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BATCH LACTIC ACID FERMENTATION ON LIGNOCELLULOSIC HYDROLYSATE: IDENTIFICATION OF PHYSIOLOGICAL MODEL*

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Abstract: The effect of lignocellulosic hydrolysate of crushed corn cobs on the fermentation kinetics of *Lactobacillus casei* and *Lactobacillus lactis* in a batch culture was studied. From computer-aided analysis of data a simple physiological model of the process was derived relating to bacterial growth, lactic acid formation, utilization of two different sources of nutrients and inoculum size. The parameters of the model represent the effect of preparation of media on the physiological activity of both used strains of bacteria.

lactic acid; batch fermentation; *Lactobacillus*; physiological model; lignocellulosic hydrolysate

Crushed corn cob hydrolysate is a promising substitute of sugar in lactic acid fermentation (Rychtera et al., 1990). A mathematical model taking into account essential physiological functions may be used as a tool of fast transfer of technology based on sugar to that employing the hydrolysate.

The first generation of models (Luedeking, Piret, 1959; Hanson, Tsao, 1972) was proposed on simple kinetics and mass balances of biomass, sugar and product. Leh and Charles (1989) applied the above mentioned model in a batch fermentation of whey permeate and found that the parameters must vary with the medium composition. Yang et al. (1988), Ishizaki et al. (1989, 1992) and Nielsen et al. (1991) successfully applied the modelling of lactic acid production on other strains than *Lactobacillus*. Although a number of other papers (Borzani et al., 1990;

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Goncalves et al., 1991; Venkatesh et al., 1993; Acuna et al., 1994) studied the kinetic of lactic acid batch fermentation by lactic bacteria, no one presented a clear cut expression from the standpoint of product inhibition and medium composition variations.

The objective of this study was to develop a simple structural model, suitable to evaluate the effect of changes of the medium on the physiology of two different strains of *Lactobacillus.*

MATERIAL AND METHODS

Organisms

Lactobacillus lactis 447 and *Lactobacillus casei,* subsp. *rhamnosus* 1753 (Culture Collection of Diary Microorganisms, Prague) were maintained by monthly subculture on the MRS medium supplemented with 2% calcium carbonate powder and afterwards stored at 4 °C.

Media

MRS medium was used for the maintaining of cultures and for biomass accumulation.

The complete medium (CM) for batch cultivations contained (g/l): glucose 50 or 100; yeast extract (Imuna, Šarišské Michalany, Slovak Republic) 20; peptone (Imuna) 10; KH_2PO_4 2; sodium acetate 5; ammonium citrate 2; $MgSO_4.7H_2O$ 0.2; $MnSO_4.4H_2O$ 0.05; pH was adjusted to 5.8.

Lignocellulosic hydrolysate (LH) was prepared by enzymatic hydrolyses. Crushed corn cobs (kernels removed) after steam expansion were mashed in citrate buffer pH 5.0 (100 g/l) and treated with 7.7 g/l enzyme preparation with 260 U/g cellulolytic activity (Cellulase VÚPP 4/88, Research Institute of Food Industry, Prague, Czech Republic) at 45 °C for 24 h. Obtained hydrolysate containing 74 g/l glucose was used as a carbon source only, glucose concentration was adjusted at 50 g/l and other components were supplemented in the same amounts like those in CM. To remove cellulolytic enzymes and macromolecular substances the part of LH was filtered through a UF-membrane CPD 20 (DDS, Denmark).

All media were sterilised by autoclaving at 120°C for 20 minutes.

Growth conditions

Organisms were grown in a glass stirred fermenter with working volume 500 ml. The pH value of the cultures was automatically maintained at 5.8 and the temperature was set at 37 °C. The fermenter was seeded by washed centrifuged cells cultured in conical flasks containing 100 ml of CM on rotary shaker (2 Hz) at 37 °C for 20 h.

Analyses

Glucose, lactate, ethanol and other fermentation products in media and culture supernatants were determined by HPLC (Laboratory Instruments Co., Prague, Czech Republic) on a column filled with Ostion LG KS 0800 (strongly acidic cation exchanger) in H-cycle which was used with 0.005M H_2SO_4 as eluent at 65 °C, and with refractive index detector (Melzoch et al., 1994).

Biomass was measured as dry weight using the method of Herbert et al. (1971).

Mathematical model development

During preliminary evaluation of experimental data we found that the production of lactic acid was proportional to the consumption of reducing sugars. The production of lactic acid ceases when the sugars are exhausted. There is the apparent sugar limitation of product formation.

On the other hand, the growth of biomass was not limited by carbon source, neither inhibited by lactic acid produced. Having analysed the specific growth rate, we have discovered that there is a short lag phase at the beginning of cultivation and the specific growth rate reaches the maximum between the second to fourth hour of fermentation, depending on inoculum size. Then, apparently, the growth is limited by some nutrient in the complex medium. The maximum cell density is dependent on the quality of used medium.

These findings can be summarised in a simple physiological model as follows.

Balance of sugars (S_1):

$$dS_1/dt = -k_1 S_1 X / (S_1 + K_{S_1}) \quad [1]$$

Balance of product (L):

$$dL/dt = -Y_{L/S_1} \, dS_1/dt \qquad [2]$$

Balance of biomass (X):

$$dX/dt = \mu X \qquad [3]$$

For the description of specific growth rate, we used the concept of "metabolic activity functional", originally introduced by Powell (1968) and later applied by Volesky and Votruba (1992) in the modelling of anaerobic production of solvents by *Clostridium acetobutylicum*.

According to this approach, the specific growth rate may be expressed as a linear function of intracellular RNA concentration.

$$\mu = \text{const} \ (\text{RNA} - \text{RNA}_{\text{min}}) \qquad [4]$$

where RNA_{min} – RNA content in nongrowing-resting cells

Roels (1980) summarised the experimental data from different cultivations and found that such a relation may be generalised for bacteria growing either in aerobic or anaerobic conditions. Equation [4] may be therefore used to express the physiological potential of unlimited growing microbial population. When a substrate limitation has to be considere, then according to Powell (1968), the right-hand side of Eq. [4] has to be multiplied by term that represents a limiting barrier. The simple hyperbolic term is often recommended (Volesky, Votruba, 1992). The final formula, describing the above discussed physiological phenomena may by rewritten in the following way:

$$\mu = k_2 (y - 1) S_2 / (S_2 + k_{S_2}) \qquad [5]$$

where y – dimensionless RNA concentration

$$y = \text{RNA} / \text{RNA}_{\text{min}} \qquad [6]$$

To complete the description of cell growth it is necessary to formulate the balance of RNA. We assume that the RNA is formed when the amount of nutrients in the medium guarantees the growth. Such a situation may be easily expressed by a direct proportionality between the concentration of limiting

substrate and by the total rate of RNA formation (note that total RNA concentration in the culture is RNA X) as follows:

$$d\,(\text{RNA}\,X)\,/\,dt = k_3\,S_2\,\text{RNA}\,X \qquad [7]$$

After simple algebraic manipulation with complex derivative on left hand side, dividing by X and introducing of for $1/X$ dX/dt we receive the final relation for dimensionless RNA concentration y:

$$dy\,/\,dt = k_3\,S_2\,y - \mu y \qquad [8]$$

The initial condition for y at the beginning of fermentation expresses the physiological state of a culture. When $y(0)$ equals 1, it means that the initial growth rate is zero and inoculum is represented by nongrowing – resting cells. When $y(0)$ is higher than 1, then it is considered that fresh and growing cells are used for inoculation. On the other hand, when $y(0)$ is lower than 1,

I. The basic characteristic of batch lactic acid fermentations

Batch	Organism	Medium	$S_1(0)$	$X(0)$	X	L
			[g.l^{-1}]			
1	*L. lactis*	CM	47	1.3	6.0	37
2	*L. lactis*	CM	93	1.9	4.4	69
3	*L. casei*	CM	54	0.4	11.3	41
4	*L. lactis*	LH	56	1.3	5.8	36
5	*L. casei*	LH	51	0.5	11.0	41
6	*L. casei*	LH(UF)	51	0.5	4.5	12
7	*L. casei*	LH	51	0.4	11.0	40
8	*L. lactis*	LH	47	0.4	5.9	27
9	*L. lactis*	CM	53	0.4	11.3	52
10	*L. lactis*	CM + E	92	1.9	3.6	74
11	*L. lactis*	CM	92	1.9	4.3	74
12	*L. lactis*	LH	47	0.5	7.3	36
13	*L. lactis*	LH(UF)	47	0.5	7.3	31

CM + E = CM with addition of cellulolytic enzymes (7.7 g.l^{-1})
LH(UF) = LH filtered through UF-membrane

then the part of cell biomass is considered as dead. In our study, we considered that $y(0)$ equals 1.1. It means, we supposed the slow growing cells at inoculum.

For simulation purposes, we have to complete the model by the balance of unknown, growth limiting substrate S_2. It was suitable to assume that the rate of unknown substrate consumption is proportional to the growth rate. Because the yield coefficient for S_2 is unknown we can assume it as one and during parameter estimation to evaluate the unknown initial value of $S_2(0)$.

Balance of growth limiting substrate (S_2):

$$dS_2 / dt = -dX / dt \qquad [9]$$

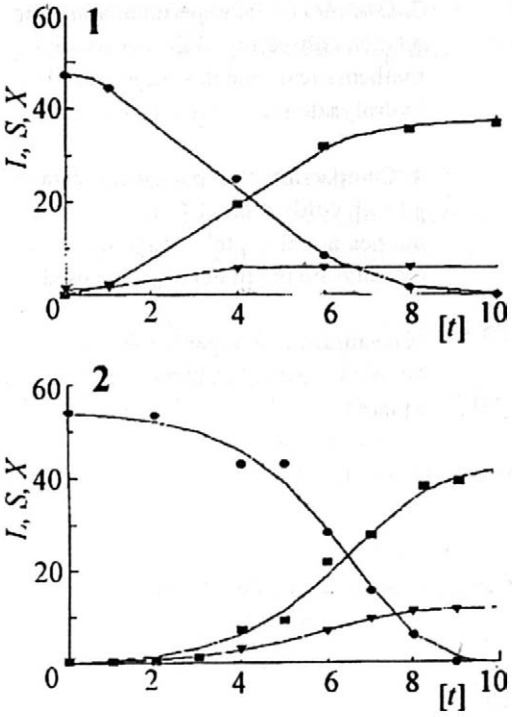
Based on the collection of 13 batch data sets (Table I), the unknown parameters of the model were estimated by computer program BIOKIN (Volesky, Votruba, 1992) and the results are summarised in Table II.

