. - PEZOLD, H. von: Über die prooxygene Wirkung von Antioxygenen. Fette, Seifen, Anstrichm., *59*, 1957 : 330-338.

HOLASOVÁ, M. - PAŘÍZKOVÁ, H. - DAVÍDEK, J. - POKORNÝ, J.: Effect of lipid hydroperoxides on pheophytin in the dark. Nahrung, *33*, 1989 : 171-176.

HOLASOVÁ, M. - PAŘÍZKOVÁ, H. - POKORNÝ, J.: Spectrophotometric determination of chlorophyll pigments in crude vegetable oils. Riv. ital. Sostanze grasse, *67*, 1990 : 521-522.

HOLASOVÁ, M. - PAŘÍZKOVÁ, H. - POKORNÝ, J.: Stanovení chlorofylových barviv v řepkovém oleji. Prům. Potr., *43*, 1992 : 30-32.
KARAHADIAN, C. - LINDSAY, R. C.: Evaluation of the mechanism of dilauryl thiodipropionate antioxidant activity. J. amer. Oil Chem. Soc., *65*, 1988 : 1159-1165.
KAWAKATSU, M. - TERAO, J. - MATSUSHITA, S.: Phospholipid oxidation catalyzed by ferrous ion and ascorbic acid. Agric. biol. Chem., *48*, 1984 : 1275-1279.
LEE, S.-H. - MIN, D. B.: Effects, quenching mechanisms, and kinetics of carotenoids in chlorophyll-sensitized photooxidation of soybean oil. J. agric. Food Chem., *38*, 1990 : 1630-1634.
MESHEHDANI, T. - POKORNÝ, J. - DAVÍDEK, J. - PÁNEK, J.: The lipoxygenase activity of rapeseed. Nahrung, *34*, 1990 : 727-734.
NIEWIADOMSKI, H.: Technologia nasion rzepaku. Warszawa, Panstwowe Wydawnictwo Naukowe 1983.
POCKLINGTON, W. D. - DIEFFENBACHER, A.: Determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. Pure appl. Chem., *60*, 1988 : 877-892.
POKORNÝ, J. - DOBIÁŠOVÁ, S. - DAVÍDEK, J. - RANNÝ, M.: Effect of synthetic phospholipids and their metal salts on the oxidative stability of edible oils. Acta Univ. carol. med., *32*, 1986 : 53-57.
POKORNÝ, J. - PAŘÍZKOVÁ, H. - HOLASOVÁ, M. - DAVÍDEK, J. - ČMOLÍK, J.: Vliv získávání oleje, rafinace a ztužování na obsah barviv v řepkovém oleji. Potr. Vědy, *7*, 1989 : 98-101.
VELÍŠEK, J. - DAVÍDEK, J. - MICHOVÁ, J. - POKORNÝ, J.: Rapid gas chromatographic determination of volatile degradation products of glucosinolates in rapeseed oil. J. Chromatogr., *502*, 1990 : 167-170.
VELÍŠEK, J. - POKORNÝ, J. - DAVÍDEK, J. - MICHOVÁ, J. - ČMOLÍK, J.: Degradation of glucosinolates and their decomposition products during the processing of Czechoslovak double-low and high-glucosinolate rapeseed. In: Sbor. VŠCHT v Praze, *E 62*, 1991 : 55-84.

Received for publication August 31, 1992

J. Pokorný, J. Velíšek, J. Pánek, J. Káňová, H. Pařízková, M. Holasová, R. Koplík, J. Čmolík (Vysoká škola chemicko-technologická – Ústav chemie a analýzy potravin a Výzkumný ústav potravinářského průmyslu, Praha; SETUZA, a. s. Ústí n. L.)

Doprovodné lipofilní složky surového řepkového oleje

Surové řepkové oleje byly vyrobeny ze semen ozimé řepky olejné (*Brassica napus* L.) sklizené v Čechách, obsahujících jen stopová množství kyseliny erukové (pod 2 %). Obsah glukosinolátů byl u části vzorků tradiční, u části snížený. Oleje byly získány kombinací lisování ve šnekových lisech a extrakcí výlisků hexanem v karuselovém průmyslovém extraktoru Extraktionstechnik. Surové oleje byly po vyčištění na sítech dále čištěny centrifugací, potom byly skladovány v ocelových nádržích smíchané s olejem získaným extrakcí v objemových poměrech 2 : 1. V surových olejích byl stanoven obsah chlorofylových a karotenoidních barviv spektrofotometricky a obsah feofytinů a chlorofylů spektrofluorometricky. Tokoferoly byly stanoveny vyso-

koúčinnou kapalinovou chromatografií, těkavé rozkladné produkty glukosinolátů kapilární plynovou chromatografií a obsah železa po zpopelnění spektrofotometricky. Stabilita olejů proti oxidačnímu žluknutí byla stanovena Schaalovou zkouškou s gravimetrickou indikací. Celkem bylo analyzováno 10 surových filtrovaných lisovaných olejů, 14 centrifugovaných lisovaných olejů, 14 olejů extrahovaných z výlisků hexanem a 9 směsí lisovaného a extrahovaného oleje z nádrže. Výsledky byly statisticky zpracovány regresní analýzou s použitím programu Statgraphics.

Oleje obsahovaly značná množství feofytinu a, doprovázeného malým množstvím feofytinu b a obou chlorofylů. Mezi tokoferoly mírně převažoval γ-derivát nad α-derivátem, zatímco δ-tokoferolu byly jen stopy. Karotenoidů bylo řádově tolik, kolik feofytinů, i když mezi obsahy obou těchto složek nebyl statisticky průkazný vztah. Obsah železa byl značný, a to hlavně v lisovaném filtrovaném oleji. Poměrně vysoký obsah rozkladných produktů glukosinolátů nebyl v jednoduchém vztahu k obsahu původních glukosinolátů v semenech. Nitrily značně převažovaly nad isothiokyanatany.

Ke stanovení stability proti oxidaci byla zvolena Schaalova zkouška při 60 °C, protože výsledky dávají dobrou korelaci s poměry při běžném skladování. Vzhledem k tomu, že se u řepkového oleje neprojevuje zřetelná indukční perioda, podobně jako u jiných značně nenasycených olejů, byla jako konec indukční periody zvolena doba potřebná k absorbování určitého množství kyslíku (200 nebo 500 mg na 1 kg oleje).

Stabilita proti oxidaci byla v pozitivním vztahu k obsahu chlorofylových barviv; hlavně stopových chlorofylů a a b, a u lisovaných olejů také (ale negativně) na obsahu tokoferolů. Za nízké teploty a nepřístupu světla mohou chlorofylová barviva (hlavně feofytiny) působit jako inhibitory oxidace, a tokoferoly naopak v přirozeně přítomných vysokých množstvích již spíše jako slabé prooxidanty. Tyto doprovodné složky však spíše sloužily jako indikátory stavu oleje a skutečně aktivních složek (např. fosfolipidů a produktů lipoxygenasou katalyzované oxidace), protože za podmínek skladování za nepřístupu světla a zvýšené teploty nemůže být vliv těchto složek tak podstatný. Kovy s proměnlivou valencí (včetně železa) působí sice jako prooxidanty, ovšem pouze ve stopových množstvích. V lisovaných olejích měl obsah železa spíše pozitivní vztah ke stabilitě, a to pravděpodobně proto, že v olejích jsou ionty železa vázány v neaktivních komplexech, např. na kyselé fosfolipidy. Stabilita olejů proti žluknutí nebyla v žádném průkazném vztahu k obsahu nitrilů a isothiokyanatanů, i když by se mohl příznivý vliv přítomné síry očekávat.

Výsledky ukazují, že jako indikátorů stability olejů proti žluknutí lze použít řady látek, z nichž některé mají přímo účinek, jiné pouze naznačují přítomnost skutečně účinných inhibitorů anebo naopak katalyzátorů oxidace.

řepkový olej; autooxidace; stabilita; doprovodné lipidy

Potr. Vědy, *11*, 1993 (3) : 197-208

GAS CHROMATOGRAPHIC ANALYSIS OF CELLULAR FATTY ACIDS IN THE IDENTIFICATION OF LACTIC ACID BACTERIA

Renata GILAROVÁ, Michal VOLDŘICH, Kateřina DEMNEROVÁ[1], Miroslav ČEŘOVSKÝ, Jaroslav DOBIÁŠ

Institute of Chemical Technology – Department of Food Preservation and Meat Technology and [1]Department of Biochemistry and Microbiology, Technická 5, CS-166 28 Praha 6, Czech Republic

A procedure including the cultivation of cells, sample preparation, conditions of the high resolution gas chromatography of fatty acid methylesters and analysis of results for the identification of lactic acid bacteria has been proposed and verified. The separated cells after precultivation were cultivated in liquid media, centrifuged cells were treated with 2% sulphuric acid in methanol, the methylesters of cellular fatty acids were separated using 50 m DB1 capillary column. The fatty acid composition of 15 different strains of *Lactobacillus acidophilus*, *L. plantarum* and *Lactococcus lactis* was analyzed, the distance coefficients σ and overlap coefficients were used for the data evaluation. The reliability and usability of the procedure are discussed.

lactic acid bacteria; cellular fatty acid; fatty acid methylesters; capillary gas chromatography

Gas chromatographic analysis of cellular fatty acids has been commonly used in bacteria taxonomy since the early 70s (Riedmann, 1972; Kondo, Nagashue, 1988; Urdaci et al., 1990; Kaneda, 1991; Richter, 1991). The development of analytical equipment, especially the introduction of high resolution gas chromatographic techniques made this procedure one of the main biophysical methods used to characterize bacterial strains (Dziedzak, 1987). Commercial identification systems based on the fatty acid pattern are also available (Hewlett Packard).

The use of fatty acid analysis for the characterization of lactic acid bacteria was already described (Decallonne et al., 1991; Rizzo et al., 1987; Schmitt et al., 1989). Depending on the species and cultivation conditions, the following main fatty acids have been found in different lactic acid bacteria and can be used for their classification: tetradecanoic, 13-methyltetradecanoic, 12-methyltetradecanoic, pentadecanoic, cis-9-hexadecenoic, hexadecanoic, hepta-

decanoic, cis-9-octadecenoic, cis-11-octadecenoic, octadecanoic, 11, 12-methylene-octadecanoic and eicosanoic acid; however, the content and composition of the main fatty acids depend not only on the microorganism strains but also they are affected by a number of factors, such as a decrease in pH value or an increase in NaCl content in the medium within the cultivation of *Leuconostoc* sp., which provokes the synthesis of lactobacillic (11, 12-methyleneoctadecanoic) acid from its precursor cis-9-octadecenoic acid (S c h m i t t et al., 1989). An important factor which has not been satisfactorily explained is at what age cultures possess the stabilized fatty acid pattern and when they are optimal for analysis (D e c a l l o n n e et al., 1991). Another factor to be explained is the way the samples are prepared before GC analysis, which could be a source of composition changes. Generally, the two main derivatization procedures which are used involve: 1. alcalic hydrolysis and 2. rapid base, and/or acid catalyzed esterification or just one step acid catalyzed transesterification (C h r i s t i e, 1982). The former is usually insufficient to hydrolyze N-acyl derivatives of long-chain bases and the saturated C_{16} and C_{18} fatty acids, which are bound there, are lost. However, on the other hand, the heating in the acidic conditions of the latter procedure usually destroys cyclopropen rings of fatty acids and thus the lactobacillic acid will not be correctly determined. These and other factors affecting the found fatty acid methylester (FAME) pattern complicate the use of the procedures and results obtained by other authors.

Therefore the aim of this paper is to propose and verify a simple procedure allowing the build up of FAME database usable together with other tests to classify lactic acid bacterial strains used in food technology.

MATERIAL and METHODS

Preparation of bacterial cultures — A list of bacterial species and strains used is given in Table I. The strains were grown under anaerobic conditions for 24 hours in 50 ml of liquid culture medium. The composition of medium is given in Table II.

Sample preparation — After 24 hours of cultivation the bacterial suspension was centrifuged, and bacteria were twice washed with distilled water. Portions of ca. 100-200 mg fresh weight of cells were suspended in 10 ml of 2% solution of sulphuric acid in anhydrous methanol. The mixture was refluxed in 2 hours, then methylesters were extracted into 3 ml of heptane after the addition of NaCl saturated solution. The separated organic phase was evaporated with a gentle stream of nitrogen; until analysis the samples were stored under nitrogen at -18 °C.

GLC analysis — The HRGC 5300 gas chromatograph (Carlo Erba, Milano) equipped with DB1 (50 m, i.d. 0.22 mm) (Hewlett Packard) capillary column was

I. A list of analysed bacterial strains

Strain		Source
Lactobacillus acidophilus	CH5	Milcom servis a.s. Praha
	Vokovice	Milcom servis a.s. Praha
	AL	Milcom servis a.s. Praha
	A	Milcom servis a.s. Praha
	12A	Milcom servis a.s. Praha
	13B	Milcom servis a.s. Praha
	14C	Milcom servis a.s. Praha
	15D	Milcom servis a.s. Praha
	16E	Milcom servis a.s. Praha
Lactobacillus plantarum		Dept. Biochem. Microbiol. Inst. Chem.Tech. Praha
Lactococcus lactis subsp. *cremoris*	1A	Milcom servis a.s. Praha
	5E	Milcom servis a.s. Praha
Lactococcus lactis subsp. *lactis*	7A	Milcom servis a.s. Praha
	10D	Milcom servis a.s. Praha
	11E	Milcom servis a.s. Praha

used. The chromatographic conditions were as follows: Carrier gas nitrogen under pressure of 135 kPa, make up gas (N_2) pressure 50 kPa, hydrogen 60 kPa, air 70 kPa. Temperatures: injector port 240 °C, detector 260 °C, column temperature program 190 - 280 °C, 2 °C/min. The split ratio was 1 : 50.

II. Composition of cultivation medium

Component	Quantity	Component	Quantity
Casein enzyme hydrolyzate	10 g	$MgSO_4.7H_2O$	0.8 g
Yeast autolyzate	5 g	$MnCl_2.4H_2O$	0.14 g
$K_2HPO_4.3H_2O$	5 g	$FeSO_4.7H_2O$	0.04 g
NaCl	5 g	Tween 80	1 g
Sodium citrate	5 g	H_2O	1000 ml
Glucose	10 g	Agar	2 %(w/w)

Analysis of results — Fatty acid methylesters were identified comparing the retention times with those of the standard mixture Bacterial mix (Supelco) and the results were expressed as a percentage of identified acids (the response factor of FID being used to equal 1). For evaluating the effects of growth conditions, methods of sample preparation and analysis and to compare the analyzed strains the "distance coefficient" (σ) calculated after D e c a l l o n n e et al. (1991) and the "similarity or overlap coefficient" (*So*) of B o u s f i e l d et al. (1983) were used. The "distance coefficients" were calculated for the six fatty acids of major relative percentage areas using the formula:

$$d(i,j) = (x_{i,k} - x_{j,k})^2 \; / (0.5x_{i,k} + 0.5x_{j,k})$$

then $\sigma 3$ and $\sigma 6$ being the sums of the d values for three or six major acids, respectively. The "overlap" coefficient was calculated with the expression:

$$So(i,j) = 100 - 0.5 \sum | x_{i,k} - x_{j,k} |$$

where: $x_{i,k}$ and $x_{j,k}$ are percentage areas of the k-th peak for the i-th and j-th organism respectively

The "overlap coefficients" were also used for the construction of similarity cluster. All the calculations were made by the Microsoft Excel Software.

RESULTS and DISCUSSION

The proposed procedure to classify the lactic acid bacterial strains could be as follows: precultivation to isolate the strain or to stabilize fatty acids composition, preliminary classification, cultivation under the standard conditions (universal medium, temperature), isolation of cells, acid transesterification, GC, data processing (comparison with database), classification or identification.

The steps beginning from the cultivation were only tested from the aforementioned procedure because the collection strains kept in standard conditions (agar medium, 4 °C - Table II) or pure strains in sterilized milk were analyzed to get primary data for the future database. Thirteen main fatty acids were separated and identified comparing with the retention times of standard mixture (Table III). The effects of the cultivation conditions were evaluated using overlap and distance coefficients. Considering our aim to propose a rapid and handy method, the analyzed strains were cultivated in 50 ml of modified APT liquid medium at 30 °C for *Lactococcus* and 37 °C for *Lactobacillus*. Within the analyses of the effect of aging on the FAME composition of cells of *L. acidophilus* CH5 and *L. plantarum* strains we found almost the same composition changes after 24, 48 and 72 hours of cultivation as those described by D e c a l l o n n e et al. (1991) (Table IV).