RESULTS AND DISCUSSION

The single batch fermentations including basic experimental data are characterised in Table I. Figures 1 and 2 compare the results of computer

II. The parameters of the model

Batch	k_1	k_2	k_3	K_{S_1}	K_{S_2}	Y_{L/S_1}	$S_2(0)$
1	2.61	0.582	0.306	15.92	0.0483	0.803	4.81
2	2.75	0.398	0.472	51.36	0.0831	0.812	2.28
3	2.67	0.348	0.282	17.53	13.48	0.738	11.88
4	1.45	0.362	0.146	16.74	15.21	0.594	4.57
5	1.91	0.487	0.211	12.95	24.89	0.835	9.08
6	2.33	0.832	0.132	34.38	24.89	0.823	9.28
7	2.64	0.437	0.115	28.55	0.076	0.684	5.92
8	2.39	1.081	0.162	12.81	21.57	0.893	6.16
9	2.58	0.880	0.229	9.12	28.49	1.030	10.13
10	3.85	0.436	0.433	51.65	1.125	0.847	1.62
11	2.23	0.581	0.242	12.35	0.708	0.869	2.23
12	2.67	1.121	0.078	26.14	4.198	0.846	7.62
13	1.88	0.389	0.186	11.29	7.772	0.910	7.41



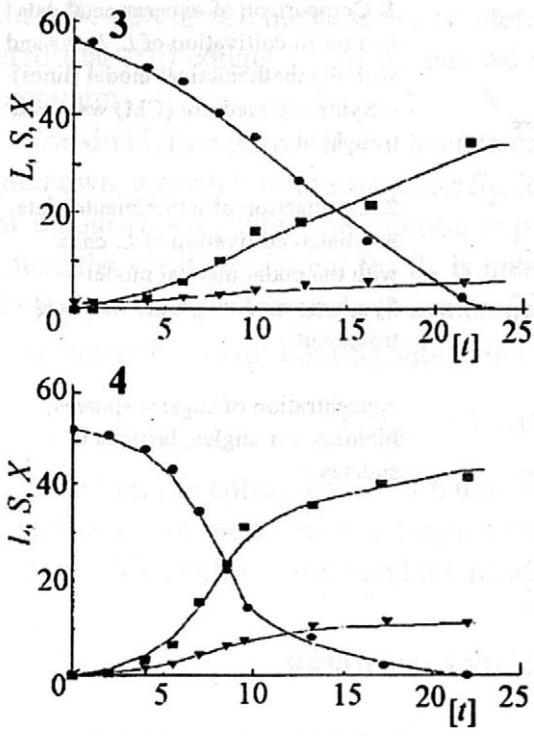
1. Comparison of experimental data in a batch cultivation of *L. lactis* and with the mathematical model (lines) – Synthetic medium (CM) was used troughout

2. Comparison of experimental data in a batch cultivation of *L. casei* with the mathematical model – Synthetic medium (CM) was used troughout

concentration of sugar – spheres; biomass – triangles; lactic acid – squares

simulation and experiments performed in synthetic medium (Batch No.1 and 3 in Table I and II). It appears that the cultivation of *L. lactis* (Fig. 1) was faster than that of *L. casei* (Fig. 2) but the difference was caused by different size of inoculum. As follows from Table II, the estimated kinetic parameters were of the same magnitude. However, the strain *L. casei* could better utilize the growth supporting substrate S_2 from synthetic medium, as shown in Table II in sixth column. Figures 3 and 4 (Batch 4 and 5 in Table I and II) compare the physiological activity of both strains when the lignocellulosic hydrolysate medium was used. Evidently, the duration of lactic fermentation is twice longer than that performed with synthetic medium, but the differences between growth parameters of both strains and yield of lactic acid were similar.

Table II summarises the results of parameter estimation. It was surprising that the kinetics and stoichiometry of product formation and sugar consumption were for both strains and different media similar. The mean yield of produced lactic acid from utilised sugar (Y_{L/S_1}) was about 0.822 ± 0.103 g per g, when compared with theoretical value which is 1g lactic acid per 1g



3. Comparison of experimental data in a batch cultivation of *L. lactis* with the mathematical model. – Lignocelullosic hydrolysate medium (LH) was used

4. Comparison of experimental data in a batch cultivation of *L. casei* with the mathematical model. – Lignocelullosic hydrolysate medium (LH) was used

concentration of sugar – spheres; biomass – triangles; lactic acid – squares

hexose. The maximum specific rate of product formation (k_1) was 2.46 ± ± 0.55 g lactic acid per 1g DW of biomass per 1 h. The mean value of K_{S_1}, which may be taken as a measure of sugar limitation of lactic acid formation was about 23 ± 15 g sugar per litre. On the other side, the kinetic of growth shows the dependence on the medium composition. Generally, the mean value of k_3, which characterises the physiological potential of growth, was for complete medium (CM) two times higher than that for enzymatic hydrolysate of corn cobs (LH). The apparent concentration of unknown growth limiting substrate $S_2(0)$ was higher for *L. casei* when compared with *L. lactis*. It may be explained by higher metabolic potential of *L. casei* to utilise the nutrients for growth from both media (CM and LH). The similar dependence was found for the value of K_{S_2} which characterises the growth limitation by unknown substrate S_2.

The addition of cellulolytic enzymes to synthetic medium (Batch No. 10) caused the decrease of both $S_2(0)$ and K_{S_2}. It may be deduced that the enzymes interact with unknown growth supporting substrate and partially in-

hibit its utilization. The experiment performed on the production LH medium, which was clarified by ultrafiltration, supports that hypothesis.

List of symbols

k_1	kinetic constant characterizing sugar uptake	$[h^{-1}]$
k_2	kinetic constant characterizing growth of microorganisms	$[h^{-1}]$
k_3	kinetic constant characterizing dynamics of RNA formation	$[m^3\ h^{-1}\ kg^{-1}]$
K_{S_1}, K_{S_2}	kinetic parameters characterizing the substrate limitation	$[kg\ m^{-3}]$
L	concentration of lactic acid	$[kg\ m^{-3}]$
RNA	mass fraction of RNA in the biomass	
S_1	concentration of sugar	$[kg\ m^{-3}]$
S_2	concentration of unknown growth supporting substrate	$[kg\ m^{-3}]$
t	time	[h]
X	concentration of biomass	$[kg\ m^{-3}]$
y	dimensionless variable defined by Eq. [6]	[–]
Y_{L/S_1}	yield coefficient	[–]
μ	specific growth rate	$[h^{-1}]$

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Produkce kyseliny mléčné ve vsádkové fermentaci na lignocelulozovém hydrolyzátu: Identifikace fyziologického modelu

Práce se zabývá studiem produkce kyseliny mléčné buňkami *Lactobacillus casei* a *Lactobacillus lactis* ve vsádkové fermentaci. Fermentace byly prováděny v bioreaktoru při konstantním pH 5,8 ± 0,1 a teplotě 37 °C na dvou rozdílných typech médií

– na kompletním médiu (CM) a na lignocelulózovém hydrolyzátu (LH). V obou případech zdrojem uhlíku a energie byla glukóza. Glukóza obsažená v LH byla získána enzymovou hydrolýzou drcených kukuřičných oklasků, které byly nejprve podrobeny parní expanzi (rozvláknění lignocelulozového komplexu a částečné naštěpení hemiceluloz) a poté byla celulosa štěpena za účasti celulolytických enzymů na glukózu.

Byl sledován vliv použití lignocelulózového hydrolyzátu připraveného z kukuřičných oklasků na kinetiku vsádkové fermentace. Na základě počítačem řízené analýzy dat získaných z 13 fermentací byl odvozen jednoduchý fyziologický model bioprocesu, který popisoval základní fyziologické funkce obou bakteriálních kmenů týkající se růstu kultury mléčných bakterií, tvorby kyseliny mléčné, utilizace glukózy v závislosti na typu použitého média (dvou rozdílných zdrojů živin) a dále na velikosti a kvalitě inokula.

Z uvedených dat vyplynulo, že růst biomasy není limitován zdrojem uhlíku (glukózou), ani inhibován produkovanou kyselinou mléčnou až do vyčerpání cukerného zdroje v médiu. V průběhu fermentace dochází k limitaci růstu biomasy neznámým substrátem. Překvapujícím faktem bylo zjištění, že kinetika a stechiometrie tvorby kyseliny mléčné a spotřeby cukru byly pro oba kmeny mléčných bakterií a na obou typech médií téměř shodné.

kyselina mléčná; vsádková fermentace; *Lactobacillus casei*; *Lactobacillus lactis*; fyziologický model; lignocelulózový hydrolyzát

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THE CHANGES IN DIETARY FIBER CHARACTER AFTER THE EXTRUSION OF CEREALS*

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Abstract: Total dietary fiber, insoluble and soluble parts of dietary fiber were determined in the samples of crisp bread and in the mixtures of raw materials for their extrusion. AOAC method was used with the Fibertec E laboratory instrument for the enzymatic hydrolysis of starch and protein. The TDF was not affected by extrusion so deeply as IDF and SDF. The samples with wheat bran showed in one set of samples the greatest, in the other set the lowest decrease of TDF content in material after extrusion. IDF content was decreased in all cases, SDF content was decreased in the sample with vegetables only, in the other samples it was increased after extrusion.

dietary fiber; extrusion; Fibertec E

Despite many discussions concerning the dietary fiber (DF) composing parts and respective methods of determination especially of soluble and insoluble fiber, two main aspects are important for the consumers and nutritionists. First, any definition should respect the fact that dietary fiber is primarily defined as non digestible part of food and that any method of determination should give the results as close to this definition as possible. Second, we need much more information concerning the dietary fiber content for the consumers as soon as possible irrespective of the methods used that do not precisely classify the chemical substance of composing parts. A study of chemical substances should be obviously a matter of scientific study for many years, but most effective information for consumer is more general information given in time and reflecting the physiological role of fiber.

This work is the first part of a larger study of the effect of processing on dietary fiber content and on its changes in some foods based on cereals.

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A dietary fiber definition

The term dietary fiber was introduced by Hipsley in 1953 to cover what had earlier been called unavailable, e.g. undigested carbohydrates of plant foods. In 1972 Trowell defined dietary fiber as the undigestible plant cell wall material, but later on restricted the definition to include only lignin and the plant polysaccharides that are not digested by enzymes secreted in the human digestive tract. On the other hand, undigestible polysaccharides not related to the cell wall, such as gums and mucilages as well as food additives of this nature, are also included in such a dietary fiber concept (Selvendran, Robertson, 1990).

As we have seen at ICC Conference 1995 in Vienna a lot of scientists are working on definition and method for determination of dietary fiber. In the United States, the Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) issued final food labelling regulations in January 1993. For nutritional labelling, AACC Methods 32-05 (corresponding to AOAC Method 985.29) and 32-07 (AOAC Method 991.43) have been recommended for DF analysis by both agencies (Lee, Prosky, 1994).

Based on scientific findings, Lee and Prosky have proposed the expansion of the definition of dietary fiber to include resistant oligosaccharides, in addition to currently included nonstarch polysaccharides, resistant starch, and lignin. They have also proposed a new term resistant oligosaccharides, which is defined as oligosaccharides that are resistant to hydrolysis by human alimentary enzymes. The term resistant oligosaccharides can be used synonymously with the term unavailable oligosaccharides.

They expect that certain types of oligosaccharides can be claimed for dietary fiber in future, if the unavailability in the healthy human upper gastrointestinal tract and the resulting physiological action similar to other dietary fiber components are fully proven. They want to point out that oligosaccharides that are hydrolysed in the upper gastrointestinal tract of normal healthy human subjects, such as lactose, maltotriose, and maltotetraose cannot be classified as resistant oligosaccharides.

Along this line analytical methodology should be tuned to fully recover resistant oligosaccharides as well as resistant starch in the dietary fiber analysis. In some previous papers, they addressed the issue of incomplete recovery of resistant starch with the current dietary fiber methods. Until a

reliable methodology is developed to meet the new definition of dietary fiber, current dietary fiber methods should continue to be used for dietary fiber labelling. They want to emphasise that this new definition would not affect the dietary fiber values of most food products.

Composition of dietary fiber

Currently, total dietary fiber (TDF) is plant material that is not digestible by appropriately chosen enzymes that mimic the human alimentary system (Stauffer, 1993). TDF is split into two components: soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). Insoluble dietary fiber is not soluble in hot water. The group of IDF mostly contains these components: cellulose, hemicellulose, lignin, products of Maillard reaction and resistant starch. Soluble dietary fiber is soluble in warm or hot water, but is reprecipitated when that water is mixed with four parts of ethyl alcohol. This group contains mainly β-glucans, pentosans, pectic substances, gums of plant origin, some bacterial polysaccharides and synthetic derivatives. Certain groups of gums have both soluble and insoluble species.

Total dietary fiber is important in calculating energetic value of foods containing these materials. Insoluble and soluble forms have somewhat different functional characteristics that influence physiological effects. The insoluble fiber fraction is known to have a mechanical effect on the intestinal tract, whereas the soluble fraction seems to be more responsible for metabolic effects, which affect primarily carbohydrates and lipid metabolism (Ralet et al., 1990). It has been speculated that dietary fiber plays a role in the reduction or prevention of diseases of the colon such as constipation, appendicitis, cancer, haemorrhoids, and ulcers. It may also be responsible for preventing or delaying the onset of metabolic diseases such as the various vascular diseases, heart disease, hypertension, obesity, and diabetes, and may ameliorate the effects of endocrine diseases, hiatal herniás and dental caries.

Methods of determination

The two general strategies to measure dietary fiber are commonly termed gravimetric and chemical methods. Both these methods employ a series of steps to selectively extract nonfiber components from the test material. In gravimetric procedures, the weight of the fiber residue is corrected for the

most likely contaminants such as ash and protein, and the remainder is assumed to be dietary fiber. In chemical procedures, the chemical components of fiber in the residue are quantified through a series of specific assays. The weight of the residue is used to calculate the amounts of the chemical components of fiber in the original material (Vollendorf, Marlett, 1991).

At present, the most widely used methods are the enzymatic-gravimetric AOAC official method (AOAC,1990) and the enzymatic-chemical Englyst method. New modifications of the AOAC method are continuously proposed by several authors and the present Englyst method is also the result of different reviews.

Effect of heat processing on the DF

Most foods containing DF are processed in one way or another before consumption, as well as the physico-chemical properties of the fiber. Being highly dependent upon such properties, the physiological effects of DF may also be altered by processing (Asp et al., 1986).

Processing may alter the content and properties of DF in different ways:

1. Leakage to the processing water of soluble DF, or ions – especially calcium – interacting with the fiber.
2. Formation of acid insoluble material such as Maillard reaction products that add to the lignin content.
3. Formation of enzyme resistant starch fractions that add to the dietary fiber polysaccharides.
4. Fragmentation of dietary fiber components by hydrolysis of glycosidic bonds within DF polysaccharides and other constituents. This may lead to increased solubility of fiber, decreased dietary fiber content if low molecular weight fragments are formed, and loss blood glucose lowering effect of guar is abolished when the polysaccharide is depolymerized.

Effect of extrusion

Extrusion cooking may change the content, composition and physiological effects of dietary fiber in various ways. First, starch could undergo modification and form enzyme-resistant fractions, which have acted *in vivo* as dietary fiber (Björck et al., 1986). Second, degradation of dietary fiber to low molecular weight fragments would diminish its content and hence re-

duce its benefits. Third, macromolecular degradation of fiber may increase the solubility and change the physiological effects of fiber (Lue et al., 1991).

Authors studying the effect of extrusion on the content of dietary fiber observed different results. Björck et al. (1984a, b) reported a slight increase in TDF after extruding white and whole meal wheat flour. The same result was found in extruded whole meal barley by Ostergard et al. (1989). In contrast, Siljeström et al. (1986) and Schweizer and Reimann (1986) found no changes in TDF content when extruding wheat flour. Fornal et al. (1987) reported decreased contents of cellulose and lignin in extruded starch mixtures of buckwheat and barley. Studying the extrusion cooking of high-fiber cereal, Sandberg et al. (1986) reported that mild extrusion conditions did not change the content of nonstarch polysaccharides but decreased the amount of Klason lignin (Lue et al., 1991). Wang et al. (1993) described that after extrusion, TDF was significantly decreased only in the wheat bran low-condition sample, but IDF was lower in extruded wheat and wheat bran than in the raw samples. Soluble dietary fiber tended to increase in the extruded samples. Decreased TDF, decreased insoluble dietary fiber, and increased soluble dietary fiber in extruded products could be the result of disruption of covalent or noncovalent bonds in the carbohydrate and protein moieties, leading to smaller, more soluble molecular fragments.

It seems that differences in dietary fiber content depend on extrusion conditions as well as on the processing material (Lue et al., 1991).

MATERIALS AND METHODS

The first set of the samples of extruded crisp bread was obtained from industrial production at the industrial one-screw extruder in EXTRUDO Company in Bečice (South Bohemia). The second set of the samples was obtained from experimental laboratory extrusion in Massy (France). Two-screw extruder was used in this case.

The complex mixtures of raw materials for extrusion were sampled at the time of feeding the extruder. An average processing time of material in extruder was checked to get the product from the same material as was fed to the extruder. The breads were ground at the laboratory mill until the size of all particles was less than 0.5 mm.

The No. 1 set of the samples: TDF, IDF, and SDF content was determined in three types of crisp bread and the raw materials used for their production: The Caraway Bread, The Graham Bread, and Setuza, Ústí nad Labem The Vegetable Bread.

The No. 2 set of the samples: TDF content was determined in crisp bread from wheat flour T550 and crisp bread from wheat flour T550 with wheat bran and in the raw materials used for their production.

A mixture for The Caraway Bread is based in main part on wheat meal with some part of rice and rye flours. The Graham Bread is based mainly on wheat meal with small addition of wheat and rye bran. The Vegetable Bread contains mainly a rice flour with the addition of some dried powdered vegetables.

The samples extruded in Massy were the traditional extruded crisp breads made either from wheat flour or from that with the addition of wheat meal.

Principle of AOAC method (985.29)

Determination of TDF. Food samples were ground, fat-extracted if they contained > 5% fat, and then weighed duplicate 1g samples into 400 ml beakers. Samples were gelatinised with Termamyl (heat-stable α-amylase, No. A3306, Sigma Chemical Co.), then enzymatically digested with protease (No. P3910, Sigma Chemical Co.), and amyloglucosidase (No. A9913, Sigma Chemical Co.), to remove protein and starch. Four volumes of EtOH (95% v/v) were added to precipitate soluble dietary fiber. Total residue was filtered, washed with 78% ethanol, 95% ethanol, and acetone. After drying, the residue was weighed. One duplicate was analysed for protein, and another was incinerated at 525 °C and ash was determined. Total dietary fiber is the weight of the residue less the weight of the protein and ash.

Determination of IDF. The method for determination of IDF is the same as for TDF. The only modification is omitting of addition of EtOH. Samples, after incubation with amyloglucosidase were filtered, washed with H_2O, 95% ethanol, and acetone. After drying the residue was weighed. One duplicate was analysed for protein, and another was incinerated at 525 °C and ash is determined. Insoluble dietary fiber is the weight of the residue less the weight of the protein and ash.

Determination of SDF. To the filtrate from the determination of IDF, 4 volumes of EtOH (95% v/v) were added to precipitate soluble dietary fi-

ber. Total residue was filtered, washed with 78% ethanol, 95% ethanol, and acetone. After drying, residue was weighed. One duplicate was analysed for protein, and another was incinerated at 525 °C and ash is determined. Soluble dietary fiber is the weight of the residue less the weight of the protein and ash.

All other conditions as described in the AOAC Method were respected.

RESULTS AND DISCUSSION

Different types of extruded crisp bread were analysed as well as the complex mixtures of raw materials used for extrusion. Two or in some cases three parallel determinations were done with each of the samples (Table I).

I. No. 1 set of samples. The results of TDF, IDF and SDF determinations (% in d.m.)

Material	TDF		IDF		SDF	
		average		average		average
1. The Caraway Bread						
– raw	6.6, 6.8	6,7	6.4, 6.6	6.5	0.5, 0.7	0.6
– product	6.7, 6.8	6.8	4.5, 5.0	4.8	1.8, 1.3	1.6
2. The Vegetable Bread						
– raw	3.7, 3.9	3.8	2.6, 2.7 2.8, 1.9	2.6	0.4, 1.2 1.3, 1.6	1.1
– product	3.5, 3.6	3.6	1.9, 1.9	1.9	0.6, 0.7	0.7
3. The Graham Bread						
– raw	10.1, 9.4 9.8, 9.7	9.8	7.5, 8.0	7.8	2.0, 2.3	2.2
– product	8.2, 7.7	8.0	6.1, 7.8	7.0	2.7, 2.6	2.7

It can be seen from Table I that extrusion had no effect on the TDF content in samples 1 and 2, but in samples 3, there is a decrease of TDF content in extruded product from graham mixture. As sample 3 contained a greater part of bran, and results have confirmed the part of conclusions of different authors that found out the decrease of TDF in extrudates with bran.