Supposing 10 hours for the lag phase and about 24 hours to reach the end of the log phase and also relatively important changes within the prolonged cultivation, the 24-hour cultivation was chosen for the standard procedure. Both tested genera were not possible to cultivate at the same temperatures, therefore two parallel cultivations should be done within the standard procedure if the isolate or unknown strain belongs to *Lactococcus*.

As it is mentioned in the introduction, it is almost impossible to find a simple and rapid esterification procedure providing correct and objective results. Considering the aim of our work it is not very important to determine the objective

III. Fatty acid methylesters found in the analyzed strains and used for similarity calculations

Fatty acid	Symbol	ECL*
Tetradecanoic	14:0	14.00
13-methyltetradecanoic	i15:0	14.63
12-methyltetradecanoic	a15:0	14.71
Pentadecanoic	15:0	15.00
cis-9-hexadecenoic	16:1	15.79
Hexadecanoic	16:0	16.00
Heptadecanoic	17:0	17.00
cis,cis-9,12-octadecadienoic	18:2	17.66
cis-9-octadecenoic	18:1(9)	17.74
cis-11-octadecenoic	18:1(11)	17.79
Octadecanoic	18:0	18.00
11,12-methyleneoctadecanoic	19:0c	18.88
Eicosanoic	20:0	20.00

*ECL - "Eqivalent chain length" (C h r i s t i e, 1982)

composition of cellular fatty acids if the proposed method allows us to obtain reproducible results usable to distinguish the different bacteria strains. Therefore the rapid and handy transesterification with two-hour boiling in 2% sulphuric acid solution in methanol, which can destroy a majority of lactobacillic acid but which is cheap, does not need to eliminate all the remaining water within the cell isolation, and where there is no danger of possible artefact formation (as e.g. the use of BF_3) (C h r i s t i e, 1986), was used. The esterification step was made three times from the same divided sample of cells and gave almost the same results ($\sigma 3 = 0.01$ and $\sigma 6 = 0.04$). Very close results were also obtained for the repeated injection ($\sigma 3 = 0.01$; $\sigma 6 = 0.02$) and for the different chromatographic conditions, the

IV. The effect of the culture age on distance coefficients σ

Strain	Cultures compared	σ3	σ6
L. acidophilus CH5	24hours - 48hours	0.68	2.46
	24hours - 72hours	2.96	10.59
	48hours - 72hours	3.16	12.13
L. plantarum	24hours - 48hours	0.42	2.49
	24hours - 72hours	1.98	4.43
	48hours - 72hours	0.80	4.25

injector temperature ranging from 240 to 260 °C (σ3 = 0.13; σ6 = 0.18), the detector temperature from 240 to 280 °C (σ3 = 0.27; σ6 = 0.30).

The whole procedure including the parallel repeated 24-hour cultivation made at different times with the collection strains kept in MRS agar was used to evaluate the reproducibility. The results are given in Table V.

The whole cultivation and subsequent analyses were repeated (at least) twice with the similarity coefficients ranging in the intervals 0 - 0.2 for σ6 and 100 - 97 in the case of the similarity coefficient *So*. Comparing the FAME composition of several times precultivated cultures, the preliminary treatment affected the

V. Evaluation of reproducibility of the whole procedure

Strain	Analysis No.	*So* (σ6)		
		2*	3	4
L. acidophilus AL	1**	95.54(1.30)	95.92(1.23)	94.86(1.61)
	2*	-	94.05(2.01)	93.27(2.53)
	3	-	-	98.84(0.09)
		5	6	7
L. acidophilus CH5	5	-	98.51(0.19)	98.94(0.13)
	6	-	-	98.55(0.15)

* twice precultivated from milk at agar plate; ** twice precultivated in liquid medium

obtained results (σ6 ranged from 1.5 to 2.87 and *So* 95 - 91.6), nevertheless the repeated 24-hour cultivation under the standard conditions could stabilize the FAME composition.

The identified FAME and their ECL coefficient on DB1 capillary column under the used chromatographic conditions are given in Table III.

VI. Fatty acid composition of the analyzed strains

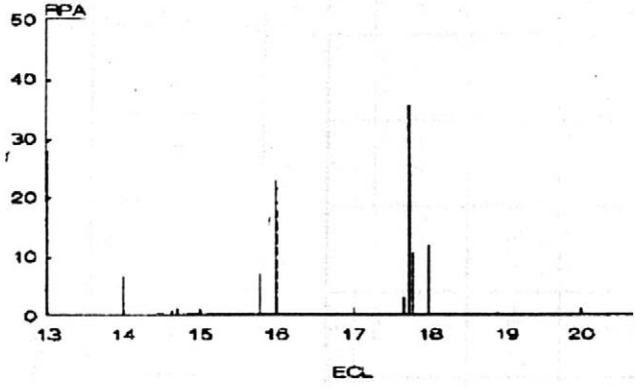
Strain	Fatty acid (% of identified acid)												
	14:0	i15:0	a15:0	15:0	16:1	16:1	17:0	18:2	18:1(9)	18:1(11)	18:0	19:0c	20:0
L. acidophilus 12A	5.12	0.16	0.31	0.62	11.63	23.88	0.47	0.93	22.79	25.58	6.2	0.93	2.02
L. acidophilus 13B	5.85	0.57	0.38	0.75	9.62	43.39	0.38	0.75	11.69	16.42	6.42	1.51	2.64
L. acidophilus 14C	6.37	0.49	0.74	0.98	6.62	22.55	0.98	2.94	35.29	10.29	11.52	0.25	0.98
L. acidophilus 15D	2.13	0.15	0.15	0.22	2.93	25,38	0.44	5.72	42.19	4.99	15.04	0.44	0.22
L. acidophilus 16E	3.61	0.00	0.00	0.00	3.01	28,33	0.00	5.42	36,75	9.04	12.65	1.2	0.00
L. acidophilus Vokovice	13.08	1.43	0.89	2.32	10.52	24.03	1.43	0.67	16.76	13.37	11.59	0.89	3.03
L. acidophilus CH5	6.39	1.68	2.02	1.68	8.08	10.44	0.00	0.00	30.64	25.59	9.43	1.35	2.69
L. acidophilus AL	7.21	1.44	0.48	2.4	5.77	21.55	0.96	0.00	24.04	26.92	7.21	0.96	1.44
L. plantarum	4.23	0.00	0.81	0.00	6.43	40.89	0.00	0.00	10.15	23.61	10.86	0.52	2.41
L. lactis subsp. *cremoris* 5E	9.52	0.00	0.68	0.68	7.48	37.41	0.68	0.00	19.05	12.24	10.88	0.00	1.36
L. lactis subsp. *cremoris* 1A	9.96	0.4	0.4	0.4	8.37	33.45	0.4	0.00	15.54	19.52	9.56	0.00	1.99
L. lactis subsp.*lactis* 10D	25.27	0.27	0.54	0.54	2.15	36.29	0.00	1.08	10.48	12.9	7.53	0.27	2.69
L. lactis subsp. *lactis* 11E	7.76	0.29	0.29	0.57	3.74	36.21	0.00	1.15	17.53	20.11	4.89	5.46	2.01
L. lactis subsp. *lactis* 7A	9.52	0.38	0.38	0.62	2.09	42.86	0.00	2.01	15.42	19.05	4.76	0.36	2.63

VII. Combination of overlap coefficients *So* and distance factors σ6 (values in brackets)

Strain		Sample No.	2	3	4	5	6	7	8	9	10	11	12	13	14
L. acidophilus	12A	1	78.64 (22.94)	79.65 (24.71)	69.03 (59.39)	74.23 (38.19)	83.53 (19.90)	85.32 (15.73)	93.06 (5.18)	78.81 (24.50)	78.08 (16.05)	82.83 (13.17)	65.82 (62.61)	82.55 (17.13)	75.87 (31.84)
	13B	2		70.40 (43.75)	62.54 (65.77)	68.71 (48.58)	79.61 (22.12)	66.92 (62.77)	74.65 (30.62)	89.76 (7.02)	58.89 (10.60)	87.31 (7.74)	80.08 (36.14)	86.98 (10.50)	90.12 (13.53)
	14C	3			86.76 (13.25)	92.00 (5.87)	83.11 (20.49)	82.19 (23.30)	82.56 (21.38)	70.10 (49.80)	81.15 (28.20)	76.41 (27.66)	65.74 (62.71)	73.93 (62.71)	68,31 (31.87)
	15D	4				91.81 (4.59)	71.42 (54.89)	68.95 (55.53)	70.17 (53.61)	62.02 (76.09)	70.74 (42.89)	62.02 (61.87)	70.74 (93.37)	66.00 (59.34)	59,02 (70.61)
	16E	5					76.62 (36.65)	74.19 (40.56)	75.58 (31.56)	70.22 (50.58)	78.93 (24.10)	74.19 (37.60)	67.14 (67.92)	74.59 (33.99)	69.70 (43.31)
	Vokovice	6						74.52 (32.19)	80.84 (20.19)	76.31 (28.75)	87.95 (8.41)	87.95 (7.20)	79.20 (27.09)	82.08 (18.56)	77.67 (30.80)
	CH5	7							87.81 (10.84)	70.92 (58.33)	71.45 (42.76)	75.92 (37.21)	57.30 (86.55)	72.69 (43.25)	66.01 (62.78)
	AL	8								78.08 (26.80)	78.39 (23.26)	82.25 (23.65)	67.84 (51.45)	82.77 (15,69)	76.88 (25.65)

Strain			Sample No.	2	3	4	5	6	7	8	9	10	11	12	13	14
L. plantarum			9									84.95 (17.18)	87.06 (9.83)	77.62 93.21)	86.13 (13.09)	86.24 (16.49)
L lactis	subsp. *cremoris*	5	10										91.30 (4.78)	82.61 (26.42)	88.46 (11.39)	86.30 (15.33)
		1	11											80.96 (26.05)	91.58 (7.58)	89.24 (13.11)
	subsp. *lactis*	10D	12												81.96 (27.24)	81,88 (20.85)
		11E	13													93.32 (2.74)
		7A	14													

Using the described procedure, 13 different bacterial strains were analyzed, and their fatty acid composition is given in Table VI. The distance factors and overlap coefficients between all the analyzed strains are presented in Table VII. The visual characterization of strains was made using the percentage area ECL "spectra" (Fig. 1).



1. FAME "spectrum" of *Lactobacillus acidophilus* 14C

Comparing the used similarity coefficient σ and *So*, both of them gave close results, for all the tested combinations the correlation coefficient between σ6 and *So* was 0.9132. The distance coefficients could be very useful especially for a rapid comparison. The limit values of both coefficients when it is possible to suppose that the two compared strains are the same could be, in agreement with Decallonne, lower or equal 3.00 for σ6 and higher or equal 90 for *So*. The analyzed strains differed in the FAME composition, an interesting result is the difference between the strains of the same genus. The number of strains which were analyzed and the number of completed experiments do not allow us to make a final conclusion, nevertheless it seems possible to use the method also to distinguish the subspecies and strains within the same genus or subspecies; however, other experiments should be done in order to:

1. make clear what is the effect of the living conditions preceding the analysis (whether the similar composition of cellular faty acids is not mainly given by a similar common history)
2. find the cultivation conditions appropriate for the wide spectrum of lactic acid bacterial strains
3. find the simplest and reproducible analytical procedure allowing standardization for several laboratories
4. build wide database.

References

BOUSFIELD, I. - SMITH, G. - DANDO, T. - HOBBS, G.: Numerical analysis of total fatty acid profiles in the identification of coryneform, nocardioform, and some other bacteria. J. Gen. Microbiol., *129*, 1983 : 375-394.

CHRISTIE, W. W.: Lipid Analysis, Isolation, Separation, Identification and Structural Analysis of Lipids. 2nd ed. Oxford, New York, Pergamon Press 1982.

DECALLONNE, J. - DELMEE, M. - WAUTHOTZ, P. - EL LIOUI, M. - LAMBERT, R.: A rapid procedure for the identification of lactic acid bacteria based on the gas chromatographic analysis of the cellular fatty acids. J. Food Protect., *54*, 1991 : 217-224.

DZIEDZAK, J.: Rapid methods for microbiological analysis of foods. Food Technol., *54*, 1987 : 56-73.

KANEDA, T.: Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol. Rev., *55*, 1991 : 288-302.

KONDO, F. - NAGASHUE, S.: Gas-liquid chromatographic determination of propionic acid production differentiates between the food-poisoning strains and toxigenic-type strains of *Clostridium perfringens*. J. Food Protect., *51*, 1988 : 283-288.

RICHTER, V.: Identifikace mikroorganismů na základě analysy mastných kyselin v biomase plynovou chromatografií. [PhD. Thesis.] Praha 1991.- UK PF.

RIZZO, A. - KORKEALA, H. - MONONEN, I.: Gas Chromatography analysis of cellular fatty acids and neutral monosaccharides in the identification of *Lactobacilli*. Appl. Environ. Microbiol., *53*, 1987 : 2883-2888.

SCHMITT, P. - MATHOT, A. - DIVIES, C.: Fatty acid composition of the genus *Leuconostoc*. Milchwissenschaft, *44*, 1989 : 556-559.

URDACI, M. C. - MARCHAND, M. - GRIMONT, P. A. D.: Characterization of 22 *Vibrio* species by gas chromatography analysis of their cellular fatty acids. Res. Microbiol., *141*, 1990 : 437-452.

HEWLETT PACKARD: Microbial Identification System. Hewlett Packard Company Avondale, 1987 PA.

Received for publication February 18, 1993

R. Gilarová, M. Voldřich, K. Demnerová, M. Čeřovský, J. Dobiáš

(Vysoká škola chemicko-technologická – Ústav konzervace potravin a technologie masa a Ústav biochemie a mikrobiologie, Praha)

Plynová chromatografie celulárních mastných kyselin v identifikaci baktérií mléčného kvašení

Možnosti využití analýzy složení celulárních mastných kyselin v taxonomii mikroorganismů jsou studovány prakticky od počátků plynové chromatografie. S rozvojem instrumentální techniky, zejména zavedením vysokoúčinné kapilární chromatografie a rozšiřujícím se možnostem využití výpočetní techniky, je analýza methy-

lesterů celulárních mastných kyselin pokládána za jednu ze základních biofyzikálních metod používaných při charakterizaci kmenů baktérií (D z i e d z a k, 1987). Přes optimistické závěry většiny publikovaných prací brání většímu rozšíření nedostatek znalostí o všech faktorech ovlivňujících složení buněčných mastných kyselin a velká variabilita výsledků různých autorů. Cílem prezentované práce bylo navrhnout jednoduchou metodu kultivace a analýzy složení celulárních mastných kyselin mléčných baktérií a ověřit její použitelnost při identifikaci baktérií mléčného kvašení.

Složení celulárních mastných kyselin včetně možností využití výsledků pro charakterizaci baktérií mléčného kvašení bylo již věnováno několik prací (D e c a l l o n n e et al., 1991; R i z z o et al., 1987). S c h m i t t et al. (1989) popsali nárůst obsahu laktobacilové (11,12-methylen-oktadekanové) kyseliny vlivem snížení pH a zvýšením obsahu NaCl v médiu při kultivaci baktérií druhu *Leuconostoc*. Vedle podmínek kultivace je nalezené složení ovlivněno také analytickou metodou, použije-li se kyselá hydrolýza (kysele katalyzovaná esterifikace) dochází k rozkladu cyklické laktobacilové kyseliny, která je jednou z majoritních složek, naopak použití alkalické hydrolýzy neumožní hydrolýzu N-acyl derivátů mastných kyselin.

Navržený postup zahrnuje kultivaci čistého nebo vyizolovaného kmene v tekutém APT médiu 24 hodin, izolaci biomasy odstředěním a její promytí destilovanou vodou, hydrolýzu a methylaci varem s 2% kyselinou sírovou v methanolu, extrakci methylesterů do heptanu, analýzu plynovou chromatografií na kapilární koloně 50 m s nepolární fází DB1.

Popsaný postup byl použit při analýze patnácti různých kmenů *Lactobacillus acidophilus*, *Lactobacillus plantarum* a *Lactococcus lactis*. Byla ověřena reprodukovatelnost jednotlivých částí postupu a vliv různých podmínek chromatografické analýzy. Podobnost analyzovaných kmenů byla posouzena dvěma různými způsoby statistického zpracování výsledků.

Všech patnáct analyzovaných kmenů se dostatečně lišilo složením mastných kyselin, získané shluky vypočtené na základě vzájemné podobnosti složení mastných kyselin neodpovídají tradičnímu třídění kmenů. Proto před dalším rozšiřováním báze dat zůstává vedle definitivní úpravy postupu kultivace a analýzy také ověřit, do jaké míry se na podobnosti složení celulárních mastných kyselin odráží případná společná předchozí historie analyzovaných kmenů.

baktérie mléčného kvašení; celulární mastné kyseliny; methylestery mastných kyselin; kapilární plynová chromatografie

Potr. Vědy, *11*, 1993 (3) : 209-215

HPLC DETERMINATION OF LACTOSE AND GLUCOSE IN CULTURE MEDIA

Vladimír FILIP, Milada PLOCKOVÁ, Jana CHUMCHALOVÁ

Institute of Chemical Technology – Department of Milk and Fat Technology, Technická 5, CS-166 28 Praha 6, Czech Republic

Lactose and glucose as fermentable sugars are used in lactic acid fermentation as a carbon source. The determination of their concentration in fermentation broth is possible by HPLC method using the Separon SGX-NH_2 column, elution with acetonitrile/water and xylose as an internal standard. In addition this method makes possible simultaneous assay of xylose, glucose (galactose), sucrose and lactose. The developed method was applied for the determination of concentration changes of glucose in MRS broth and for concentration changes of lactose in skim milk during cultivation of the strain *Lactobacillus acidophilus* (Laktoflora 92). Trichloracetic acid was used for precipitation. HPLC (calibration with external and internal standard) was compared with the titrimetric method (IDF) and the recovery obtained was 95 - 105 %.

galactose; glucose; lactose; sucrose; xylose; HPLC; *Lactobacillus acidophilus*

Lactose, a disaccharide composed of glucose and galactose, occurs in milk in the amount approx. 5 % w/w. Lactic acid bacteria use it for energy production and cell growth. A lot of processes in dairy industry are based on biochemical activity of lactic acid bacteria. It is believed that the first step of lactose metabolism in mesophilic and thermophilic lactic acid bacteria includes the hydrolysis of lactose or lactose-6-phosphate to glucose and galactose or glucose and galactose--6-phosphate (B i s s e t t, A n d e r s o n, 1974). Glucose is then metabolized by glycolysis while galactose is either in the case of gal$^-$ strains of genus *Lactobacillus* unmetabolized or transformed to glucose-1-phosphate by the Leloir pathway (C o g a n, D a l y, 1987). The determination of the utilization rates of lactose, glucose and galactose is important in food industry research as well as for the characterization of the individual strains of lactic bacteria.

One of the most important issues of the determination of sugars is usually the necessity to separate the accompanying substances (e.g. proteins, lipids). This step, known as clarification, is needful both in the classical and modern analytical

methods. The chromatographic methods give the option to separate several carbohydrates in a mixture, however, the separation of the accompanying substances cannot be solved by this single step. Thus various chemical methods are used to remove these interfering substances. As an example may be given the application of chlorous acid (W e s t, L l o e n t e, 1981), 2-propanol (K w a k, J e o n, 1988), or the reverse phase technique (J e o n et al., 1984).

The isolation of lactose, glucose and galactose from milk using the strong acid cation exchange (Ca^{2+} form) column may be realized with good reproducibility. An Aminex Microguard Anion/OH cartridge was used as a guard column to remove interfering acids and salts (R i c h m o n d et al., 1982). Recently, the sugar from Cheddar cheese were separated by the Aminex HPX-87 column in the H^+ form (B o u z a s et al., 1991). R i e l et al. (1986) suggested separation of mono- , di- and trisaccharides on a cation exchange (Pb^{2+} form) column. Selectivity of the separation was influenced by varying the Ag^+ and Pb^{2+} ratio. The post column chemical detection (D e r u y et al., 1988), or enzyme detection using NAD^+ glucose dehydrogenase (M a r k o - V a r g a, 1987) were recomended. Another assay is based on anion exchange chromatography with a pH gradient elution (O l i e m a n, 1991) and subsequent amperometric detection.

The silica gel NH_2-propyl bounded-phase column, acetonitrile/water as an eluent and a refractometric detection (Y a n g et al., 1981) system is often used for the determination of sugars in food products. These types of column were used for lactose determination in milk (W e s t, L l o r e n t e, 1981), in dried milk (R u g g e r i, F o n s e c a, 1985) and for determination of purity of lactose (S a u c e r m a n, W i n s t e a d, 1984).

The authors proposed rapid simultaneous determinations of lactose, glucose and galactose in media used for cultivation of the lactic acid bacteria by means of HPLC using the calibration either by the external or internal standard and compare it with the chemical method routinely used in dairy industry.

MATERIALS and METHODS

Lactose and glucose were determined in MRS broth, medium routinely used for cultivation of lactobacilli (M a n et al., 1960) (initial concentration of glucose was approx. 2 % w/w) and in skim milk enriched with the yeast extract (initial concentrations of lactose 5.5 % w/w, glucose and galactose were not present). The decrease in sugar concentration was monitored during the cultivation of the strain *Lactobacillus acidophilus*, Laktoflora 92 (obtained from the Collection of Milk Microorganisms of Milcom, Ke dvoru 12, Praha 6, Czech Republic) in both culture media at 37 °C for less than 12 hours.

Prior HPLC determination the samples (approx. 5 or 10 g of culture medium) were treated with 5% w/w trichloracetic acid in the ratio 1 : 1 (v/v). After an internal standard addition, adjustment of sample volume with distilled water and subsequent filtration (Whatman 3) the filtrate was used both for the direct injection into the liquid chromatograph and for the iodometric determination of lactose (IDF, 1974).

The chromatographic system consisted of HPP 5001 High Pressure Pump (Laboratorní přístroje Praha), Differential Refractometer RIDK 101 (Laboratorní přístroje Praha), Line Recorder TZ 4200 (Laboratorní přístroje Praha), Rheodyne 7125 syringe loading sample injector with a 20 µl loop (Rheodyne, Berkeley, Cf.). Analyses were performed on the Separon SGX-NH_2 150 x 3 mm column (Tessek Ltd., Praha) with an average particle size of 5 µm. Mobile phase acetonitrile/water (75 : 25 v/v), flow-rate was 1 ml/min. The peak heights were measured for the purpose of quantitative determination of tested sugars. The internal standard with xylose and the external standard calibration methods were used. The response factors for tested sugars were calculated from the equation:

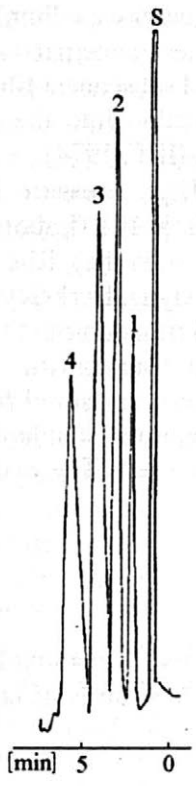
$$f_i = \frac{c_i}{s_{st}} \frac{v_{st}}{v_i} \qquad [1]$$

where c resp. v are the concentrations of tested components (i) [standard (st)], resp. the peak heights of the component (i) [standard (st)]. Standards of lactose, sucrose, glucose, galactose and xylose were p.a. grade (Lachema, Brno).

RESULTS and DISCUSSION

The sugars were eluated in the described system in following order: xylose (internal standard), glucose, galactose, sucrose, lactose (Fig. 1). The elution rate and the distribution of the individual components depend on the acetonitrile : water ratio. With the increasing volume of acetonitrile the elution rate rises and the separation worsens (M a c r a e, D i c k, 1981). Using the conditions described for the separation on Separon SGX-NH_2 the acetonitrile : water at suitable ratio 75 : 25 (v/v) optimal separation and elution rate was achieved. The precipitation agent, trichloracetic acid does not influence the separation of sugars. This finding is important from the viewpoint of the method application in real samples (e.g. milk and milk products). The detector applied had not been sensitive enough under the experimental conditions used. Thus the signals from the line detector were approximately five time simplified and then the noise level and the base line drift were found satisfactory.

The relationships between the response factors and the concentrations of the individual sugars (in distilled water) were evaluated. The injection volume (20 µl)



1. Typical chromatogram of a standard mixture of glucose, lactose, sucrose, and xylose

Peaks: S - solution of CCl_3COOH in water
1 - xylose
2 - glucose
3 - sucrose
4 - lactose

were held on the constant level during the experiment. Using the peak height better results were achieved compared with the peak areas. The linear dependence of the peak height upon the sugar concentration was found (for the standard concentration 0-5 % w/w). Further it was found, that the response factors related to the unit amount of the individual components, varied. The numerical values of the response factors decreased in the following order: xylose, glucose, sucrose, galactose, lactose (Table I). Presented dependence of the peak height upon the sugar concentration can be at the same time used as a calibration curve for the determination of sugars in the culture media by the external standard calibration method.

I. The relationship between the peak height and sugar concentration (c)

Sugar	a_0	s_{a_0}	a_1	s_{a_1}	r
Xylose	0.089	0.276	54.25	0.979	0.999
Galactose	-0.016	0.610	30.566	1.240	0.987
Glucose	-0.295	0.240	45.246	0.608	0.999
Sucrose	-0.122	0.281	32.013	0.727	0.998
Lactose	-0.673	0.632	24.954	2.143	0.964

$v = a_0 + a_1 . c$ [cm; w/w], a_0, a_1 – linear constants, s_{a_0}, s_{a_1} – standard deviations, r – correlation coefficient

Since the precision of the estimation by using the external standard calibration technique depends on the constant conditions of analysis the internal standard

II. The response factors (f_i) of xylose, galactose, glucose, sucrose and lactose and their standard deviations ($s_{f\,i}$)

Sugar	f [1]	s_f [1]
Xylose	1.00	0.04
Galactose	1.54	0.35
Glucose	1.29	0.03
Sucrose	1.49	0.08

III. The glucose concentration (c_{glu}) estimated by the HPLC/ES technique and by the HPLC/IS technique in MRS broth during the cultivation of *Lactobacillus acidophilus*, 92 at 37 °C for the time (τ)

τ [h]	pH	c_{glu} [% w/w]	
		ES	IS
0	6.29	1.71	1.97
3	6.18	1.55	1.63
6	5.46	1.49	1.53
9	4.74	1.23	1.33

IV. Lactose concentration (c_{lac}) estimated by the titrimetric method (T), HPLC/ES method and HPLC/IS method in skim milk enriched with 0.5 % w/v of yeast extract during the cultivation of *Lactobacillus acidophilus*, 92 at 37 °C for the time (τ)

τ [h]	pH	c_{lac} [% w/w]		
		T	ES	IS
0	6.43	5.35	5.08	5.20
3	6.22	5.57	5.46	5.06
6	5.36	5.34	5.68	5.56
9	4.43	4.49	4.40	4.45

(xylose) technique was also tested. The response factor of xylose was defined as one unit. The response factors of individual components present in the mixture [xylose, glucose (galactose), sucrose and lactose] were then estimated using the constant internal standard concentration and the changing concentrations of sugars (Table II). The relative standard deviation of the method was found to be less than 5 % (lactose concentration 5 % w/w).

The described HPLC method has been also used for the measurement of the glucose consumption during the fermentation of *Lactobacillus acidophilus*, 92 in MRS medium (Table III). The decrease in lactose concentration during *Lactobacillus acidophilus*, 92 growth in the skim milk was also monitored (Table IV). In this case the lactose concentrations were determined both by the titrimetric method (IDF, 1974) and by the HPLC external and internal standard calibration methods. If the recovery of the titrimetric method was declared as 100 %, it was possible to compare the recoveries of the both HPLC techniques. It was proved that the both HPLC technique recoveries usually lie in the interval 95 - 105 %. This is in a good agreement with the relative standard deviation in the value of 4 % (lactose concentration 5 % w/w) evaluated.

References

BISSET, D. L. - ANDERSON, R. L.: Lactose and D-galactose metabolism in group N Streptococci: Presence of enzymes for both the D-galactose 1-P and D-tagatose 6-P pathways. J. Bacteriol., *117*, 1974 : 318 - 320.

BOUZAS, J. - KANTT, C. A. - BODYFELT, F. - TORRES, J. A.: Simultaneous determination of sugars and organic acids in Cheddar cheese by HPLC. J. Food Sci., *56*, 1991 : 276-278.

COGAN, T. M. - DALY, C.: Cheese Starter Cultures. In: FOX, P. F. (Ed.): Cheese: Chemistry, Physics, and Microbiology. New York, Elsevier Appl. Sci Publishers 1987.

DERUY, G. - LESCURE, J. P.: Determination of reducing sugars by HPLC with post-column colour development. Sucrerie Fr., *129*, 1988 : 117-124.

JEON, I. J. - GALITZER, S. J. - HENNESSY, K. J.: Rapid determination of lactose and its hydrolysates in whey and whey permeate by HPLC. J. Dairy Sci., *67*, 1984 : 884-887.

KWAK, H. S. - JEON, I. J.: Comparison of HPLC and enzymatic method for the measurment of lactose in milk. J. Food Sci., *53*, 1988 : 975-976.

MAN, J. C., de - ROGOSA, M. - SHARPE, M. E.: A medium for the cultivation of lactobacilli. J. Appl. Bact., *23*, 1960 : 130- 131.

MARKO-VARGA, G.: HPLC separation of some mono- and disaccharides with detection by a post column enzyme reactor and a chemically modified electrode. J. Chromatogr., *408*, 1988 : 157-170.

OLIEMAN, C.: Determination of sugars by HPLC in combination with electrochemical detection. Voedingsmiddelentechnologie, *24*, 1991 : 10-11.

RIEL, J. A. M., von - OLIEMAN, C.: HPLC of sugars on a mixed cation-exchange resin column. J. Chromatogr., *362*, 1986 : 235-242.

RICHMOND, M. L. - BARFUSS, D. L. - HARTE, B. R. - GRAY, J. I. - STINE, C. M.: Separation of carbohydrates in dairy products by HPLC. J. Dairy Sci., *65*, 1982 : 1394-1400.
RUGGERI, P. - FONSECA, G.: HPLC determination of sugars in some powdered milks. Latte, *10*, 1985 : 1052-1053.
SAUCERMAN, J. R. - WINSTEAD, C. E.: Liquid chromatographic determination of lactose purity. J. Assoc. Off. Anal. Chem., *67*, 1984 : 899-901.
WEST, L. G. - LLORENTE, M. A.: HPLC determination of lactose in milk. J. Assoc. Off. Anal. Chem., *64*, 1981 : 805-807.
YANG, M. T. - MILIGAN, L. P. - MATHISON, G. W.: Improved sugar separation by HPLC using porous microparticle carbohydrate columns. J. Chromatogr., *209*, 1981 : 316-322.
IDF: International Dairy Federation Document 28A/1974. Determination of the lactose content of milk. 50 BEF : 40 p.

Received for publication February 9, 1993

V. Filip, M. Plocková, J. Chumchalová (Vysoká škola chemicko-technologická – Ústav technologie mléka a tuků, Praha)

Stanovení laktosy a glukosy v kultivačních médiích metodou HPLC

Na koloně Separon SGX-NH_2 s mobilní fází acetonitril/voda 75 : 25 (v/v) s refraktometrickou detekcí probíhá eluce sacharidů v pořadí: xylosa, (IS), glukosa, galaktosa, sacharosa, laktosa. Při koncentraci složky v nástřiku 0 - 1 μg je závislost odezvy detektoru lineární. Odezva detektoru na jednotkové množství jednotlivých složek je různá. Klesá v pořadí xylosa, glukosa, sacharosa, galaktosa, laktosa. Jako vnitřní standard byla vybrána xylosa, jejíž odezvový faktor byl zvolen jako jednotkový. Hodnoty odezvových faktorů byly stanoveny pro směsi xylosy, glukosy (galaktosy), sacharosy a laktosy při konstantní koncentraci vnitřního standardu a proměnné koncentraci dalších složek (tab. II). Pro laktosu byla zjištěna chyba metody při koncentraci laktosy 5,00 % hm. ve vzorku, která nepřesahuje 5 %. Je významné, že použité srážecí činidlo - kyselina trichloroctová - nemá vliv na dělení uváděných sacharidů v popisovaném chromatografickém systému.