Compared with the IDF contents of the raw materials in samples 1, 2, 3 IDF decreased after extrusion in all three samples. The SDF content decreased slowly in sample 2, but increased in samples 1 and 3.

The decrease of IDF content in samples 1 and 2 was similar (approximately 25%) and was considerably higher than the decrease in sample 3. In general, a conclusion that the content of IDF decreasing in the extruding process has been confirmed, but the degree of decrease is considerably affected by the components of extruded mixture.

The main difference between sample 3 and remaining two samples was in the content of flours (in samples 1 and 2 more flours in total including a part of rice flour) and bran (in sample 3 almost no flour, mostly wheat meal and small part of bran). Surprisingly, thermal treatment and pressure caused degradation of meal and bran fiber components was lower than the IDF degradation in other materials with lower part of bran fiber.

The increase of SDF in samples 1 and 3 can be considered as a consequence of partial degradation of IDF that could be turned to SDF. The composition of SDF in sample 2 probably considerably differed from that of the other samples. Much greater part of pectins can be expected in vegetable components in this sample and extrusion caused changes led probably to their destruction.

The results of TDF determination in extrudate based on wheat flour or wheat flour and bran are given in Table II.

II. No. 2 set of samples from ENSIA Massy. Average TDF content (% in d.m.).

Material	TDF	
	number of samples	average
Wheat – raw	2	2.3
– product	2	1.5
Wheat with bran – raw	8	2.4
– product	2	2.3

In the samples from two-screw extruder TDF was determined only. A mixture of wheat flour with bran was used to testify a reproducibility of TDF determination. In this case eight repeated measurements were carried out (Table III). In the other cases two repeated measurements were done.

III. Reproducibility of TDF determination (% in d.m.)

Number of measurements	1	2	3	4	5	6	7	8
Content of TDF	2.8	2.4	2.2	2.1	2.4	2.3	2.2	2.6

The results showed different effects than with one-screw extruder regarding the changes in TDF content in comparison with raw mixtures. On the other hand TDF content of wheat flour mixture decreased considerably (approximately to 2/3 of original content).

The accuracy of the method of determination of TDF content was 0.3% TDF of dry solid matter ($P = 99\%$).

Conclusions

Significant changes in IDF, SDF, and in some cases in TDF content were found out as a consequence of extruding process, but the changes considerably differed due to the composition of mixtures. The results obtained with one-screw or two-screw extruder differed too.

TDF content decreased significantly after extrusion only in crisp bread Graham from one-screw extruder and in wheat-flour extrudate without bran from two-screw extruder.

IDF content decreased in all cases after extrusion of crisp bread. A decrease was similar in Caraway and Vegetable crisp bread (approx. 25%) but less significant in Graham (approx. 10%).

SDF content was decreased after the extrusion of Vegetable crisp bread only, in the other samples it was increased after extrusion.

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Změny v charakteristice vlákniny potravy po extruzi obilovin

Ve vzorcích extrudovaného křehkého chleba a v surovinách pro jeho výrobu byly stanoveny obsahy celkové vlákniny potravy (TDF), nerozpustné vlákniny potravy (IDF) a její rozpustné části (SDF). Ke stanovení bylo použito přístroje Fibertec E (Tecator) a metody AOAC (985.29). V podstatě jde o enzymovou hydrolýzu škrobu (termostabilní α-amylsou a amyloglukosidasou) a bílkovin (proteasou) a následné gravimetrické stanovení obsahu TDF. Po enzymové hydrolýze se ke vzorku přidá čtyřnásobný objem etanolu (95 % obj.) ke sražení rozpustných složek vlákniny potravy a vzorek se přefiltruje. Zbytek na filtračním kelímku se promývá a suší a stanovuje se v něm popel a zbytkové bílkoviny. Hodnota TDF se vypočte podle následujícího vzorce: TDF = hmotnost zbytku – (hmotnost bílkovin ve zbytku + + hmotnost popela).

IDF se stanoví modifikovaným postupem: analyzovaný vzorek se po enzymové hydrolýze odfiltruje a zbytek na filtračním kelímku je vlastně IDF, která ještě obsahuje popel a zbytek bílkovin. Vzorek se dále zpracovává podle AOAC metody pro TDF. Pro stanovení SDF se k filtrátu, který obsahuje SDF, přidá etanol za účelem sražení rozpustných složek. Vzorek se potom opět přefitruje a dále se postupuje jako u metody pro stanovení TDF a IDF.

Vzorky pro stanovení byly získány z jednošnekového extruderu z běžné výroby firmy EXTRUDO, s.r.o. Bečice (křehký chléb kmínový, zeleninový a Graham) a z dvoušnekového extruderu v laboratoři ENSIA v Massy (Francie). Výsledky ukázaly, že obsah TDF nebyl významně změněn vlivem extruzního procesu s výjimkou vzorku s vysokým obsahem pšeničných otrub (chléb Graham) od firmy Extrudo (tab. I) a vzorku z pšeničné mouky z ENSIA Massy (tab. II). Přitom však obsah TDF ve vzorku s otrubami (ENSIA) se příliš nezměnil.

Obsahy IDF se ve všech případech při extruzi snižovaly, u křehkého chleba kmínového a zeleninového cca o 25 % a u Grahamu cca o 10 %. Obsahy SDF se po extruzi výrazně zvýšily u kmínového chleba, méně u Grahamu. U zeleninového křehkého chleba, který obsahuje určitý podíl kořenové zeleniny, se obsahy SDF snížily.

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Pro stanovení TDF byla zjišťována přesnost měření při osmkrát opakovaném stanovení. Pro zvolenou pravděpodobnost 99 % byla přesnost měření ± 0,3 % TDF. Pro stanovení SDF a IDF nebyla přesnost dosud stanovena, lze však předpokládat, že zejména u stanovení SDF bude chyba stanovení větší.

vláknina potravy; extruze; Fibretec E

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MIGRATION OF POLYETHYLENE TEREPHTHALATE OLIGOMERS FROM PACKAGING INTO FOOD SIMULANT LIQUIDS*

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Abstract: Phthalic acid isomers migrating into water simulants were determined after their isolation and silylation by gas chromatography. PET oligomers in water food simulants were hydrolysed by alkali, the released isomers of phthalic acid were quantified by the same procedure as above. To determine the PET oligomers in olive oil the simulant containing PET oligomers was hydrolysed in methanolic KOH solution, the liberated phthalic acid isomers were methylated and analysed by gas chromatography. The migration of phthalic acid isomers of analysed PET samples into water food simulants ranged from 0.9 to 4.7 μg/dm^2. The PET oligomers migration into water food simulants was in the range 0.10 to 0.81 mg/m^2, in the case of olive oil it was from 1.15 to 3.46 mg/dm^2.

packaging; plastics; polyethylene terephthalate; migration; oligomers

Polyethylene terephthalate (PET) is a polymer widely used in food packaging. Considering its generally low content of low molecular weight components, which are capable of migration into a package content, it is utilised for production of bottles for packaging of mineral water, soft drinks, wine and recently also spirits. Due to its excellent thermal stability up to 220 °C PET is suitable for packaging of conventional and/or microwave cooked foodstuffs in the form of thermoformed trays and dishes as well as films for oven roasting bags.

The largest group of substances transferring from PET into food consists of PET oligomers (Castle et al., 1989). These compounds are low molecular substances, from trimers up to nonamers. Shino (1979a, b) found that cyclic trimer forms about 81% of it, the rest contains mainly cyclic tetramer and pentamer, but traces of higher cyclic oligomers from hexamer up to

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nonamer were also proved in simulating liquids and/or foods after migration tests. Besides those cyclic oligomers small amounts of linear oligomers from dimers up to pentamers were found, but possibility of migration of higher homologues is not excluded (Castle et al., 1989; Begley et al., 1990; Begley, Hollifield, 1990). The amount of other migrants, i.e. acetaldehyde, ethylene glycol and terephthalic acid, etc., is much lower.

In our laboratory we systematically study the migration from food package materials (Kalačová et al., 1995; Voldřich et al., 1995). The aim of presented work was to evaluate the PET oligomers migration from several packaging materials used in the Czech Republic.

METHODS AND MATERIAL

Samples

The following packaging materials were tested:

i) Tairilin PET FCB4® (Nan Ya Plastic Corporation, Taiwan) PET virgin resin in granules used for bottle blowing,

ii) Stretchblown bottles (2 l) for carbonated beverages made of Tairilin PET FCB4® (TOMA s.r.o., Nehvizdy, Czech Republic),

iii) PET biaxial oriented film (thickness 420 μm) for tray production (Technoplast a.s., Chropyně, Czech Republic).

Determination of Migration of Terephthalic and Isophthalic Acid into Water Food Simulating Liquids

2 dm^2 of PET bottle wall or film cut to pieces 2 x 2 cm as well as 7.25 g of PET granulate (weight equivalent of 2 dm^2 of PET bottle wall) were refluxed in 100 ml of distilled water, 15% ethanol and 3% acetic acid for 60 minutes. The same samples were stored in distilled water for 10 days at 40 °C. After this treatment polymer materials were removed and the simulants were acidified to pH 2. The isomers of phthalic acid were extracted by diethyl ether (3 x 30 ml). Mixed extracts were reduced in volume by evaporation in a water bath under the Snyder column and then dried in a vacuum rotary evaporator at laboratory temperature. The residue was dissolved in 100 μl of dry pyridine and 100 μl of benzoic acid solution in pyridine (20 mg/ml) were added as the internal standard. After addition of 200 μl of BSTFA

(N,O-Bis(trimethylsilyl)-trifluoroacetamide) the phthalic isomers were silylated for 20 minutes at laboratory temperature. Then 1 µl of reaction mixture was injected into a gas chromatograph. Analysis conditions were as follows:

Chromatograph: capillary gas chromatograph HRGC 5300 (Fisions Instr., UK)

Detector: FID

Column: capillary column HP1 (Hewlett Packard, UK), length 50 m, internal diameter 0.25 mm

Chromatographic software: CSW (DataApex s.r.o., Czech Republic)

Carrier gas: nitrogen, flow rate 0.6 ml/min

Temperatures: injector port 250 °C
detector 280 °C
column 200–280 °C, 8 °C/min

Split ratio: 1 : 20.

Results were calculated using internal standard method.

Determination of PET Oligomers Migration into Water Simulants

Migration tests were carried out as described above. The simulant with released PET oligomers was evaporated using a vacuum rotary evaporator to volume 20 ml at 40 °C. Potassium hydroxide (5 g) was then added and sample was closed in flasks and stored at 95 °C for 48 hours with occasional shaking. Then the solution was neutralised using 15 ml of 6M sulphuric acid, 500 µl of benzoic acid in 0.01M KOH (0.1 mg/ml) were added as the internal standard and solution was extracted by 40 ml of diethyl ether. The organic phase was dried over sodium sulphate and evaporated in a vacuum rotary evaporator at laboratory temperature. The residue was dissolved in 100 µl of dry pyridine and the following process was as mentioned above.

Determination of PET Oligomers Migration into Olive Oil

The method according to Castle et al. (1989) was used. The samples of packaging materials (2 dm^2 or 7.25 g of granulate) were in contact with olive oil (100 ml) under following conditions: 10 days at 40 °C, 60 minutes at 100 °C, 121 °C, 150 °C and 175 °C. After migration test the aliquot of olive oil (6 g) along with 10 µl of benzoic acid in 0.01M aqueous potassium hydroxide (20 mg/ml) as an internal standard was hydrolysed with 0.2M potassium hydroxide in methanol (10 ml) at 65 °C for 5 hours. Then boron

trifluoride etherate was added (2 ml) and the solution was heated again at 65 °C for 5 hours. Then 40 ml of diethyl ether were added to the methylation mixture, sample was twice extracted by water (2 x 50 ml) and once by aqueous solution of sodium sulphate (50 ml of a half-saturated solution). The organic phase was then dried over sodium sulphate and the solvent was evaporated under vacuum. The residue was dissolved in acetonitrile (50 ml) and three times washed by hexane (3 x 50 ml). The acetonitrile phase was evaporated under vacuum, the rest was dissolved in 1 ml of diethyl ether and 1 µl of this solution was injected into gas chromatograph. The analysis conditions were as described for determination of migration of phthalic acid isomers into water food simulating liquids, only the temperature programme of the chromatograph oven was as follows: 150–250 °C, 8 °C/min.

RESULTS AND DISCUSSION

The results mentioned in this article are based on five parallel tests and data given in all tables are in the form $x \pm sd$, where x is an arithmetical mean and sd is a standard deviation.

The results of migration of terephthalic acid and isophthalic acid into water food simulating liquids are summarised in Tables I and II. The migration levels for monomers of terephthalic acid were found in the range 0.9 to 4.7 µg per dm^2, and for monomers of isophthalic acid from 0.2–0.7 µg/dm^2.

I. Migration of terephthalic acid and isophthalic acid into water food simulating liquids [µg.dm^{-2}] after 60 minutes at 100 °C

Sample	Simulant	Isophthalic acid	Terephthalic acid
PET granulate	distilled water	0.4 ± 0.2	0.9 ± 0.3
	15% ethanol	0.5 ± 0.3	3.6 ± 1.1
	3% acetic acid	0.5 ± 0.2	2.1 ± 1.5
PET bottle	distilled water	0.4 ± 0.1	2.0 ± 1.4
	15% ethanol	0.7 ± 0.2	4.7 ± 2.1
	3% acetic acid	0.4 ± 0.1	1.8 ± 0.9
PET film	distilled water	0.3 ± 0.2	1.1 ± 0.8
	15% ethanol	0.3 ± 0.2	2.1 ± 0.5
	3% acetic acid	0.2 ± 0.1	1.4 ± 0.8

II. Migration of terephthalic acid and isophthalic acid into distilled water [$\mu g.dm^{-2}$] after 10 days at 40 °C

Sample	Isophthalic acid	Terephthalic acid
PET granulate	0.4 ± 0.1	0.7 ± 0.3
PET bottle	0.4 ± 0.2	0.9 ± 0.3
PET film	0.2 ± 0.1	0.9 ± 0.3

These values are slightly higher than generally published data, when migration of terephthalic acid into food simulants was found at level < 2 $\mu g/dm^2$ for water, acetic acid and 15% ethanol, and about 3 $\mu g/dm^2$ for 50% ethanol and about 5 $\mu g/dm^2$ for olive oil (Tice, McGuinness, 1987; Steiner, 1990).

The levels of migration of PET oligomers into food simulants are given in Tables III and IV. It is obvious that under different test conditions the migration levels for distilled water changed from 0.20 to 0.41 mg/dm^2, for 15% ethanol from 0.62 to 0.81 mg/dm^2 and for 3% acetic acid in the range 0.44 to 0.72 mg/dm^2. As expected the migration into olive oil was much greater, i.e. from 1.15 to 1.98 mg/dm^2. Higher temperature caused an increase of PET oligomers transfer into olive oil so that after 1 hour at 175 °C migration levels up to 3.5 mg/dm^2 were found (Table V). Considering the obtained results they are in good agreement with published data on migration of PET oligomers into food simulants (Castle et al., 1989; Begley et al., 1990).

The method of determination of PET oligomers on the basis of their conversion in food simulant to the monomer terephthalic acid was found to be very useful for study of migration processes in PET packaging materials. It

III. Migration of PET oligomers into food simulating liquids [$mg.dm^{-2}$] after 60 min at 100 °C

Simulant	PET granulate	PET bottles	PET film
Distilled water	0.31 ± 0.10	0.41 ± 0.22	0.20 ± 0.15
15% ethanol	0.79 ± 0.21	0.81 ± 0.60	0.62 ± 0.22
3% acetic acid	0.44 ± 0.18	0.72 ± 0.40	0.71 ± 0.29
Olive oil	1.82 ± 0.93	1.98 ± 0.89	1.15 ± 1.05

IV. Migration of PET oligomers into distilled water and olive oil [mg.dm^{-2}] after 10 days 40 °C

Simulant	PET granulate	PET bottles	PET film
Distilled water	0.10 ± 0.62	0.32 ± 0.73	0.15 ± 0.31
Olive oil	1.94 ± 0.88	1.79 ± 0.48	1.83 ± 0.84

provides results precise enough for practical purposes and it is much simpler than chromatographic methods for individual oligomers monitoring.

Obtained results for PET oligomers migration are very close to the overall migration level that is characteristic of polyethylene terephthalate (Kalačová et al., 1995; Tice, McGuinness, 1987). It is confirmation of the fact that PET oligomers form the majority of substances migrating from the polymer into food during storage and/or handling of food products.

V. Migration of PET oligomers into olive oil [mg.dm^{-2}] after 60 minutes at different temperatures

Temperature [°C]	PET granulate	PET bottles	PET film
100	1.82 ± 0.93	1.98 ± 0.89	1.15 ± 1.05
121	1.95 ± 0.78	2.11 ± 1.14	1.35 ± 0.85
150	2.53 ± 1.13	3.02 ± 1.32	1.77 ± 0.97
175	3.32 ± 1.55	3.46 ± 1.81	2.11 ± 1.76

The obtained results confirmed the good thermal stability of PET packaging materials.

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Migrace oligomerů polyetylenteraftalátu z obalů do modelových roztoků potravin

Cílem práce bylo sledování specifické migrace izomerů kyseliny ftalové a oligomerů polyethylentereftalátu (PET) u obalů na bázi PET, tj. lahví, granulátu použitého pro jejich výrobu a biaxiálně orientované fólie určené pro výrobu tepelně tvarovaných misek, do potravin za použití modelových roztoků a jejich vzájemné porovnání. Migrace byla stanovována do destilované vody, 15% etanolu a 3% kyseliny octové jako vodných simulantů a do olivového oleje jako simulantu tučných potravin za podmínek doporučovaných směrnicemi Evropské unie.

Izomery kyseliny ftalové uvolněné do vodných simulantů byly stanoveny po izolaci a převedení na silylestery plynovou chromatografií. Oligomery PET po migraci do vodných modelových roztoků byly hydrolyzovány v alkalickém prostředí a uvolněné izomery kyseliny ftalové byly analyzovány postupem uvedeným výše. Oligomery PET v olivovém oleji byly hydrolyzovány v alkalickém prostředí za

přítomnosti metanolu, uvolněné izomery kyseliny ftalové byly převedeny na metylestery, izolovány a stanoveny plynovou chromatografií.

Nalezené hodnoty migrace izomerů kyseliny ftalové do vodných simulantů se pohybovaly v rozmezí 0,9–4,7 μg/dm^2, u oligomerů PET byla zjištěna migrace do vodných simulantů v rozmezí 0,10–0,81 mg/dm^2 a do olivového oleje v rozmezí 1,15–3,46 mg/dm^2.

obaly; plasty; polyetylentereftalát; migrace; oligomery

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Potrav. Vědy, *14*, 1996 (1) : 33–41

FILTRATION OF FINE PARTICLES FROM DENSE SUGAR SOLUTIONS*

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Abstract: The work deals with filtration of fine particles from dense sugar solutions which are further used for crystallization of white sugar. Use of continuous thickening filters with the continuous creation of filter cake from fine particles of calcium carbonate belongs to new design of this filtration operation. The results of application in industrial operations are complemented with laboratory investigations the properties of filtration cake and the ability of precipitated particles to create the filter cake.

filtration of sugar solutions; sugar technology; fine particles; thickening filters

Filtration of Dense Sugar Solutions in Sugar Manufacture

Filtration of dense sugar solutions before crystallization is a significant operation of sugar technology. The perfect separation of insoluble particles is a condition for the high quality of the final product and requires efficient filtration devices including subsidiary filtration materials.