Popisovaná chromatografická technika byla použita ke stanovení úbytku sacharidů v kultivačních médiích v závislosti na době kultivace. Při kultivaci kmene *Lactobacillus acidophillus*, 92 v MRS bujónu byl sledován pokles koncentrace glukosy, v odstředěném mléce byl sledován pokles koncentrace laktosy. Při srovnání s klasickou titrační metodou (IDF, 1974), jestliže se předpokládá její výtěžnost 100 %, pohybuje se výtěžnost metody HPLC v modifikaci absolutní kalibrace a s použitím vnitřního standardu zpravidla v intervalu 95 - 105 %.

galaktosa; glukosa; HPLC; *Lactobacillus acidophilus*; laktosa; sacharosa; xylosa

Potr. Vědy, *11*, 1993 (3) : 217-224

RELATIONS BETWEEN CHARACTERISTICS OF HYDROGENATED RAPESEED OIL DURING FRENCH FRYING

Lenka KOUŘIMSKÁ, Jan POKORNÝ, Petr ŠTERN[1]

Institute of Chemical Technology – Department of Food Chemistry and Analysis, Technická 5, CS-166 28 Praha 6;
[1]Institute of Hydrodynamics, Czech Academy of Sciences, Podbabská 13, CS-166 12 Praha 6, Czech Republic

Hydrogenated rapeseed oil was used for French frying of fresh potatoes for six consecutive days. The degree of deteriorative reactions was rather low. The content of total polar compounds was correlated with spectrophotometric and rheological data better than the content of free fatty acids or of oxidized products. The static yield value at 20 °C was more suitable for calculating the correlations than the static yield value measured at other temperatures or than the apparent viscosity. Close correlations were obtained between the appearance and the colour of fries or between the crunchiness and the intensity of crunching sounds. Correlations between flavour characteristics were significant but not sufficiently high to allow combinations of descriptors and simplification of the flavour profile.

French frying; hydrogenated rapeseed oil; quality assessment; analysis chemical and sensory

Deep fat frying is increasingly used for the preparation of food because it is very easy, and because fried products have agreeable flavour.

During repeated frying the frying oils grow deteriorated by both hydrolytic and oxidative processes. It is very important analytical task to evaluate the degree of frying oil deterioration in order to be able to replace used oil by fresh one after the optimum frying time. In several countries in different parts of the world, the use of different analytical methods was investigated (Al-Kahtani, 1991; Croon et al., 1986; Hau et al., 1986; Maksimets et al., 1987; White, 1991; Wu, Nawar, 1986; etc.). The content of polar compounds is most frequently used, the viscosity, colour changes, and various empirical methods (Croon et al., 1986; Kouřimská et al., 1992) are common as well.

The frying oil evaluation should be carried out with the minimum analytical methods in order to make the analysis cheaper and more rapid. The analyses which give similar, highly correlated results are redundant, and can be eliminated. For this reason, we have studied correlations between various methods used in the routine practical examination.

MATERIAL and METHODS

Material — Commercial hydrogenated rapeseed oil (melting point 34.6 °C) contained 2.7 % dienoic acids and 0.3 % trienoic acids. Fresh potatoes were peeled, cut into slices which were cut into pieces 40 to 50 x 10 x 10 mm.

Procedure — The household fryer Philips Nova (Model 2802.00) was filled with 2.5 kg frying oil, which was then preheated to 170 °C within 7 min, and 42 fryings were performed in the same oil during six working days. Batches of 200 g potato were added, reducing thus the temperature to 130 °C, which increased again to 170 °C during 5 min frying. After seven fryings, oil was cooled down, and stored for another day. French fries were left to drip, and they were immediately tested while still warm.

Analytical Methods — The acid value and the content of total polar compounds were determined after IUPAC (1987), total oxidation products were determined by reversed-phase HPLC (P á n e k, 1989). The colour was determined by measuring the spectrum between 400 and 700 nm, and by calculating the trichromatic luminance, dominant wavelength and saturation (ASTM, 1991).

Rheological properties were determined using the HAAKE Rotovisco RV 20 Viscometer (the cone diameter 28 mm, angle 2°) equipped with a flattened apex. Flow curves were determined in the shear rate range of 0 - 500 s^{-1}. The cone achieved its maximum rate in 1 min (the upward flow curve), and came back to rest in another 1 min (the downward flow curve). The samples were measured at 15.0, 20.0, and 25.0 °C with the accuracy of ±0.02 °C. The static yield value was determined as the maximum value of shear stress on the upward flow curve, the apparent viscosity at the shear rate of 500 s^{-1} (P o k o r n ý et al., 1984).

The sensory profile of French fries was determined under standard conditions (ISO, 1985) by a panel of trained and experienced assessors. The sensory profile consisted of eight descriptors, evaluated with use of unstructured graphical scales, represented by straight lines 145 mm long, provided with verbal descriptions at each end: acceptability of appearance (0 % = excellent, 100 % = very bad), colour acceptability (0 % = excellent, 100 % bad), intensity of crunchiness (0 % = excellent, 100 % = completely plastic), intensity of crunching sounds (0 % =

inaudible, 100 % = very strong), acceptability of overall flavour (0 % = excellent, 100 % = very bad), intensity of fried flavour (0 % = very strong, 100 % = hardly perceptible), intensity of rancid off-flavour (0 % = imperceptible, 100 % = very strong), and intensity of total off-flavours (0 % = non perceptible, 100 % = very strong).

The programme Statgraphics was used for statistical evaluations. The probability level was $P = 0.95$. The correlation coefficients of the linear ($y = a.x + b$) or of the semilogarithmic (either $y = a.\log x + b$, or $\log y = a.x + b$) or a double-logarithmic plot ($\log y = a.\log x + b$) were calculated on the basis of two sets of the total of 42 analyses, the R^2 expresses the degree, in which the relation explains the overall variability. Among the above four expressions, only those with the highest correlation coefficient are shown in Tables.

RESULTS and DISCUSSION

Values of correlation coefficients between variables concerning the frying oil are given in Table I. The content of free fatty acids could be correlated with the content of polar compounds or the colour changes but not with changes of rheological parameters. The content of total polar compounds was much better related to physical characteristics than the content of oxidized compounds. This phenomenon is probably due to lower content of oxidized products compared with

I. Relations between characteristics of frying oils (relation: $y = a.x + b$)

Dependent variable (y)	Independent variable (x)	Correlation coefficient (r)	Square of the correlation coefficient (r^2) p. c.
A	log B	0.6103	37.2
	D	0.6069	36.8
	log E	-0.6615	43.8
B	C	0.6648	44.2
log B	E	-0.8761	76.8
	F	0.6343	40.2
	K	0.7759	60.2
D	log E	0.7984	63.7
E	log K	-0.7175	51.5
log F	log K	0.9981	99.6

A = free fatty acids, B = total polar compounds, C = oxidized products, D = trichromatic luminance, E = dominant wavelength, F = trichromatic saturation, K = static yield value at 20 °C

hydrolytical products (B l u m e n t h a l, 1987), and to their relatively lower polarity and lower activity in forming hydrogen bonds than that of the hydrolytical products. Correlation coefficients were not very high (with the exception of the last expression, which is particular for these experiments and was not confirmed with frying oil of another type) so that no variable could be eliminated as superfluous.

Contrary to non-heated hydrogenated oils, the temperature dependence of frying fats was rather high as the results depended not only on changes of the solid phase, but also on those of the hydrogen bonding.

The static yield value (Table II) was in slightly closer correlation with other parameters than the apparent viscosity, and the measurement at 20 °C was preferable to either higher or lower temperatures (Table II). At 15 °C, the consistency of frying fat was hard so that wall slip was observed, and at 25 °C, differences between values measured after different frying times became small (both in the static yield value and in the apparent viscosity) because of low content of solid crystals. The temperature of 20 °C thus resulted as optimal for the determination of rheological characteristics.

II. Relationships between colour characteristics and rheological characteristics (linear relations: $y = a.x + b$, correlation coefficient r)

Dependent variable y	Independent variable x			
	dominant wavelength		trichromatic saturation	
	(r)	(r^2) p.c.	(r)	(r^2) p. c.
log J	-0.7157	51.2	0.6023	36.3
log K	-0.7175	51.5	0.9981	99.6
log L	-0.5776	33.4	0.9425	88.8
log M	-0.5885	35.8	0.9756	95.2
log N	-0.6327	40.0	0.9837	96.8
log P	-0.5412	29.3	0.9473	89.7

Static yield value: J = at 15 °C, K = at 20 °C, L = at 25 °C
Apparent viscosity: M = at 15 °C, N = at 20 °C, P = at 25 °C

The pronounced temperature dependence of frying oils as confirmed by correlations between rheological measurements at different temperatures are given in Table III. Static yield value measured at 15 °C deviated from those observed at higher temperatures and from the apparent viscosities. Semilogarithmic plots gave

III. Correlations between rheological parameters (values: r^2 p.c.) (relation: $y = a.x + b$, correlation coefficient r)

Dependent variable	Correlation coefficients with independent variables				
	log P	log N	log M	log L	log K
J	96.3	99.2	99.2	96.0	97.7
log J	79.0	87.2	86.8	74.5	97.1
log K	96.4	98.9	99.3	96.3	
log L	97.3	99.0	98.9		
log M	98.2	99.0			
log N	97.4				

Explanation of symbols see Table II

the best fitting curves in this case while log-log relations were more suitable in other cases. The apparent viscosity of frying fat is perceived rather at the end of chewing and during swallowing when fat present in the morsel has been heated in the oral cavity and became nearly liquid. Its determination at low temperature is thus of little practical significance.

IV. Correlation coefficients between sensory characteristics of French fries fried in hydrogenated rapeseed oil (linear regression: $y = a.x + b$, correlation coefficient r, probability level: $P = 95$ %, $N = 42$)

Charactistic	Correlation coefficients of the characteristic						
	R	S	T	U	V	W	X
Q	0.9072	0.4712	-0.3589	0.5715	0.1505	0.3636	-0.2477
R		0.4410	-0.2950	0.5189	0.0825	0.3530	-0.2517
S			0.9596	0.1920	0.5259	-0.0231	-0.3489
T				-0.1850	-0.5667	0.0474	0.2848
U					-0.3967	0.7282	0.2828
V						-0.2919	-0.1507
W							0.7022

Codes of the sensory characteristics: Q = appearance, R = colour, S = crunchiness, T = crunching sounds, U = overall flavour, V = fried flavour, W = rancid flavour, X = total off-flavours

Correlation coefficients of linear correlations between the sensory characteristics of French fries are given in Table IV. As expected, very high correlation coefficient was observed between the appearance and the colour of fries. Therefore, these two characteristics could be combined. The correlation between the crunchiness and the intensity of crunching sounds was still higher, so that these two characteristics could be combined in the profile as well. Other two significant correlations (flavour intensity and intensity of rancid flavour, and between the intensities of the rancid off-flavour and the overall off-flavours) are not so high to allow their combination into one descriptor. The lack of high correlation coefficient is caused by relatively short frying time. The relatively stable hydrogenated oil was deteriorated only in small degree, and highly objectionable off-flavours did not develop.

In case of household frying, the oxidation of frying oil during the storage between fryings is very important. For these reason we have interrupted the frying relatively often, and stored frying oil till the other day.

The results show that in case of relatively short use (as it is common in household fryers), the deterioration of frying fat does not proceed to any advanced degree. Therefore a single indicator is not sufficient for safe assessment of the degree of degradation of frying oil. The determination of the degree of deteriorative changes should thus be based on several characteristics which cannot be reduced to one or a few variables.

References

Al-KAHTANI, H. A.: Survey of quality of used frying oils from restaurants. J. amer. Oil Chem. Soc., *68*, 1991 : 857-862.

BLUMENTHAL, M. M.: Optimum Frying: Theory and Practice. 2nd ed. Piscataway, Libra 1987.

CROON, L.-B. - ROGSTAD, A. - LETH, T. - KIUTAMO, T.: A comparative study of analytical methods for quality evaluation of frying fat. Fette, Seifen, Anstrichmittel, *88*, 1986 : 87-91.

HAU, L. B. - YOUNG, P. K. - HWANG, L.-S.: Quality assessment of oils during heating and frying. J. chinese agric. chem. Soc., *24*, 1986 : 397-405.

KOUŘIMSKÁ, L. - POKORNÝ, J. - VELÍŠEK, J.: Effect of prefrying on sensory characteristics of French fries. Potrav. Vědy, *10*, 1992 : 205-214.

MAKSIMETS, V. P. - SAVGIRA, Y. A. - KRAVCHENKO, E. F. - KONONENKO, L. V. - OSINSKAYA, L. I. - FETISOVA, M. M.: Thermooxidation of culinary fat. Izv. Vyssh. Ucheb. Zaved. Pishch. Tekhnol., *3*, 1987 : 80-81.

POKORNÝ, J. - DAVÍDEK, J. - DOBIÁŠOVÁ, S. - ŠTERN, P. - ČMOLÍK, J.: Comparison of sensory analysis and rotational rheometry in the texture evaluation of margarines. J. Texture Stud., *15*, 1984 : 395-406.

WHITE, P. J.: Methods for measuring changes in deep-fat frying oils. Food Technol., *45*, 1991 : 75-76 and 78-80.
WU, P.-F. - NAWAR, W. W.: A technique for monitoring the quality of used frying oils. J. amer. Oil Chem. Soc., *63*, 1986 : 1363-1367.
ASTM: Standards on Color and Appearance Measurement. 3rd ed. Philadelphia, American Society for Testing and Materials 1991.
ISO 6658: Sensory analysis - Methodology - General guidance. Geneva, International Organization for Standardization, 1985.
IUPAC: Standard methods for the analysis of oils, fats and derivatives [PAQUOT, C. - HAUTEFENNE, A. (Eds.)], 7th ed. Oxford, London, Edinburgh, Boston, Palo Alto, Melbourne, Blackwell Sci. Publ. 1987.

Received for publication April 4, 1993

L. Kouřimská, J. Pokorný, P. Štern (Vysoká škola chemicko-technologická – Ústav chemie a analýzy potravin a Ústav hydrodynamiky, Česká akademie věd, Praha)

Vztahy mezi charakteristikami hydrogenovaného řepkového oleje během smažení bramborových hranolků

Bramborové hranolky byly postupně smaženy ve ztuženém řepkovém oleji ve fritéze pro domácí smažení po dobu šesti dnů bez doplňování oleje. Byly sledovány změny čísla kyselosti, obsahu veškerých polárních produktů, oxidačních produktů, trichromatických charakteristik barvy a reologických charakteristik, k nimž patřilo stanovení statické meze toku a zdánlivé viskozity při 15, 20 a 25 °C. Výsledky byly statisticky zpracovány s použitím programu Statgraphics. Korelační koeficienty uvedené v tab. I ukazují, že obsah volných kyselin byl v lineárním vztahu ke změnám obsahu veškerých polárních produktů, avšak celkový obsah oxidačních produktů, stanovený vysokoúčinnou kapalinovou chromatografií, byl méně vhodný.

Statická mez toku a zdánlivá viskozita značně závisely na teplotě (více než u nezahřátých tuků) a měření při teplotě 20 °C se osvědčilo pro sledování vztahů s ostatními charakteristikami lépe než při vyšších nebo nižších teplotách (tab. II). Logaritmické a semilogaritmické vztahy se ukázaly jako vhodnější pro hodnocení závislostí za různých teplot měření (tab. III).

Smažené bramborové hranolky byly hodnoceny metodou sensorického profilu a velmi těsné vztahy byly zjištěny mezi vzhledem a barvou a mezi chrupavostí a zvuky při konzumu. Méně těsné byly korelace mezi chuťovými charakteristikami, k nimž patřily: celková chuť, intenzita žluklé chuti a celkových pachutí (stanovené s použitím nestrukturovaných grafických stupnic), což bylo způsobeno poměrně malými oxidačními změnami tuku během poměrně krátkého smažení, jaké je při domácím smažení běžné.

Vzhledem ke krátkému používání oleje při domácím smažení nestačí k jeho hodnocení jediný ukazatel, ale je potřebí kombinovat dva nebo tři ukazatele, z nichž lze učinit bezpečné závěry při použití mnohonásobné regrese k vyhodnocení výsledků. Konkrétní

bezpečné závěry při použití mnohonásobné regrese k vyhodnocení výsledků. Konkrétní vztahy pro výpočet stavu oleje po smažení je třeba stanovit pro každý druh oleje, typ smažiče a druh smaženého pokrmu.

smažení bramborových lupínků; hydrogenovaný řepkový olej; ukazatel kvality; chemická a senzorická analýza

Potr. Vědy, *11*, 1993 (3) : 225-232

INHIBITION OF NITRATE REDUCTASE ACTIVITY OF MICROBIAL CONTAMINANTS IN HOPPED WORT

Alena BOKVAJOVÁ, Kateřina DEMNEROVÁ, Martina ŠŮCHOVÁ, Blanka KRÁLOVÁ, Milan KRÁL[1]

Institute of Chemical Technology – Department of Biochemistry and Microbiology and [1]*Department of Analytical Chemistry, Technická 5, CS-166 28 Praha 6, Czech Republic)*

Two microbial strains with nitrate reductase activity, yeast *Hansenula* sp. and bacteria *Xanthomonas maltophilia* were isolated from non-pasteurized hopped wort. The effect of five different inhibitors (riboflavin, L,D-tyrosin, ferric-ammonium citrate, niacin, nicotinamide, phenazinemethosulphate) on reduction of nitrate reductase activity (NRA), detected in microbial cells, was tested. The best inhibitory effect on NRA was obtained with riboflavin. Limiting inhibitory concentration of riboflavin was determined as 2.5 ppm. Other tested inhibitors applied in the same concentration partially reduced nitrate reductase activity of both microorganisms, too.

microbial nitrate reductase; inhibition; riboflavin; brewery industry

The occurrence of inorganic nitrates in beer has become a serious problem. Their ability to participate in the formation of carcinogenic nitrosocompounds appears to be the most hazardous aspect. These compounds are synthesized by the action of nitrous acid on secondary amines. Nitrous acid is formed by the action of denitrifying bacteria on nitrates. These bacteria are genetically diverse and metabolically versatile. Two pathways of denitrification are known. One uses nitrate as the only nitrogen source for microbial growth. The other utilizes nitrates as a hydrogen acceptor instead of oxygen under anaerobic conditions. These processes are known as the nitrate assimilation and nitrate dissimilation, respectively.

The final products of denitrification by the dissimilatory (respiratory) pathways are N_2O or N_2. This process occurs only in bacteria and low eukaryotes. But some microorganisms produce N_2O as the end product, ammonia is also sometimes developed during this anaerobic reduction. This is connected with assimilation for instance in *Escherichia coli*, *Desulfotomaculum desulfuricans*, *Clostridium* sp. Some bacteria produce a mixture of gases NO, N_2O and N_2. NO or NH_2OH

intermediates are however formed during assimilatory processes. An assimmilatory pathway was described in bacteria, fungi and plants.

P i c h i n o t y et al. (1969) elucidated the difference between denitrification processes. They identified two classes of nitrate reductases, nitrate reductase A, taking part in the dissimilation pathway and nitrate reductase B, participating in the assimilation pathway. They differ in the reaction with chlorate. Nitrate reductase A may use chlorate as a substrate instead of nitrate, while nitrate reductase B is inhibited by chlorate. The existence of both pathways was found in some bacteria. Only one type of the enzyme is present in other denitrifying bacteria. For example in the genus *Pseudomonas* all combinations of nitrate reductases A and B were observed. This genus comprises nonfermenting bacteria in which the dissimilatory reduction of nitrate is coupled with ATP formation (W a l t e r, 1978). *Pseudomonas aeruginosa* and *P. stutzeri* have both nitrate reductases A and B. *P. fluorescsens*, *P. multivorans*, *P. acidivorans* and *P. maltophilia* have nitrate reductase A and perhaps some of B-enzyme. *P. putida*, *P. testosteroni* and *P. pseudoalcaligenes* have only nitrate reductase B (P i c h i n o t y, 1965).

Nitrate reductase B and assimilatory nitrite reductase are repressed by ammonia, which is the end product of the assimilatory reduction.

Generally, dissimilatory processes are known better in comparison with assimilatory ones.

Respiratory nitrate reductases are located in the cytoplasmic membrane in *E. coli*. This enzyme contains four Mo atoms per mole and four to eight Fe-S clusters.

In the present work bacteria and yeast strains with the high nitrate reductase activity were isolated from the fermented hopped wort of a brewery. Several cofactors were tested for the inhibition of their nitrate reductase activity.

MATERIAL and METHODS

Analytical methods — Nitrate reductase activity was detected by the determination of nitrites formed during the growth of microorganisms on the medium containing KNO_3. The determination was based on the ability of nitrites to react with iodide in the acidic pH. Evolved I_2 gives with starch blue colour. The medium for estimation of nitrate reductase activity contained: peptone 5.0 g, beef extract 3.0 g, glucose 3 g, glycerol 5.0 g, KNO_3 1.0 g, Na_2HPO_4 2.5 g, agar 20.0 g, H_2O 1000 ml.

Nitrates and nitrites were determined according to P o s t e l (1976).

Microorganisms — Nitrate reducing microorganisms were isolated from non-pasteurized 10% hopped wort of a brewery. Their nitrate reductase activity

was tested on the nitrate medium as a decrease of nitrate contents. One bacterial strain chosen from 50 isolates and forming gramnegative rods was identified as *Xanthomonas maltophilia*. The isolated yeast strain was identified as *Hansenula* sp.

Experimental arrangements — The occurrence of nitrate reductase activity was followed in two types of microorganisms (bacteria and yeasts). They were isolated from non-sterile hopped wort and had high nitrate reductase activity as tested by starch reaction on Petri dishes.

Samples of sterile 10% hopped wort (100 ml) were inoculated by loopful with 24 h old bacterial or yeast culture. The nitrate content was adjusted to 50 mg/l by KNO_3 before sterilization. Cultivations were carried out at 28 °C under shaking.

Inhibition of nitrate reductase activity — The inhibitory effects of riboflavin, D,L-tyrosin, ferric-ammonium citrate, niacin, nicotinamide and phenazinemethosulphate were tested. The amount of inhibitors added into the medium was adjusted to 0.001 % based on the preliminary tests. The concentration of nitrates in the medium was 0.5 g/l of hopped wort. The medium was inoculated with 24 h cultures and cultivated as indicated above. Samples were analysed after 48 and 72 hours. Results were compared with those obtained without the inhibitor addition.

RESULTS

Nitrate reductase activity of selected microorganisms — Three samples of non-pasteurized hopped wort of individual brewing were taken and nitrate concentration was estimated immediately in five parallel determinations. The average values were 18.08 mg NO_3^-, 15.50 mg NO_3^- and 9.2 mg NO_3^- per 100 ml, respectively. The nitrite content in wort was 0.1 - 0.2 mg/l. The low concentration of nitrates in the third sample was caused by a higher contamination by microorganisms with nitrate reductase activity. The content of NO_3^- in all three samples was adjusted to 50 mg per 100 ml by KNO_3 and the nitrate reductase activity of selected microorganisms was tested after 24 and 48 hours of cultivation.

The yeast strain *Hansenula* sp. exibited higher nitrate reductase activity in comparison with the bacterial strain. The yeast strain decomposed 80 % of nitrates during 48 hours. The bacterial strain decomposed only 50 % of nitrates from media during the same time.

Inhibition of nitrate reductase activity — Six different inhibitors (riboflavin, L,D-tyrosin, ferric-ammonium citrate, niacin, nicotinamide and phenazinemethosulphate) were used to reduce nitrate reductase activity. All of them were used at the concentration of 0.01 %. Tables I and II show the effect of inhibitors on the nitrate reductase activity of yeast and bacteria, respectively.

I. Inhibition of nitrate reductase activity of *Hansenula* sp.

Inhibitor	Nitrate concentration in wort (average values) [mg NO_3^- . 100 ml^{-1}]					
	Sample 1		Sample 2		Sample 3	
	Cultivation time [h]					
	24	48	24	48	24	48
R	48.93	45.26	48.00	45.30	49.80	48.33
T	49.18	48.76	49.80	48.20	49.92	49.73
C	50.00	49.90	50.00	49.19	50.00	48.56
N	-	-	49.72	37.47	50.02	32.68
NA	49.54	48.65	49.56	46.65	49.74	49.62
PM	49.40	49.20	49.40	49.00	48.80	48.20
None	9.20	0	9.85	0	10.20	0

Holds for Tables I, II and V:
R - riboflavin, T - L,D-tyrosin, C - ferric-ammonium citrate, N - niacin, NA - nicotinamide, PM - phenazinemethosulphate

II. Inhibition of nitrate reductase activity of *X. maltophilia*

Inhibitor	Nitrate concentration in wort (average values) [mg NO_3^- . 100 ml^{-1}]					
	Sample 1		Sample 2		Sample 3	
	Cultivation time [h]					
	24	48	24	48	24	48
R	49.90	48.00	49.40	47.50	49.90	49.80
T	50.00	49.50	47.10	45.80	32.70	-
C	49.90	49.90	47.90	42.65	23.60	-
N	50.00	49.00	49.60	41.80	46.20	42.80
NA	50.00	49.80	50.20	49.00	41.40	40.80
PA	50.00	49.30	50.00	47.00	50.00	48.10
None	21.10	17.40	23.70	19.30	20.30	18.40

The results show that all compounds inhibited the nitrate reductase activity of selected microbial strains. The use of riboflavin appears to be the most convenient for food technology. The limit concentrations of riboflavin inhibitory effect to yeast and bacterial nitrate reductases were tested. These results are summarized in Tables III and IV.

III. Estimation of the limit riboflavin concentration inhibitory to *Hansenula* nitrate reductase activity (cultivation time: 48 hours)

Riboflavin concentration [mg . 100 ml^{-1}]	Nitrate concentration in wort [mg NO_3^- . 100 ml^{-1}]		
	Sample 1	Sample 2	Sample 3
0.25	50.00	50.00	50.00
0.05	50.00	50.00	49.00
0.025	45.00	48.00	40.00
0.0125	18.00	22.00	10.50
0.00625	12.60	18.50	10.50
0	12.00	14.00	8.20

Both strains exhibited the same sensitivity to riboflavin. The limit concentration of riboflavin appeared to be 0.25 mg/100 ml. Other inhibitors were applied at the same concentrations as riboflavin in order to compare their effect with riboflavin

IV. Estimation of the limit riboflavin concentration inhibitory to *X. maltophilia* nitrate reductase activity (cultivation time: 48 hours)

Riboflavin concentration [mg . 100 ml^{-1}]	Nitrate concentration in wort [mg NO_3^- . 100 ml^{-1}]		
	Sample 1	Sample 2	Sample 3
0.25	50.00	50.00	50.00
0.05	50.00	38.00	50.00
0.025	49.80	15.00	50.00
0.0125	8.70	9.20	45.10
0.00625	7.50	8.70	34.50
0	7.30	8.00	12.20

V. Estimation of the inhibitory effects on yeast and bacterial nitrate reductase activity at inhibitor concentration of 0.25 mg per 100 ml of the medium (cultivation time: 48 hours)

Inhibitor	Nitrate concentration in wort [%]*	
	Hansenula sp.	*X. maltophilia*
T	47.90	67.90
C	39.30	83.00
N	22.90	45.40
NA	28.90	52.80
PM	15.20	39.80

T – L,D-tyrosin; C – ferric-ammonium citrate; N – niacin, NA – nicotinamide; PM – phenazinemethosulphate

*The results represent percentual values of nitrate residual concentrations in medium after cultivation in the medium containing nitrates 50 mg per 100 ml

on the limiting concentration. Table V summarizes this comparison as percentages of residual nitrate concentrations after cultivation.

All inhibitors at concentrations of 0.25 mg/100 ml partially reduced nitrate reductase activity. The best effects on the yeast and bacterial nitrate reductases were obtained with riboflavin. As the assimilation nitrate reductase pathway is known to operate in *Hansenula* but *X. maltophilia* contains mainly nitrate reductase A (P i c h i n o t y, 1969), it can be concluded that riboflavin is effective against both types of nitrate reductases.

These obtained results suggest that riboflavin may be used as an inhibitor of microbial reductase activity in brewing industry. But our further intensive tests with pitching yeasts after their fourth or fifth use in brewery (the paper is in preparation), showed that some bacterial contaminants of brewer's yeast were more resistant to the inhibitory action of riboflavin, so that the 100% inhibition was not achieved in all cases.

CONCLUSION

It has been found that riboflavin used at 2.5 ppm final concentration in hopped wort inhibits nitrate reductase activity of certain bacterial and yeast contaminants occurring during bottom fermentation in breweries.

References

PICHINOTY, F.: Methods of investigation of bacterial nitrate reductase A and B. Bull. Soc. Chim. Biol., *47*, 1965 : 1526-1528.

PICHINOTY, F.: Bacterial nitrate reductases III. Properties of enzyme B. Bull. Soc. Chim. Biol., *51*, 1969 : 875-890.

POSTEL, W.: Nitratbestimmung und Nitratgehalt in Bier und Brauereirohstoffen. Brauwissenschaft, *29*, 1976 : 39-44.

WALTER, B. - SIDRANSKY, E. - KRISTJANSSON, J. K. - HOLLOCHER, T. C.: Inhibition of denitrification by uncomplers of oxidation phosphorylation. Biochemistry, *17*, 1978 : 3039-3045.

Received for publication February 19, 1993

A. Bokvajová, K. Demnerová, M. Šůchová, B. Králová, M. Král (Vysoká škola chemicko-technologická – Ústav biochemie a mikrobiologie a Ústav analytické chemie, Praha)

Sledování nitrátreduktásové aktivity kontaminující mikroflóry pivovarských surovin a ověření možnosti její inhibice

Problém přítomnosti dusíkatých sloučenin anorganického původu v pivě je stále více aktuální, obzvláště z pohledu možného vzniku potencionálně karcinogenních nitrosaminů. Na vzniku těchto látek se mohou podílet i mikroorganismy, již přítomné v pivovarských surovinách, které mají enzym nitrátreduktasu redukující dusičnany na dusitany, přeměňované dále na nitrosaminy.

V této práci jsme se zabývali výběrem mikroorganismů s vysokou nitrátreduktasovou aktivitou izolovaných z nesterilované mladiny, schopností některých látek inhibovat aktivitu nitrátreduktasy a stanovením nejnižší možné koncentrace inhibující tento enzym.

Nitrátreduktásová aktivita byla sledována u dvou typů mikroorganismů (kvasinka a baktérie), které byly vybrány z 50 izolátů v předcházejících pokusech izolovaných z 10% nesterilované mladiny a vykazující značnou nitrátreduktasovou aktivitu. Tyto mikroorganismy byly identifikovány – baktérie jako *Xanthomonas maltophilia* a kvasinka byla zařazena k rodu *Hansenula*. U obou mikroorganismů byla sledována jejich schopnost redukovat dusičnany na dusitany.

Ve třech vzorcích nepasterované mladiny byl nejdříve metodou podle Postela stanoven obsah dusičnanů. Stanovené koncentrace 18,08, 15,50 a 9,2 mg NO_3^- na 100 ml byly dále upraveny na koncentraci 50 mg NO_3^- na 100 ml. Po sterilaci mladiny a inokulaci výše zmíněnými dvěma mikroorganismy byl sledován úbytek dusičnanů po 24 a 48 hodinách kultivace. Bylo zjištěno, že kvasinka vykazuje vyšší nitráreduktasovou aktivitu než baktérie, vzhledem k tomu, že kvasinky odbouraly po 48 hodinách kultivace 80 % z výchozího obsahu dusičnanů, zatímco baktérie pouze kolem 50 %.