Different types of filters are currently being used for industrial filtration of concentrated sugar solutions. Especially the following ones belong to them: automated filter presses (Muchin, 1986; Twaite et al., 1983), disc flooding filters, candle filters (Junker, 1984), low pressure strainers and some others (Schult, Schoppe, 1981; Bubník et al., 1989). Kieselguhr is commonly used as a filtration aid, often combined with adsorption decolorizing material, e.g. activated carbon. Great demands are imposed on effectivenes even on the operation of the filtration station due to the high concentration of filtered solutions (60–75% dry substance), their high viscosity and view to fine colloids difficult to separate. Similar high requirements on filtration of dense sugar solutions are posed in processes with

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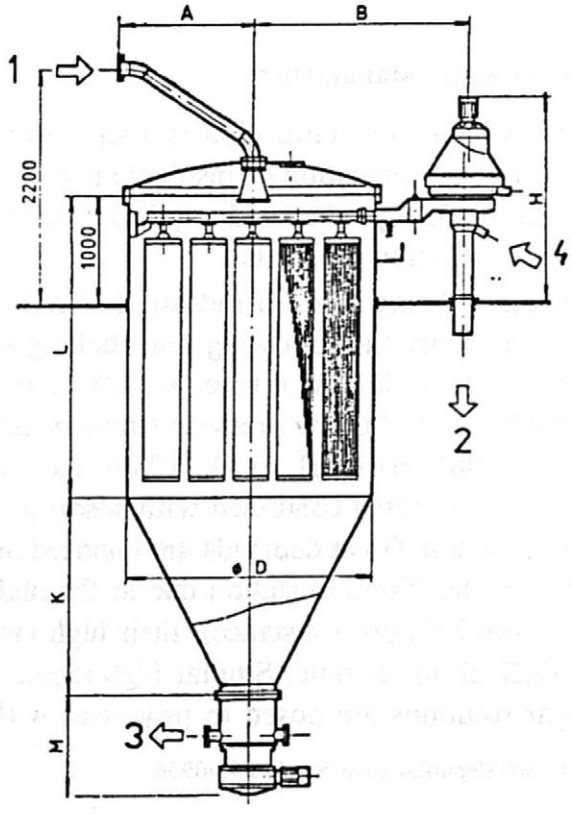
decolorization by ion exchange. If technical sugar solutions are used for modern industrial chromatographic separation (e.g. system Simulated Moving Bed), next filtration degree must follow which is realized mostly by means of ceramic membranes.

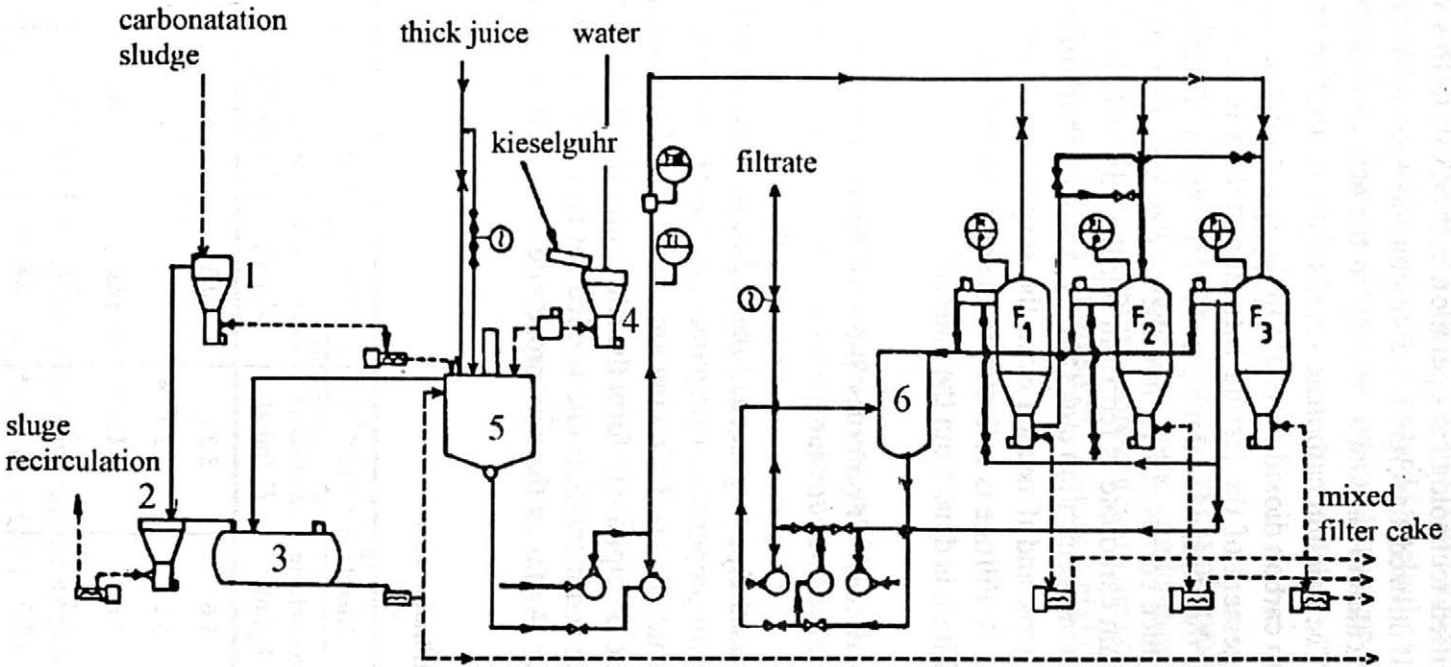
Design of a New Filtration Procedure

One of the new ways presented in this work is utilization of continuous thickening filters commonly used in sugar production and other technologies. These devices are used especially as the first degree of continuous filtration of sugar juices after carbonatation, i.e. juices containing less than 20% dry substance. 4–5% of these juices are represented by suspension of calcium carbonate and precipitated and adsorbed impurities. The tested continuous filters KZF-50 were manufactured by ZVU Hradec Králové, a.s. (Czech Republic) and they are illustrated in Fig. 1. They are usual products with the filtration area 50 m^2, their construction and function is well known from the literature.



1 - juice input
2 - filtrate output
3 - mixed filter cake output
4 - reverse washing

1. Filter KZF 50



F1, F2 and F3 – filters KZF 50; 2 – carbonatation sludge recirculation; 3 – suspension reservoir; 4 – homogenization tank; 5 – mixing tank; 6 – filtrate collector

2. Filtration station

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Another modified technological operation carried out in this work is replacement of still utilized kieselguhr. Continuous creation of filter cake from fine particles of calcium carbonate belongs to the new design of this filtration operation. The calcium carbonate results from controlled precipitation of lime milk with carbon dioxide directly in the sugar solution.

All the arrangement of the testing filtration station is shown in Fig. 2. The basic part of the station are three pieces of thickening filters KZF-50 (F_1 to F_3). Filtered juice comes at first into the mixing tank (No. 5) where suspension of calcium carbonate is fed from purification. It is previously concentrated in the homogenization tank (No. 1). Activated carbon is also added into the mixing tank and if needed even the suspension of kieselguhr prepared in tank No. 4. Filtrate is collected in the collector (No. 6). The reverse washing of the filters is done from the collector.

Laboratory Experiments – Properties Study of Filtered Solutions and of Utilized Carbonatation Sludge

Laboratory measurements preceded the full-scale experiments. In the experiments filtration properties of prepared suspensions and used carbonatation mud were studied. The following are considered: filtration rate, ability of used carbonate precipitate to form the filtration cake of necessary properties and the cake sedimentation rate needed for successful operation of the thickening filters. As far as the new procedure is concerned, all the labora-

I. Properties of the filtration cake

Weight m_k [g]	Kieselguhr CELIT		Carbonatation cake		K ratio
	cake volume V_k [ml]	coefficient K_c [ml/g]	cake volume V_k [ml]	coefficient K_k [ml/g]	K_c/K_k
0.50	1.6	3.2	0.6	1.2	2.7
1.00	3.2	3.2	1.3	1.3	2.5
1.50	4.6	3.1	2.0	1.3	2.3
2.00	6.3	3.2	2.2	1.1	2.9
2.50	7.6	3.0	2.8	1.1	2.7
3.00	9.2	3.1	3.2	1.1	2.9

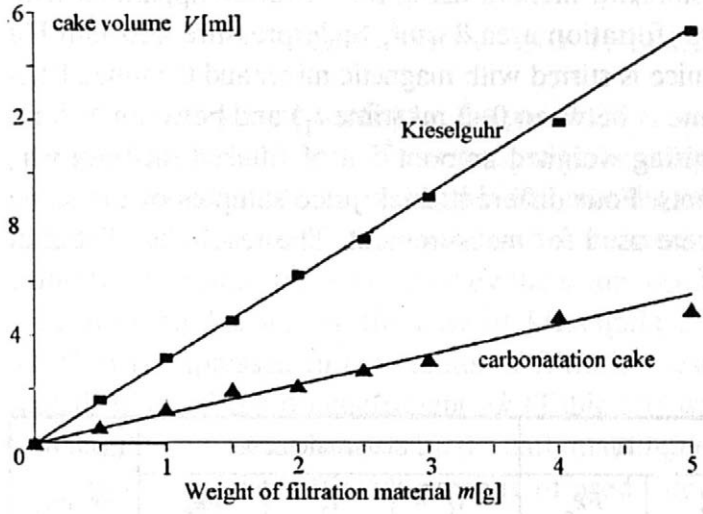
tory experiments were carried out in parallel with application of the carbonatation sludge as well as with the standard kieselguhr application, aimed at comparing them. The conditions modelling the sugar factory operation were selected for measurements.

Ability of Formation of Filtration Cake

The ability of sludge to form the filtration cake is of crucial importance for its final properties (especially porosity) in the application in the process of filtration. We may define this ability represented by the coefficient K [ml/g] as volume of the cake V_k [ml] formed by the unit weight (m_k = 1 g) of the filtration material:

$$K = V_k / m_k \qquad [1]$$

The sludge from the 2nd carbonatation was used for measurements, which is an almost pure precipitate of calcium carbonate. The experiments run at the temperature 80 °C with the sample of thick juice of the dry substance content 56.7%. Their results are summarized in Table I and Fig. 3.



3. Ability to form the filter cake

Sedimentation Ability of the Filtration Cake

Sedimentation ability of the filtration cake and/or its parts is important for control of the filtration cycles of the thickening filter. This ability was measured by means of the determination of sedimentation rate of the different

II. Sedimentation rates of filtration cake (cake sedimentation)

Cake weight [g]	0.5	1.0	1.5	2.0	2.5
Kieselguhr [m/s]	0.075	0.072	0.078	0.073	0.083
Carbonatation sludge [m/s]	0.081	0.083	0.081	0.074	0.089

parts of the filtration cake weighing from 0.5 to 2.5 g in the sample of the thick juice of dry substance 56.7% and temperature 80 °C. The results are summarized in Table II.