Schopnost inhibovat nitrátreduktasovou aktivitu byla testována u šesti potenciálních inhibitorů, riboflavinu, L,D-tyrosinu, citrátu železito-amonného, kyseliny nikotinové, nikotinamidu a fenazinmetasulfátu, přidávaných v koncentraci 0,01 %. Stanovením koncentrace dusičnanů (tab. I a II) po 24 a 48 hodinách kultivace mikroorganismů na médiích obsahujících inhibitory a porovnáním s výsledky po kultivaci bez inhibitorů bylo zjištěno, že všechny sledované látky inhibují nitrátreduktasu obou izolovaných mikroorganismů. Jako nejlepší inhibitor byl vybrán riboflavin, u něhož byla dále testována limitní koncentrace potlačující aktivitu mikrobiální nitrátreduktasy. Růstem mikroorganismů na médiích s různou koncentrací riboflavinu (0 až 10 mg riboflavinu na 100 ml) byla pro oba mikroorganismy stanovena stejná limitní koncentrace 0,25 mg riboflavinu na 100 ml zcela inhibující enzym nitrátreduktasu (tab. III a IV). Při použití ostatních inhibitorů ve stejné koncentraci, byla porovnána jejich účinnost s účinností riboflavinu (tab. V). U obou mikrobiálních kmenů byla nitrátreduktasová aktivita zčásti inhibována všemi inhibitory aplikovanými v koncentraci 0,25 mg na 100 ml, úplné inhibice však byla dosaženo pouze s riboflavinem.

mikrobiální nitrátreduktasa; inhibice; riboflavin; pivovarský průmysl

Potr. Vědy, *11*, 1993 (3) : 233-242

INFLUENCE OF PECTIC SUBSTANCE EXTRACTION INTO THE BRINE ON THE TEXTURE OF CANNED FRUIT

Jaroslav DOBIÁŠ, Dušan ČURDA, Vladimír VÁŇA[1], *Petra ŽÁKOVÁ*

Institute of Chemical Technology – Department of Food Preservation and Meat Technology and [1]*Department of Biochemistry and Microbiology, Technická 5, CS-166 28 Praha 6, Czech Republic*

The texture changes of canned peach, Red Haven variety, and apricot, Velkopavlovická variety, as well as the extraction of pectic substances during heat treatment at 70, 80, 90 and 95 °C were studied. The content of pectic substances in the liquid portion of both kinds of canned fruit is proportional to the intensity of used heat treatment and in good correlation with the their texture. The lack of pectic substances in plant tissues after heating is evident also from microscopic photographs.

peach; apricot; texture; pectic substances; heating

The influence of pectic substance structure on the texture of plant tissues is known. Considering that pectic substances bind the cells together as a component of middle lamella, their hydrolysis is regarded as one of the main causes of softening of plant tissues (S t e r l i n g, 1955; P a u l u s, S a g n y, 1980).

In the framework of our studies on the influence of heating on the changes in the texture of processed fruit and vegetables we were interested in the relation between the texture of canned peaches and apricots and the content of soluble pectic substances in the brine after different heating conditions. The changes in the structure of plant tissues after heat treatment were also followed under a microscope.

MATERIAL and METHODS

Peaches of the Red Haven variety, soluble solid content 10.2 % (w/w), acids content (as citric acid) 0.80 % (w/w), and apricots of the Velkopavlovická variety (soluble solids 12.6 %, acids 1,49 %) in canning ripeness were used for our experiments.

Halved and destoned fruits (250 g) were filled into tin cans (volume of 450 ml), poured on with sugar brine, i.e. 30 % (w/w) sucrose solution, sealed and subjected

to heating in a hot water bath. The temperature in cans was measured in the least heated place (i.e. inside of fruit parts) with thermocouple. The F values for each treatment were calculated using T_{ref} = 80 °C and z = 20 °C which is typical of texture changes in plant tissues (K a r e l et al., 1975). Samples of cans after 20 different heating treatments (Table I) were cooled and stored at room temperature for six months. Then they were opened and their content was tested.

I. Time-temperature conditions of samples taken for texture tests

Temperature [°C]	Holding time [min]/corresponding F_{80}^{20} value				
70	30/11.5	45/16.2	60/21.0	75/25.7	90/30.5
80	10/16.0	15/21.1	20/25.8	25/30.8	30/35.7
90	2/35.3	4/41.7	6/48.1	8/54.3	10/60.6
95	1/49.0	2/55.1	3/60.6	4/66.5	5/72.1

The canned fruits were homogenized by a liquidizer under constant conditions and the mixture was centrifuged for 15 min at 3000 rpm. The relative viscosity of the supernatant and its pectin content were determined.

The fruit texture was determined by the reverse extrusion method using Instron 1152 apparatus. The extrusion vessel made of stainless steel (inner diameter 40 mm) was filled with fruit pieces (80 g). The sample was then pressed with a piston (diameter 32 mm), the speed of which was 125 mm/min. From the extrusion diagram, i.e. the stress-time curve, the maximum force F_{max} [N] was read (K y z l i n k et al., 1982).

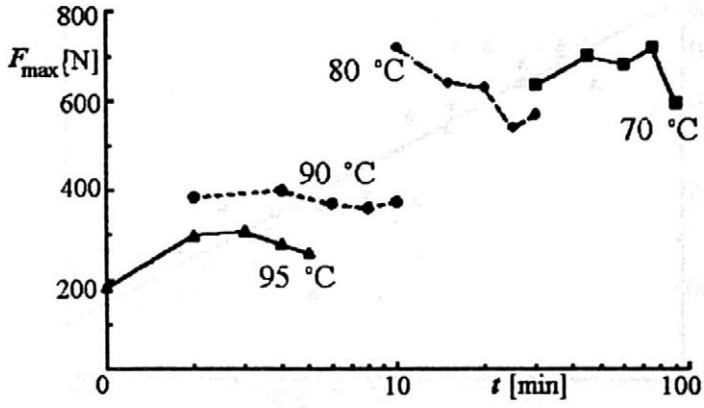
Viscosity measurements were performed using the Ubbelohde viscometer calibrated with distilled water at 20 °C. The relative viscosities of tested solutions against distilled water were determined on the basis of corrected time of outflow.

The pectin content (determined as anhydrogalacturonic acid) was analyzed colorimetrically after the reaction with 3-hydroxy-biphenyl (B l u m e n k r a n t z, A s b o e - H a n s e n, 1973).

The morphological changes of apricot tissues during heating were also studied using a microscope (NVZ, Carl Zeiss Jena, BRD; enlargement 300 - 500 times). The staining methods for starch, cellulose, pectic substances and cell walls were used (N ě m e c, 1962; G a h a n, 1984).

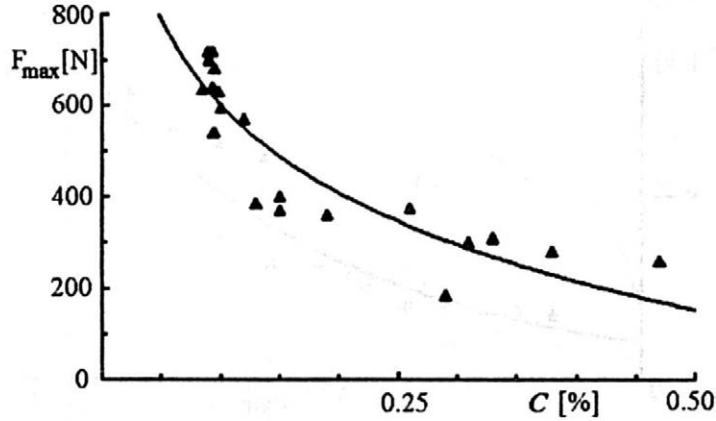
RESULTS and DISCUSSION

The relation between the peaches texture (F_{max} value) and the time of heating for each temperature is shown in Fig. 1. It is obvious that there was no significant

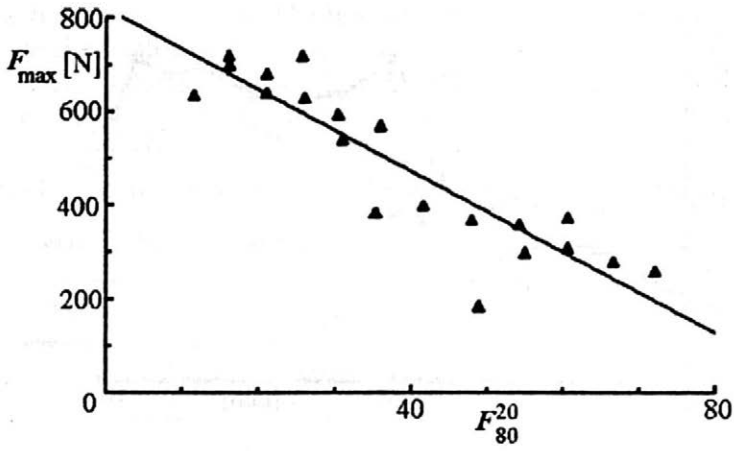


1. Texture of canned peach (F_{max} value) after different heat treatments

difference for heating under lower temperatures, i.e. 70 °C and 80 °C. This is in a good agreement with results of pectin content in the liquid portion of canned fruit. The higher F_{max} value, the lower content of pectic substances. This correlation is given in Fig. 2.

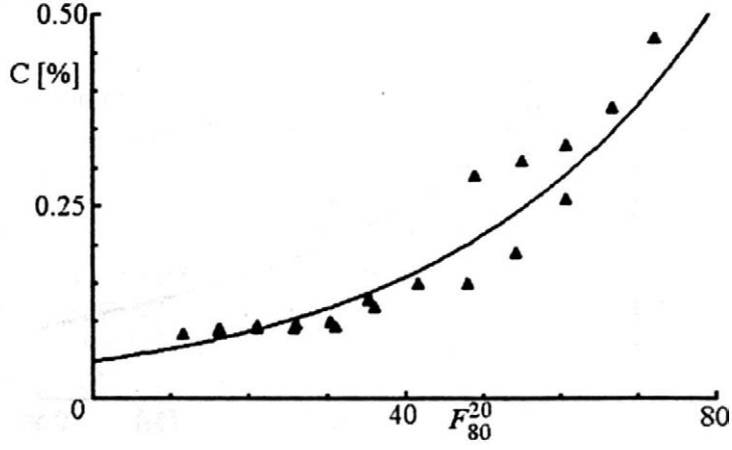


2. Correlation between texture (F_{max} value) and pectin content in the liquid portion of canned peach for all heat treatments
(F_{max} = -277.7 . ln c - 38.8; r = 0.9026)

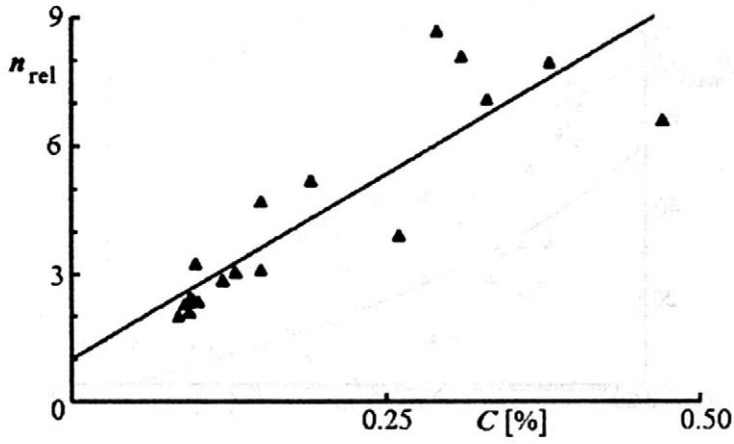


3. Correlation between texture (F_{max} value) and the intensity of heating (F_{80}^{20} value) of canned peach (F_{max} =-8.63 .F_{80}^{20} + 818.4; r = 0.8997)

Compared with the time of heating, the F value is more suitable for expressing the intensity of sterilization treatment. Figs. 3 and 4 show the close correlation between the texture of heated peaches, and/or amount of solubilized pectin, and the intensity of heating expressed by means of F_{80}^{20} value

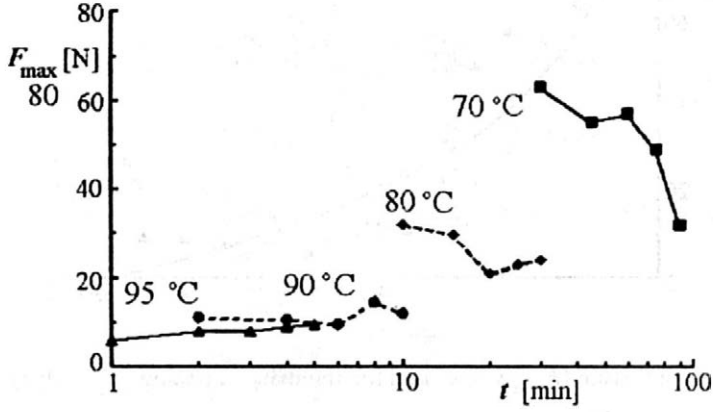


4. Correlation between the content of soluble pectin and the intensity of heating (F_{80}^{20} value) of canned peach (c = 0.0483 . exp (0.0292 . F_{80}^{20}); r = 0.9495)

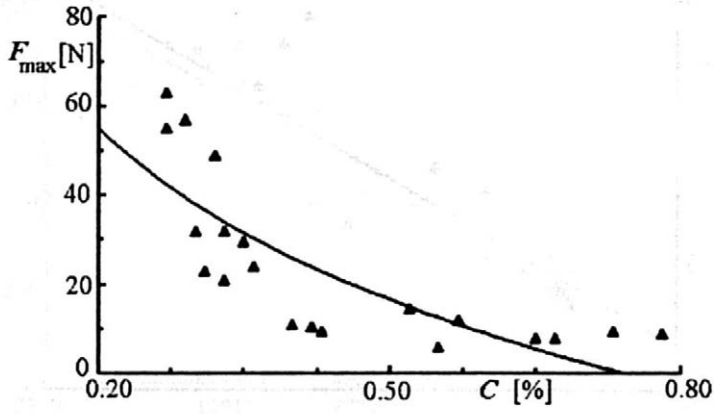


5. Correlation between relative viscosity and pectin content of the liquid portion of canned peach for all heat treatments ($n_{rel} = 17.2 \,.\, c + 1.0$; $r = 0.8767$)

The highest amount of pectin was released into the brine during the most intensive heat treatment (95 °C for 5 min) when we found nearly 76 % of the total pectin content of canned peaches in the solution, i.e. 0.46 % (w/w) of soluble pectin from total 0.61 % (w/w). Solubilized pectic substances increased viscosity of liquid portions of canned fruit. Rather a close correlation between the viscosity of tested samples and their pectin content is also evident from Fig. 5.

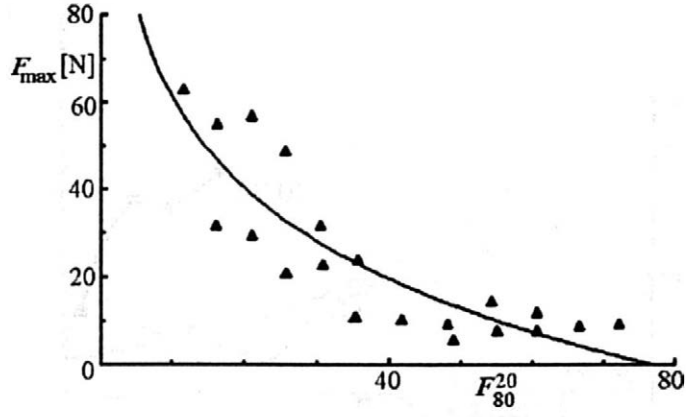


6. Texture of canned apricot (F_{max} value) after different heat treatments

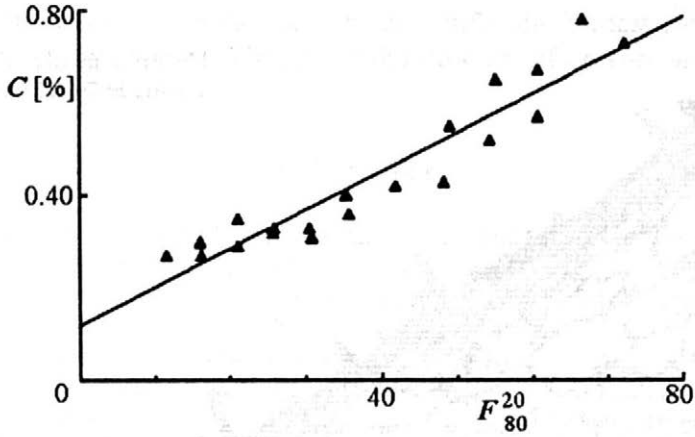


7. Correlation between texture (F_{max} value) and pectin content in the liquid portion of canned apricot for all heat treatments (F_{max} = -42.1 . ln c - 12.7; r = 0.7953)

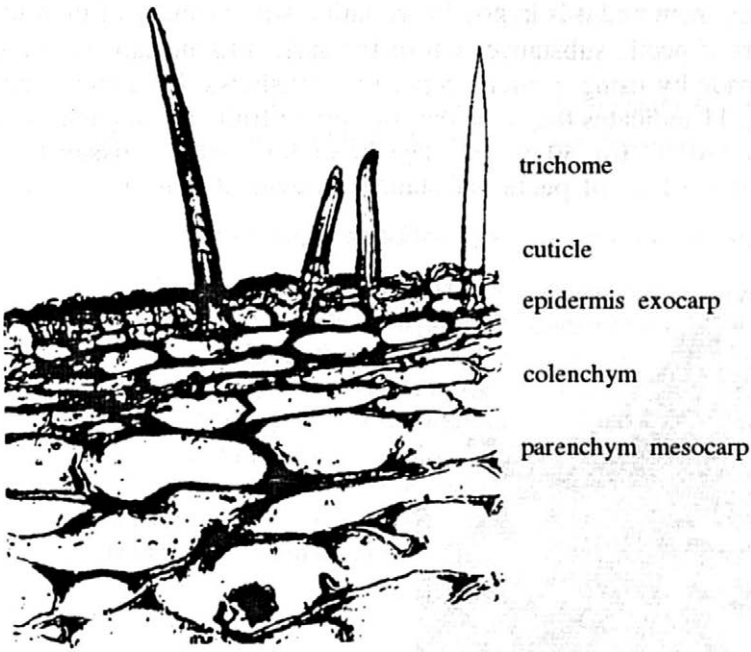
Analogical results for canned apricots are summarized in Figs. 6 - 9. Compared with canned peaches we found the more significant influence of lower temperature during heating (70 °C and 80 °C) on the texture changes and the higher ratio of soluble and total pectin after heating. For example we determined about 92 % of pectin in soluble form in fruit heated at 95 °C for 5 min, i.e. 0.52 % (w/w) of soluble pectin from total 0.56 % (w/w).



8. Correlation between texture (F_{max} value) and the intensity of heaing (F_{80}^{20} value) of canned apricot (F_{max} = -30.0 . ln F_{80}^{20} + 13t0.4; r = 0.8669)



9. Correlation between the content of soluble pectin and the intensity of heating (F_{80}^{20} value) of canned apricot ($c = 0.0084 \,.\, F_{80}^{20} + 0.117$; $r = 0.9455$)



10. Structure of fresh apricot tissue

11. Structure of apricot tissue after heating at 70 °C for 30 minutes

It can be concluded from the described results that the content of pectic substances in the liquid portion of both kinds of canned fruit is proportional to the intensity of used heat treatment and it is in good correlation with changes of their texture.

The transport of pectic substances out of the apricot tissue can also be seen in photographs made by using a microscope. Fig. 10 shows the tissue structure of fresh fruit. Fig. 11 indicates the same part of apricot fruit after the least intensive heat treatment (70 °C for 30 min). The breakdown of the tissue texture in consequence of the lack of pectic substances is evident mainly in cell corners

12. Structure of apricot tissue after heating at 90 °C for 5 minutes

(especially in mesocarp). In the tissue heated under the hardest condition (95 °C for 5 min) the pectin is nearly absent and the structure of exocarp and mesocarp is completely destroyed (Fig. 12).

References

BLUMENKRANTZ, N. - ASBOE-HANSEN, G.: New method for quantitative determination of uronic acid. Analyt. Chem., *54*, 1973 : 484.
GAHAN, P. B.: Plant Histochemistry and Cytochemistry. London, Academic Press 1984 : 256.
KAREL, M. - FENNEMA, O. R. - LUND, D. B.: Principles of Food Science. Part II. Physical Principles of Food Preservation. New York, Marcel Dekker, Inc. 1975 : 53.
KYZLINK, V. - ČURDA D. - SLAVÍČKOVÁ, A. - PARMOVÁ, M.: Effect of temperature and time of heating on the consistence of apples. Sci. Papers Prague Inst. Chem. Technol., *E53*, 1982 : 215.
NĚMEC, B.: Botanická mikrotechnika. Praha, ČSAV 1962 : 125.
PAULUS, K. - SAGNY, J.: Effect of heat treatment on the quality of cooked carrots. J. Food Sci., *45*, 1980 : 239.
STERLING, C.: Effect of moisture and high temperature on cell walls in plant tissues. Food Res., *20*, 1955 : 474.

Received for publication February 5, 1993

J. Dobiáš, D. Čurda, V. Váňa, P. Žáková (Vysoká škola chemicko-technologická – Ústav konzervace potravin a technologie masa a Ústav biochemie a mikrobiologie, Praha)

Vliv extrakce pektinových látek do nálevu na konzistenci kompotovaného ovoce

Vliv struktury pektinových látek na konzistenci rostlinných pletiv je znám a hydrolýza pektinů je považována za jednu z hlavních příčin měknutí rostlinných pletiv při tepelném opracování (S t e r l i n g, 1955; P a u l u s, S a g n y, 1980). Cílem práce bylo hledání vztahu mezi konzistencí kompotovaných broskví a meruněk a obsahem rozpustného pektinu v nálevu při různých podmínkách sterilace.

Půlené a odpeckované meruňky (odrůda Velkopavlovická) a broskve (odrůda Red Haven) zalité nálevem a uzavřené v plechovkách o objemu 450 ml byly sterilovány ve vodní lázni při teplotách 70, 80, 90 a 100 °C. Bylo použito dvacet různých sterilačních režimů, intenzita tepelného účinku každého z nich byla sledována pomocí termočlánku. Vysterilované kompoty byly skladovány šest měsíců při laboratorní teplotě a poté byl jejich obsah analyzován.

Viskozita tekutého podílu homogenizovaného kompotu byla stanovena za použití Ubbelohdeho viskozimetru a obsah rozpustných pektinů v něm kolorimetricky po reakci s 3-hydroxy-difenylem (B l u m e n k r a n t z, A s b o e - H a n s e n, 1973) a dále

pak konzistence ovoce metodou zpětné extruze (K y z l i n k et al., 1982). Morfologické změny struktury pletiva meruněk přitom byly sledovány i mikroskopicky.

Z výsledků vyplynulo, že obsah rozpustných pektinových látek v obou typech kompotů je úměrný intenzitě tepelného zákroku a těsně koreluje se změnami konzistence plodů. Při sterilaci meruněk byly na rozdíl od broskví zjištěny podstatnější změny konzistence již při nižších teplotách záhřevu, tj. při 70 a 80 °C. Uvolňování pektinových látek z pletiv zahřívaných meruněk bylo zřejmé i z mikroskopického sledování.

broskve; meruňky; pektinové látky; sterilace

Potr. Vědy, *11*, 1993 (3) : 243-250

SURVEY

THE ROLE OF RADIONUCLIDES IN MODERN FOOD RESEARCH, ANALYSIS AND CONTROLLING OF TECHNOLOGICAL PROCESSES

Pavel RAUCH, Jan KÁŠ

Institute of Chemical Technology – Department of Biochemistry and Mikrobiology, Technická 5, CS-166 28 Praha 6, Czech Republic

Radionuclides and various compounds labelled with them have represented an irreplaceable research means in many branches of biological and chemical sciences for decades (Tables I and II). Their utilization in the mentioned scientific branches is however very different. While biochemistry, organic chemistry, physiology, agriculture, pharmacology, human and veterinary medicine may serve as examples of their intense application, food research started to utilize their benefits in the eighties, if we do not consider very scare studies in the sixties and seventies (R a u c h, 1991; R a u c h et al., 1991). The reason for such a delay in their application is most probably to be a certain conservative approach of food scientists, unsufficient knowledge of radionuclide merits (the improvement of specificity and sensitivity of applied procedures, etc.).

I. Nuclides most frequently used in food research, analysis and controlling of food allied processes

Radionuclides	^{3}H, ^{14}C, ^{22}Na, ^{32}P, ^{35}S, ^{45}Ca, ^{55}Fe, ^{57}Co, ^{59}Fe, ^{75}Se, ^{125}I
Stable nuclides	^{2}H, ^{13}C, ^{15}N, ^{18}O

The goal of this paper is to bring in closer contact those producing labelled compounds and those utilizing them in various food branches. The paper avoids food irradiation and fully agricultural problems which are described elsewhere. It is concerned mainly with the utilization of radionuclides in tracer studies, in investigation of mutual interactions among various food components, testing of the availability of food nutrients and essential factors, studies of changes and interractions of various compounds during food processing, use of radionuclides in food analysis and finally in the testing of technological procedures and functioning of technological apparatuses.

Many of these approaches are not replaceable by other methods or procedures.

II. Most frequently used radiolabelled compounds in food research and analysis

Food components	Contaminants
$^{3}H_2O$, $^{14}CO_2$, H_{332PO4}	^{14}C-pesticides
^{14}C-organic acids, amino acids	^{14}C-organic pullutants
^{14}C-carbohydrates	^{14}C-mycotoxins
^{14}C-lipids	^{32}P-pesticides
^{14}C-proteins	^{35}S-pesticides
^{14}C-anthocyanins	components of packaging materials
^{14}C-vitamins (^{57}Co-cyanocobalamin)	
^{32}P-casein	
^{35}S-methylmethionine	
^{35}S-adenosylmethionine	
^{75}S-N^{5}-methyltetrahydrofolate	

Tracer studies in food research

Compounds labelled with radionuclides are suitable for the investigation of transport and distribution of various naturally occurring compounds and contaminants in the food chain (Table III). They make it possible to trace the pathway of the tested compound in the experimental object and not only the final distribution. This advantage facilitates to recognize the processes and changes taking place in food raw materials, semiproducts and final foodstuffs under various conditions.

A few examples will illustrate such approaches. For instance, the distribution of emulsifying phosphate salts, calcium, iron and other biogenic metals, as well as milk clotting enzymes in whey and curd, lipoprotein lipase and some vitamins in casein, whey and lipid fraction were studied in dairy technology (S u n d h e i m, B e n g t s s o n - O l i v e c r o n a, 1986). Likewise the knowledge of the distribution of biogenic elements (ions) and lipids in relation to various conditions of their processing is important for cereal chemistry and technology. Similar examples may be presented for studies in other food branches, e.g. for meat technology. The distribution

III. Fields of radionuclide applications in food research, analysis and processing

Feature	Examples
Distribution and transport	Ions, vitamins, proteins, enzymes, pesticides and other organic pollutants, toxins
Interactions	Protein-protein, protein-lipid, protein—contaminants, dietetic fibre-vitamins or contaminants
Bioavailability testing Metabolic fate	Amino acids, proteins, lipids, biogenic elements, role of fibre, food additives, pesticides
Component changes during processing	Chemical and biochemical reactions, Maillard reaction, wine aging, haze formation during beer storage, enzyme processes
Assessment of food origin Genuineness and age	Origin of vinegar, fruit juice and honey, age of alcoholic beverages
Method	**Analytes**
Isotope dilution	Low molecular weight compounds: Ions, amino acids, aldehydes, ketones, vitamins, contaminants
Enzyme conversion	Sugars, organic, acids, vitamins
Microbiological assay	Contaminating microflora
Competitive protein binding analysis	Vitamins, hormones
Immunoassay of haptens	Toxins, pesticides, organic pollutants, anabolic hormones, veterinary drugs, vitamins, glycoalkaloids
Immunoassay of immunogens	Proteins, enzymes, toxins

and the diffusion course of sodium ions and water into smoked meat products may be measured using ^{3}H and ^{22}Na radionuclides.

In spite of the importance of the investigation of technological processes, great attention is now paid to the transport and distribution of residual contaminants, especially to pesticides in raw materials of plant and animal origin, during food

processing and also in food products. Labelled compounds may be utilized mainly in various model experiments oriented to the investigation of contaminant penetration, distribution, halflife and breakdown in various raw food materials. Application of labelled compounds may overcome experimental difficulties associated with other analytical approaches which are, in general, affected by the problem of a nonextractible (bound) portion of the contaminant in food raw materials (S t r a t t o n, W h e e l e r, 1986). Extraction, enzyme hydrolysis and techniques utilizing higher temperatures are never quantitative and in addition, they are always laborious and time consuming. In contrast, the methods using compounds labelled with radionuclides are more simple and enable to detect contaminants at very low concentrations. The sample processing is usually limited to their wet or dry mineralization which may be performed automatically. Very often it is even possible to detect the traced compounds directly without subsequent sample treatment. An investigation of contaminant distribution in stored potatoes and cereals including the course of their transport and degradation during storage and processing was described by D r a u s c h k e et al. (1981).

Analogically the distribution of contaminants has been studied in foodstuffs of animal origin. Attention was paid especially to pesticides in milk, anabolic hormones in meat, mycotoxins in cereals, various organic compounds in fish, as well as to migration of contaminants from packaging materials into foods (G o t o, H s i e h, 1985; M i r c h i et al., 1989).

These problems have been studied very intensively in recent years. Attention is now paid mainly to deeper investigation of mutual interactions between contaminants and food components. These processes may seriously affect the nutritional value of foods and sometimes they form toxic compounds which deteriorate foods. These interactions may be easily studied using labelled compounds, e.g. for the studying of protein balances in casein micelles, investigation of interactions between proteins and lipids during production of instant infant milk food, etc. (R o w l e y, R i c h a r d s o n, 1985). Interactions of dietetic fibre with vitamins and proteins with contaminants (O m a y e et al., 1983) may be presented as examples of studies concerned with interactions of low- and high-molecular weight substances.

Radiolabelled compounds are widely used for studying the bioavailability of various important food components from the nutritional viewpoint. The number of papers concerning investigations of the effects of food component availability in dependence on various physical and chemical treatments has been increasing in recent years . Great attention is also paid to the availability of food supplements owing to the frequent application of additives in contemporary technologies. The spectrum of substances, the availability of which was studied by using radiolabelled compounds, is broad. Investigations of the availability of lysine, proteins, lipids and minerals, especially of iron, may be mentioned as examples. Recently, the effect of dietetic fibre on the availability of various food components has been studied. Attention was also paid to the nutritional effect of food emulsifiers, for instance lecithins, esters of organic acids with glycerol and sucrose esters (R a u c h, 1991).

Radiolabelled compounds are also suitable to investigate their participation in various chemical reactions taking place in food raw materials during storage and processing, as well as during storage of the final products. The examples are: investigations of stepwise changes of lysine during Maillard reaction (S a i t o et al., 1980), studying of red and brown condensation products resulting during red wine aging or observing of the reactions leading to chill haze formation within beer storage. The reaction catalyzed by enzymes and taking place in foodstuffs may also be examined by means of using labelled compounds.

The number of radiolabelled compounds used for tracer studies is high (see Table II) and the contribution of this methodological approach is indisputable. There is still an open field for the preparation of new labelled compounds needful for the solution of newly arising problems, as well as for the improvement of presently applied procedures. Of course, it is necessary to remember that the introduction of the radionuclide marker cannot change the original chemical and biological properties of the labelled compound.

Assays of food components and contaminants

Food analysis represents undoubtedly the widest area of radionuclide application in food science. The method of isotopic dilution, the application of radiolabelled substrates in enzymatic and microbiological assays and the utilization of radiolabels in immunochemical and other biorecognition methods are the most important approaches from the methodological viewpoint (Table III).

The main advantage of the application of labelled compounds in food analysis lies in the improvement of specificity and sensitivity. It also helps to replace the laborious methods which are suitable only for analyzing a few samples by procedures designed for large-scale monitoring.

The method of isotopic dilution is suitable mainly for the determination of low molecular substances either naturally occurring (biogenic metal ions, amino acids, organic acids, vitamins, lipids, etc.) or contaminants (pesticides, organic pollutans, etc.). For this purpose the labelling with ^{14}C, ^{3}H, ^{35}S, and ^{32}P is most frequently used (M o o d y, P a u l s e n, 1988).

Many of these compounds labelled with ^{14}C are suitable not only for the determination of their concentration in foodstuffs but also for the detection of unfavourable contamination. The labelling with ^{14}C makes it possible to evaluate all these experiments with high sensitivity on the basis of created $^{14}CO_2$ (H a t c h e r et al., 1977). The detection of microbial contamination in this way is very sensitive and fast and allows to detect even one contaminated microbial cell (e.g. *Salmonella*).

A quite exceptional analytical approach was developed by parallel utilization of radiolabelling (e.g. with ^{125}I and ^{3}H) and biorecognition systems (e.g. binding protein-ligand, enzyme-substrate, antibody-antigen). While the radiolabelling improves remarkably the sensitivity, the biorecognition systems warrant extraordinary

specificity. The mutual interaction between binding protein and the ligand may be utilized now only for the determination of some vitamins and hormones. In contrast, the interaction between antibody and antigen is almost universally applicable in analysis. The latter method (called radioimmunoassay) has been known for more than 30 years and it has been modified within these years in many aspects.

Radioimmunoassays are used mainly for distinguishing individual proteins (e.g. meat and milk proteins) and for proving foreign additive proteins (e.g. milk proteins in meat products) in food analysis. Immunoassays are also suitable for the detection and assessment of enzymes. For instance, the assay of extracellular proteinases and lipases makes it possible to forecast the storage shelf-life of UHT-milk (R a u c h et al., 1991).