Filtration Properties of Thick Juice Suspension

Filtration properties of juices and suspensions in sugar analyses are expressed by means of so called Filtration coefficient *Fk* which is defined by the relation:

$$Fk = 0.5\ (t_1 - t_2) \qquad [2]$$

where t_1 and t_2 are times needed for getting a certain volume of filtrate

Fk is measured by a standard method using the filtration apparatus with the following parameters: filtration area 2 cm^2, underpressure 400 mm Hg and temperature 65 °C. Juice is stirred with magnetic mixer and the time of filtration of the juice volume is between 0–2 ml (time t_1) and between 4–6 ml (time t_2). The upper limiting weighed amount 5 g of filtered medium was chosen for the experiments. Four different thick juice samples of the same dry substance content were used for measurement. The results are listed in Table III.

III. Filtration rate

Juice	Kieselguhr CELIT			Carbonatation cake			Ratio *Fk*
	t_1	t_2	Fk_c	t_1	t_2	Fk_k	Fk_k/Fk_c
1	18.0	30.2	6.1	39.4	71.8	16.2	2.7
2	22.8	35.4	6.3	55.0	90.5	17.8	2.8
3	22.2	35.8	6.8	47.2	83.1	18.0	2.6
4	25.5	41.3	6.9	57.3	105.2	23.9	3.0

Full scale Experiments – Evaluation of Operation of the Testing Filtration Station

All weight and concentration data allowing the complete weight balance were continuously observed during evaluation of the filtration station (Fig. 2). Values characterizing quality of filtered solutions and their filtration properties were further observed. Parameters of the filtration station as to its energy consumption, control, regulation and personnel requirements were also investigated.

Filtered juice temperature ranged between 76 and 80 °C, temperature decrease in the filtration station was about 10–15 °C, juice dry substance varied between 56 and 64%. Carbonatation cake addition was chosen in the amount so the final insoluble dry substance content in juice ranged from 2 to 5%. Because of the high color of the juices activated carbon has been added.

The station performance has been maintained in the continuous run always for several days when the station output varied between 10 and 15 m^3/h at the filtration pressure up to 45 kPa. When the pressure exceeded this value the output dropped sharply (even to 3 m^3/h) and the filter has been put out of operation and regenerated.

DISCUSSION AND RESULTS

The laboratory tests confirmed the possibility to utilize the filtration properties of carbonatation cake from the 2nd carbonatation for filtration of thick juice. Compared to the so far used kieselguhr the carbonatation cake shows the same sedimentation rate (Table II). It is important for utilization of thickening filters. Cake volume formed by the same weight of kieselguhr and carbonatation sludge was in the case of kieselguhr 3 times higher (Table I), which was expressed in the results of filtration tests. Filtration rate represented by the filtration coefficient *Fk* (Table III) was in the observed juice samples 2.6–3.0 times lower with the carbonatation cake compared to kieselguhr. In view of the sufficient capacity of used filters, this filtration rate decrease is not a limiting factor.

The results of full-scale experiments in the sugar factory proved in the tests of continuous thickening filters with carbonatation cake from the 2nd carbonatation, a possibility to apply this procedure. The main requirement, removing of fine dispersed particles before crystallization was fulfilled. The

obtained filtrate was clear. Compared to using kieselguhr the filtration cycle got shorter as it was expected. To keep the filtration rate it was necessary to increase more the filtration pressure even to 100 kPa. From the initial average filtration output 15 m^3/h this value got as low as 3–5 m^3/h when it was necessary to regenerate the filter. Due to the juice delay in the device for 2–7 h it is necessary to maintain its good thermostability. Otherwise increased content of colorants appears. When using the carbonatation cake it is also required to evaluate regularly its filtration properties and to keep the standard way of precipitation in the purification.

The application of described filtration procedure will depend in individual cases on the equipment and capacity of the concerned filtration station and further on the economic profit from replacement of kieselguhr with waste lime cake.

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Filtrace jemných částic z hustých cukerných roztoků

Práce se zabývá filtrací jemných částic z hustých cukerných roztoků, které se dále používají ke krystalizaci bílého cukru. Dokonalá separace nerozpustných částic je podmínkou pro dosažení vysoké kvality produktu a vyžaduje náročná a účinná filtrační zařízení včetně pomocných filtračních materiálů. Jedním z nových řešení, prezentovaném v této práci, je využití kontinuálních zahušťovacích filtrů běžně

používaných při výrobě cukru i v jiných technologiích. K novému řešení této filtrační operace patří i kontinuální vytváření filtračního koláče z jemných částic uhličitanu vápenatého, tvořeného řízeným srážením hydroxidu vápenatého oxidem uhličitým přímo v cukerném roztoku. Je popsán návrh a možnosti aplikace uvedeného filtračního procesu v provozních podmínkách. Výsledky jsou doplněny laboratorními výzkumy vlastností filtračního koláče a schopností srážených částic uhličitanu tvořit filtrační koláč potřebných vlastností.

filtrace cukerných roztoků; cukerné technologie; jemné částice; zahušťovací filtry

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s Hungarian Central Food Research Institute – KEKI

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uspořádá

ve dnech 11. až 13. září 1996 v Budapešti sympozium

FOOD PACKAGING

Ensuring the Safety and Quality of Foods

Program sympozia věnovaný širokému spektru problému týkajících se balení potravin bude rozdělen do těchto tematických celků:

- Balení – vliv na zlepšení kvality potravin
- Novinky v balení potravin – modifikovaná atmosféra, poživatelné, biodegradovatelné a recyklovatelné obaly, atd.
- Fyzikální transportní procesy v balení
  - a) difúze plynů
  - b) pronikání par organických látek
  - c) migrace složek do potravin
  - d) povrchové děje
- Vliv obalu na organoleptické vlastnosti potravin
- Funkční bariéry – teoretické podklady a způsoby zkoušení
- Použití vratných a recyklovaných obalů – hygienické aspekty, důsledky pro kvalitu potravin
- Migrace složek obalů do potravin během záhřevů
- Alternativní metody zkoušení a nové analytické postupy
- Aktivní balení

Uzávěrka přihlášek referátů a posterů je 31. ledna 1996.

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Potrav. Vědy, *14*, 1996 (1) : 43–54

EFFECT OF SOAKING AND COOKING ON THE MECHANICAL PROPERTIES AND SENSORY TEXTURE OF LENTILS*

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Abstract: The effects of the soaking time (1 h, 2 h, 3 h) and the cooking time (15 min, 30 min, 45 min) on the weight increase, mechanical properties and sensory texture of Canadian green lentils were determined. Both the soaking and the cooking contributed to the weight increase, but the concentration of salt during cooking (0%, 1%, 2%) had no importance. The colour, the odour and the flavour were not affected by soaking and cooking conditions. Mechanical properties (Instron measurements) depended on the weight increase, but the regressions were strictly nonlinear. The results obtained at the compression of 20% were linearly related to the sensory hardness, while those corresponding to the 40% compression were linearly related to the disintegration during chewing. In both case, individual seeds differed in their hardness so that the Instron measurements had to be repeated 19 up to 33 times in order to improve the reproducibility.

lentils; soaking; cooking; hardness; texture

The texture of legumes is usually rather hard so that proper soaking and sufficient cooking time are necessary (Bhatty, 1988). In the literature, the addition of salt at the end of boiling is recommended to improve the texture of cooked legumes. In the case of lentils, the seed coat contains outer palisade and inner parenchyma layers like other legumes, but the subepidermal layer is formed by hourglass cells, particularly surrounding the hilum, and the entire seed coat of lentils is thinner than seed coats of most other legumes (Hughes, Swanson, 1986). The surface of seed coat is uneven with conical papillae. The unique structural characteristics may contribute to the decreased hardness of lentils.

* The project within the frame of the programme CIPA-CT92-4020, and partially supported by the Grant Agency of the Czech Republic, No. 101/93/0625.

The soaking reduces the cooking time in the case of both beans and lentils. The soaking in sodium bicarbonate solution reduced the necessary cooking time for lentils, but increased it for faba beans (Singh et al., 1988). Dehulling reduced the cooking time of lentils to a greater extent than soaking. The soaking in water, and to a greater extent, in 4% salt solution – for the study of texture, lentils were soaked in deionized water (Bhatty et al., 1983) - increased the hydration coefficient, seed weight, protein solubility, *in vitro* digestibility, and reduced the cooking time as well (Abou-Samaha et al., 1985). Lentil starch has high water binding activity because of high relative content of amylose (Schoch, Maywald, 1968).

The cooking quality was influenced by high levels of mineral nutrients, especially sodium and potassium ions in the seed (Wassimi et al., 1978). Phytic acid seems to have great effect on the cooking quality of lentils (Bhatty, 1989) by binding polyvalent metal ions, however, the phytic acid phosphorus was not correlated with cookability (El-Said, El-Shirbeeny, 1981). Perhaps, the available phosphate, produced by hydrolysis of phytic acid, might improve the cooking quality of lentils (Bhatty, Slinkard, 1989). Calcium and magnesium ions released from phytates are bound to pectins in the cell wall, and the resulting bond can bind cells together, increasing thus the hardness (Vindiola et al., 1986). These factors should be further studied.

We were interested in the effect of soaking and cooking time of lentils on mechanical properties of cooked product, and on the effect of mechanical hardness on the sensory texture of cooked lentils. Shorter soaking time resulted in lower content of imbibed water, and thus, longer cooking time was necessary to achieve proper texture (Antunes, Sgarbieri, 1979).

MATERIAL AND METHODS

Lentils were a commercial product with large green grains (First quality), packed by Senguar® Co. in December 1994. The composition (g/kg dry weight): protein 235, carbohydrates 580, fat 22, ash 25, dietary fibre 39.

Determination of mechanical properties

The apparatus INSTRON 1140 was used, equipped with the HP-85B unit. The geometry of grain compression between two parallel disks (diameter 48 mm) was used. The rate of movement of the upper disk was 50 mm/min,

while the lower disk was not moving. The upper (mobile) disk was connected to the force measuring device. The initial distance between the two disks was 8 mm. The force necessary for compression by 20% and 40% of the initial height of sample, respectively, was measured. The height of non-compressed sample was measured at the same time (indicated by an increase of force from 0 to 1% scale), using the intervals of 50 N, 100 N, and exceptionally, 200 N, respectively.