In recent years there have been efforts to replace the radionuclide labels by various enzymes. The preparation of radiolabelled compounds maintaining better the original and biological properties than those labelled with enzymes, improvements of separation steps and overall performance of radioimmunoassays may prove their benefits in comparison with methods using other markers.

Application of radionuclides in food processing

Radiolabelled compounds were also successfully applied in investigations of several technical problems. For instance some authors used them to test the proper function of sucrose crystallizer and other sugar-plant and bakery equipment (D o l i n i n, S a k h a r o v, 1976). The measurement of natural ^{3}H or ^{40}K radioactivity by means of the whole body radiometer was utilized in meat industry during the development of steaming technology in slaughter line and for the indirect assessment of mass, fat and protein content of slaughter animals (J o n e s et al., 1984). Radiolabelled compounds were also used to control the washing of bottles and sanitation level in food plants, as well as to test the proper mixing of food and feed additives, investigation of corn contamination with various insects, etc. For this purpose the radionuclides with short halftime were utilized.

Conclusions

The paper brings a short survey of the application of radionuclides in food research, analysis and in controlling the performance of technological equipment and procedures. Radionuclides are utilized in tracer studies, monitoring of processes taking place in food raw materials, semiproducts, and during processing and storage of final products. Very important is their role in analysis of naturally occurring food components and contaminants. The main target of this paper is to stimulate wider utilization of radionuclides and compounds labelled with them in indicated areas of food research, analysis and technology.

References

DOLININ, V. A. - SAKHAROV, E. V.: Use of radioisotope instruments in the bakery. Khlebopekar. i Konditer. Prom., *9*, 1976 : 30-32.

DRAUSCHKE, W.- STEINBEISS, C.-D. - HOHNSTADTER, L.: Studies on the use of nonylalcohol as a temporary sprout-inhibiting agent in plant potatoes. Nahrung, *25*, 1981 : 35-38.

GOTO, T. - HSIEH, D. P.: Fractionation of radioactivity in the milk of goats administered by 14-C-aflatoxin B1. J. Assoc. off. analyt. Chem., *68*, 1985 : 456-458.

HATCHER, W. S. - DI BENEDETTO, S. - TAYLOR, L. E. - MURDOCK, D. I.: Radiometric analysis of frozen concentrated orange juice for total viable microorganisms. J. Food Sci., *42*, 1977 : 636-639.

JONES, B. - NILSSON, T. - SORQUIST, S.: Contamination of pig carcasses with scalding water. Fleischwirtschaft, *64*, 1984 : 1226-1228.

MIRCHI, R. - KMÍNKOVÁ, M. - KOCOUREK, V.: Distribuce olova v mléce. Potr. Vědy, *7*, 1989 : 53-58.

MOODY, J. R. - PAULSEN, P. J.: Isotope dilution spark-source mass spectrometric determination of total mercury in botanical and biological samples. The Analyst, *113*, 1988 : 923-927.

OMAYE, S. T. - CHOW, F. I. - BETSCHART, A. A.: *In vitro* interactions between dietary fiber and ^{14}C-vitamin D or ^{14}C-vitamin E. J. Food Sci., *48*, 1983 : 260-261.

RAUCH, P.: Značené sloučeniny při sledování složek poživatin. Potr. Vědy, *9*, 1991 : 65-70.

RAUCH, P. - FUKAL, L. - KÁŠ, J.: Značené sloučeniny v analýze poživatin. Potr. Vědy, *9*, 1991 : 155-160.

ROWLEY, B. O. - RICHARDSON, T.: Protein-lipid interactions in concentrated infant formula. J. Dairy Sci., *68*, 1985 : 3180-3188.

SAITO, S. - KOJIMA, K. - MORI, B.: Epsilon-fructoselysine in urine of rats fed ^{14}C-lysine-labelled casein browned by amino-carbonyl reaction. Agric. biol. Chem., *44*, 1980 : 1327-1331.

STRATTON, G. D. Jr. - WHEELER, W. B.: Characterization of Bound Residues in Plants. Quantification. In: Nature and Bioavailability of Bound ^{14}C-Pesticide Residues in Soil, Plants and Food. Gainesville, Florida 25.-29. 3. 1985 : 71-81.

SUNDHEIM, G. - BENGTSSON-OLIVECRONA, G.: Iodine-125-labelled lipoprotein lipase as a tool to detect and study spontaneous lipolysis in bovine milk. J. Dairy Sci., *69*, 1986 : 1776-1783.

P. Rauch, J. Káš (Vysoká škola chemicko-technologická – Ústav biochemie a mikrobiologie, Praha)

Úloha radionuklidů v moderním potravinářském výzkumu, analýze a řízení technologických procesů

Tento článek přináší krátké sdělení o aplikaci radionuklidů v potravinářské analytice a při řízení technologických postupů. Radionuklidy jsou využívány při sledování

(stopování) pomocí značených sloučenin, při monitorování pochodů, které probíhají v potravinářských surovinách, v meziproduktech a při výrobě a skladování finálních produktů. Významnou roli mají v analýze přirozeně se vyskytujících složek potravin a kontaminantů. Hlavním cílem tohoto článku je stimulovat širší využití radionuklidů a jimi značench sloučenin v potravinářském výzkumu, analýze a technologii potravin.

Potr. Vědy, *11*, 1993 (3) : 251-253

INFORMATION

Collaboration of Faculty of Food and Biochemical Technology, Institute of Chemical Technology Prague within the European Network

Previous agreements and contracts with universities abroad on mutual scientific and teaching collaboration, exchanges of teachers, scientific workers and students are at present replaced by joint projects the applications of which are evaluated in international competitions and in case of positive results a financial grant can be awarded. Faculty of Food and Biochemical Technology (FFBT) was successful in four projects, two in scientific programme of EEC and two in Trans-European Mobility Scheme for University Studies (TEMPUS) oriented above all on the cooperation and mobility in higher education between Central/Eastern Europe and the European Community. The first two of mentioned projects are aimed at scientific collaboration within up-to-date research topics and EEC support expresses an appraisal of high scientific standard and especially of successful hitherto research activity of FFBT which is one of prerequisites of a grant award.

Project "Post-harvesting metabolism of plant organs and tissues" was elaborated and now being accomplished in collaboration of the Department of Biochemistry and Microbiology FFBT (J. Káš) and Laboratoire de physiologie de organes vegetaux apres recolte (J. Daussant) CNRS in Medon (near Paris, France). Programme is oriented on postharvest enzyme changes and their consequences in selected plant materials (rape and tomatoes) and even on innovation of educational programmes in enzymology and immunochemistry. In the first phase of research an attention was paid to enzymes affecting membrane functions, i.e. phospholipase A and D. New sensitive methods for enzyme activity determination were developed. For instance phospholipase D activity was determined by designed biosensor based on oxygen probe and on membrane immobilised cholinoxidase.

Second scientific project to which a grant was awarded in programme "Cooperation in Science and Technology with Central and Eastern European Countries" (COST) was entitled "Biodegradation of surface-active systems". In this project participate with the Department of Biochemistry and Microbiology (B. Králová, K. Demnerová) following institutions: Department of Biochemistry, University of Wales, Cardiff, UK (N. Russell, G. White), Institute of Ecobiology, Academy of Science of Slovac Republic, Bratislava (D. Tóth), Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest (T. Cserhatti), and Institute of Physical Chemistry, University of Salamanca, Spain (M. Roig). The aim of the project is to design bioreactors with immobilized microorganisms for

bioremediation of industrial wastes and municipal waste waters especially those polluted by surfactants, alkanes and fatty acids.

In programme TEMPUS is successfully solved project "University Education of Food Specialist" oriented on standardisation of European educational programmes esp. in France and Great Britain (J. K á š).

Second project in TEMPUS network is entitled AGROTEMPUS. Coordinator of this project is prof.G. M e d i n a, director of Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto. FPBT is an ordinary mamber of this programme since September 1992. Other partners are:

EC countries

1. C.E.R.I.A - Centre d'Enseignement et Recherche des Industries Alimentaires et Chimiques, Bruxelles, Belgium
2. TU Denmark, Lyngby, Denmark
3. ENSIA, Ecole Nationale Supérieure des Industries Agricoles et Alimentaires, Massy, France
4. TU München, Weihenstephan-Freising, Germany
5. Universita degli Studi di Milano, Italy
6. University College Cork, Cork, Ireland
7. Wageningen Agricultural University, Wageningen, The Netherlands
8. Catholic University of Portugal, Porto, Portugal
9. University of Reading, Reading, United Kingdom

Other countries G 24

10. Universität für Bodenkultur, Wien, Austria
11. University of Lund, Lund, Switzerland

Eligible countries

12. Institute of Chemical Technology Prague, Czech Republic
13. Technical University of Budapest, Hungary
14. University of Horticulture and Food Industry, Budapest, Hungary
15. University College of Food Industry, Szeged, Hungary
16. Warsaw Agricultural University, Warsaw, Poland
17. University of Galati, Galati, Romania (not yet an ordinary member, it is expected to become in next academic year).

Generally TEMPUS is a programme for longer period (max. five years) but the coordinators must every year apply for prolongation giving reports about the results and plan for following year. The main task of AGROTEMPUS JEP (joint European Programme) is collaboration and mobility of teachers and students in area of food high education which should firstly contribute to improvement and restructuralization of food technology and engineering education in different countries on a similar basis

and secondly to affect in the future efficiently the structure of European food industry. Nevertheless the main goal of TEMPUS acitivity is education, however, research work of students is included in all programmes. During the existence of AGROTEMPUS four working groups are gradually preparing materials on

1) Unit operations and Transport Phenomena in Food Engineering (coordinator: ENSIA),

2) Marketing and Business Management (coordinator: University of Reading),

3) Quality Control and Quality Assurance (coordinator: FFBT, Department of Food Chemistry and Analysis)

4) University - Enterprise Collaboration (coordinator: University of Horticulture and Food Industry, Budapest).

The results obtained from these studies are of great importance for further transformation of food specialists education at FFBT. The first year of our participation in AGROTEMPUS gave us possibility to enable students and teachers to spend some time at collaborating institutions (students incl. doctorands from three till six months, teachers 14 days).

Jan Káš and Mojmír Rychtera

Instructions for Authors Contributing to the Scientific Journal Potravinářské vědy

Original scientific papers, short communications, and selectively reviews, that means papers based on the study of technical literature and reviewing recent knowledge in the given field, are published in this journal. Published papers are in Czech, Slovak or English. Each manuscript must contain a short or a longer summary.

The authors is fully responsible for the originality of his paper, for its subject and formal correctness. The author shall make a written declaration that his paper has not been published in any other information source.

The board of editors of this journal will decide on paper publication, with respect to expert opinions, scientific importance, contribution and quality of the paper.

Technical layout of the manuscript – Manuscript layout shall be as follows: quarto, 30 lines per page, 60 strokes per line, double-spaced typescript. Tables, figures and photos shall be enclosed separately, not no paste-board. A figure or graph number shall be written on the reverse, along with the author's name. Texts to figures and graphs shall be indicated on a separate sheet. Tables shall be given Roman numerals. The text must contain references to all these annexes. The paper extent shall not exceed ten typescript pages, including tables, figures and graphs. In the text it is necessary to use such units that are in agreement with the International System of Units.

Layout of the paper – The title of the paper shall not exceed 85 strokes. It is necessary to avoid in the title the usage of common expressions such as A study of ..., A contribution to....., An attempt at ...; each paper shall be a finished treatise, papers in continuation denoted e.g. Part I, II, III,etc., cannot be published; subtitles of the papers are not allowed either.

Summary - a brief one – The summary is an information selection of the contents and conclusions of the paper, it is not a mere description of the paper. It must present all substantial information contained in the paper, but its role is not to replace this paper. It shall not exceed 170 words. It shall be written in full sentences, not in form of keynotes. It is published in the language the paper is written in, it should comprise base numerical data including statistical data.

Summary - a longer one – This summary is a basis of English translation, it should contain comments on the results of the study, references to tables and figures, or to the most important literary citations. Its extent shall be two to three typescript pages. It may be submitted in English, if it is submitted in Czech or Slovak, an annexed English vocabulary of technical and agricultural terms will be appreciated.

Key words, index terms – They follow after the brief and longer summary on the next but one line (both summaries shall have the same keywords).

in small letter, semicolons shall be used between these words. Their number depends on the subject-matter of the paper, it should not be smaller than three and higher than 12 words.

Introduction – This section has to present the main reasons why the study was conducted, and the circumstances of the studied problems should be described in a very brief form. Extensive historical reviews should be avoided. It does not have a title. This section include review of literature; only literary citations with close relation to the treated problem. It is rexommended to cite the lowest possible number of authors.

Material and Methods – Only original methods shall be described, in other cases it is sufficient enough to cite the author of the used method and to mention modifications of this method. This section shall also contain a description of experimental material. A description of the method should enable any specialist to repeat the study on the basis of this description and mentioned citations.

Results – In the section Results figures and graphs should be used rather than tables for presentation of quantitative values. A statistical analysis of recorded values should be summarized in tables. This section should not contain either theoretical conclusions or deductions, but only factual data should be presented here.

Discussion – This section contains an evaluation of the study, potencial shortcomings are discussed, and the results of the study are confronted with previously published results (only those authors whose studies are in closer realtion with the published paper should be cited). The sections Results and Discussion may be presented as one section only.

References – The citations are arranged alphabetically according to the surname of the first author: surname (capital letters), first name abbreviation, full title of the paper, official abbreviation of the journal, volume, year of publication, first page, last page; in book citations place of publication, publisher and year should be given. References in the text to these citations comprise the author's name and year of publication. Only the papers cited in the text of the study shall be included in the list of references. All citations shall be referred to in the text of the paper.

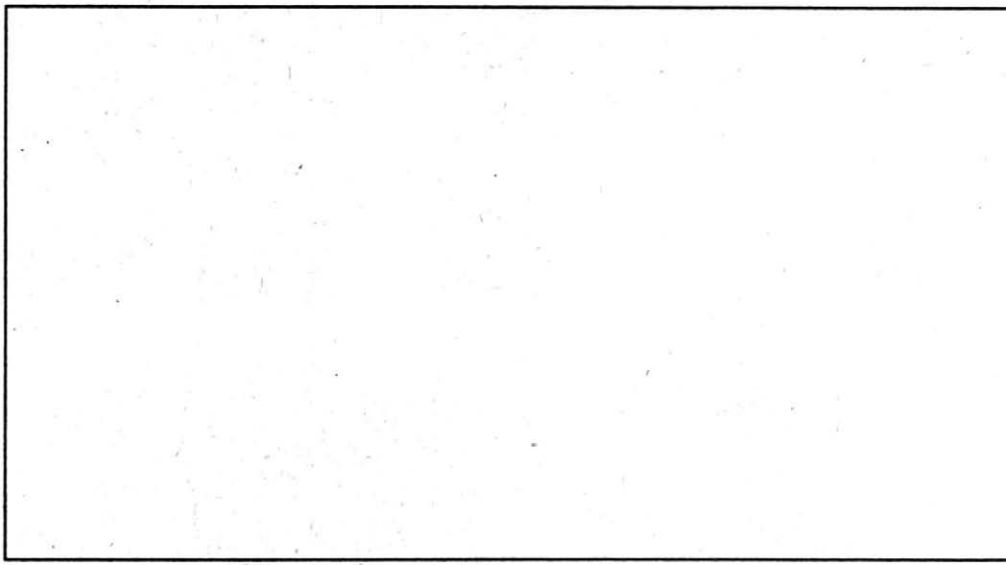
Address – The author shall give his full name (and the names of other collaborators), academic, scientific and pedagogic titles, full address of his workplace and postal code.

Use of abbreviations – If any abbreviation is used in the paper, it is necessary to mention its full form at least once to avoid misunderstanding. The abbreviations should not be used in the title of the paper nor in the summary.

CONTENTS — OBSAH

SURVEY — PŘEHLEDY

INFORMATION — INFORMACE



Vědecký časopis POTRAVINÁŘSKÉ VĚDY ◆ Vydává Česká akademie zemědělských věd
a Slovenská akadémia pôdohospodárskych vied– Ústav zemědělských a potravinářských
informací ◆ Vychází šestkrát ročně ◆ Redaktorka RNDr. Marcela Braunová
◆ Redakce: 120 56 Praha 2, Slezská 7, telefon 02/251 098 ◆ Sazba a tisk ÚZPI ◆

Rozšiřuje PNS. Informace o předplatném podá a objednávky přijímá každá administrace PNS
doručovatel tisku a Administrace centralizovaného tisku, Hvožďanská 5 - 7, 149 00 Praha 4