Sensory evaluation

Sensory characteristics were determined under conditions specified by the international standard (ISO, 1985) in standard test booths (ISO, 1988). The panel of sensory assessors consisted of persons trained according to the international standard (ISO, 1987) with the experience of at least 6 months in texture evaluation; their immediate fitness to assess was tested every day. They were instructed on the type of method used, and they were trained with use of the particular procedure in at least 5 sessions. Unstructured graphical scales (100 mm long) were used (ISO, 1978), whose orientation was defined by verbal description at the two ends of the scale. The following characteristics were evaluated: colour acceptance (0% = excellent, 100% = rather bad), odour acceptance, and flavour acceptance (the same hedonic descriptions). The texture was evaluated using intensity scales: hardness (0% = very soft, 100% = very hard) and disintegration of the sample during chewing (0% = easily, 100% = only with difficulty).

Sociological study

The acceptability of legumes was tested among college students, using preprinted questionnaires. Respondents had to rank peas, beans and lentils according to the acceptability. From the total of 415 respondents, 20 did not consume legumes and 19 gave erroneous responses (Ms M. Novosadová collaborated in the survey).

The statistical softwares Excell and STATISTICA were used. The probability level was $P = 0.95$. Linear correlation coefficients are expressed as r.

Procedure

Samples of lentils (100 g) were soaked in 300 ml water for 1 h, 2 h, or 3 h, respectively. The soaked samples were cooked in water containing 0 g, 10 g

or 20 g sodium chloride per kg of dry lentils, respectively, for 15 min, 30 min or 45 min. The weight increases were determined both after soaking and after cooking.

RESULTS

Results on the acceptability of legumes are shown in Table I (the number of responses: $N = 376$). Cooking experiments were performed in the duplicate under identical conditions. The determination of INSTRON hardness, measuring the minimum of 19 separate seeds, and the sensory assessment was based on 12–18 responses. The results on weight changes, INSTRON

I. Preference of legumes among students

Ranking	Peas	Beans	Lentils
1	68	116	192
2	132	133	111
3	176	127	73
Sum	860	763	633

and sensory analyses are summarized in Tables II and III, respectively. The respective standard deviations are given in Table IV and Table V for instrumental and sensory measurements, respectively (average values are given in the tables, i.e. dependence of standard deviations on the soaking time is the mean for all cooking times, and dependence of standard deviations on the cooking time is the mean for all soaking times investigated). They are nearly the same in all cases.

DISCUSSION

The consumption of legumes is very low in the Czech Republic, which is not desirable from the standpoint of protein consumption and diversification of the diet. Therefore, the acceptability of legumes was studied in the population of students who belong to the most progressive groups of consumers. Lentils were found the most perspective legume (Table I). Therefore, their changes during cooking were studied.

II. Effect of soaking and cooking conditions on the weight increase and mechanical properties of lentils

Salt content [%]	Soaking time [h]	Cooking time [min]	Weight increase [%]	Compression by		Height *h* [mm]
				20 % (N)	40 % (N)	
0	1	15	84	12.1	43.0	3.0
		30	112	11.8	36.7	3.1
		45	125	7.7	12.5	3.4
	2	15	111	8.1	17.0	3.4
		30	134	5.6	12.0	3.5
		45	144	4.0	6.9	3.7
	3	15	116	6.4	13.1	3.6
		30	140	5.3	8.2	3.6
		45	156	4.0	6.4	3.6
1	1	15	82	11.2	35.4	3.1
		30	122	9.1	24.5	3.0
		45	160	9.0	13.6	3.3
	2	15	97	9.0	19.6	3.3
		30	140	10.0	19.3	3.3
		45	159	5.3	8.3	3.5
	3	15	118	5.4	10.9	3.5
		30	146	3.2	6.9	3.6
		45	167	2.8	5.7	3.5
2	1	15	84	10.3	31.6	3.2
		30	124	8.0	19.0	3.2
		45	160	5.9	8.4	3.4
	2	15	102	10.0	19.9	3.2
		30	142	4.4	10.3	3.5
		45	152	4.4	10.3	3.5
	3	15	113	7.5	18.2	3.3
		30	144	3.9	7.6	3.6
		45	169	2.9	5.3	3.7

III. Effect of soaking and cooking on sensory characteristics of lentils

Salt content [%]	Soaking time [h]	Cooking time [min]	Acceptability [%]			Hardness [%]	Chewing [%]
			colour	odour	flavour		
0	1	15	41	40	45	63	64
		30	46	36	42	53	46
		45	39	41	42	30	26
	2	15	35	34	36	45	41
		30	35	30	28	37	30
		45	36	31	40	37	29
	3	15	38	33	36	49	47
		30	35	34	35	35	30
		45	34	28	31	26	20
1	1	15	42	29	55	71	71
		30	29	23	39	50	46
		45	32	27	31	24	18
	2	15	31	31	49	66	61
		30	33	23	35	28	27
		45	22	26	26	37	17
	3	15	32	25	39	54	47
		30	32	23	23	34	24
		45	31	22	32	20	12
2	1	15	41	30	43	65	64
		30	35	31	33	53	49
		45	33	26	30	30	25
	2	15	32	29	42	64	63
		30	30	25	28	36	30
		45	28	26	29	33	28
	3	15	30	31	35	48	44
		30	31	26	27	35	31
		45	30	23	23	17	13

The soaking and cooking times of green Canadian legumes were relatively short, compared with older brown varieties produced in this country. Lentils increased in weight by 84–169% during the relatively short soaking and

IV. Coefficient of variation in the determination of mechanical properties of cooked lentils

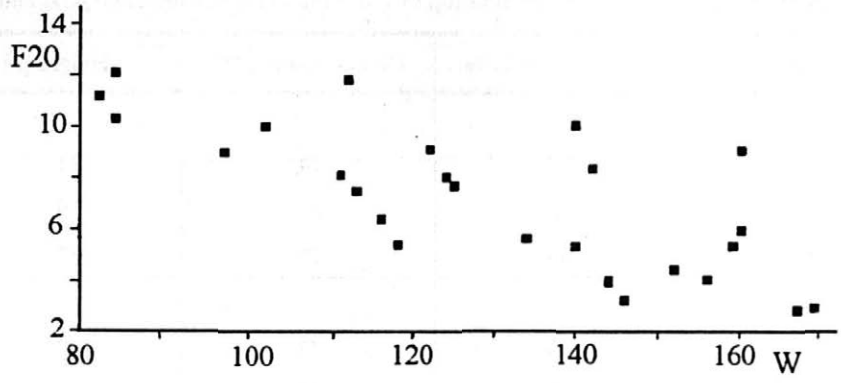
Conditions	Compression 20%	Compression 40%	Height [h]
Soaking			
1 hour	50	74	11
2 hours	55	81	9
3 hours	63	72	10
Cooking			
15 min	40	72	8
30 min	54	82	11
45 min	80	72	10

V. Standard deviations of means of sensory assesments

Conditions	Colour	Odour	Flavour	Hardness	Chewing
Soaking					
1 hour	3	3	3	3	3
2 hours	3	3	3	3	4
3 hours	3	3	3	3	3
Cooking					
15 min	3	3	4	3	4
30 min	3	3	3	3	4
45 min	3	3	3	3	3

VI. Correlation coefficients between the mechanical and sensory characteristics

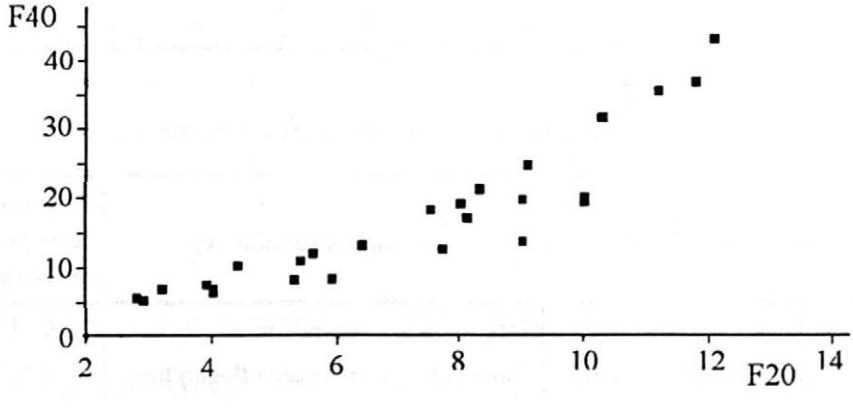
Dependent variable (y)	Independent variable (x)	Linear correlation coefficient (r)
Sensory hardness	force at 20% compression	0.717
Resistance on chewing (logarithm)	force at 20% compression (logarithm)	0.727
Sensory hardness	force at 40% compression (logarithm)	0.800
Resistance on chewing	force at 40% compression (logarithm)	0.805



F20 = the force needed for the compression by 20% (N) ; W = the weight increase (%)

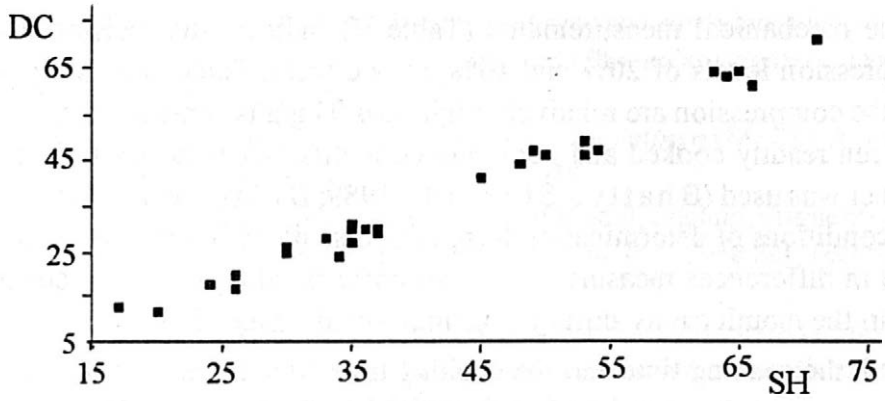
1. Relation between the weight increase and the force needed for the 20% compression

cooking (Table II), which is in agreement with the data reported for lentils (135%) in the literature (Pequignot et al., 1975). As expected, the conditions of soaking and cooking had no effect on the colour; the odour and on the flavour of cooked lentils (Table III). Surprisingly, the concentration of salt during the cooking was found unimportant (at least in the range of 1–2%), contrary to peoples experience. Modern readily cooking varieties are not probably influenced by lower concentrations of sodium chloride during cooking. Higher salt concentrations would perhaps have more effect (Abou-Samaha et al., 1985), but they would not be desirable because of their unfavourable effect on blood pressure.



F40 = force needed for the 40% compression [N]; F20 = force needed for the 20% compression [N]

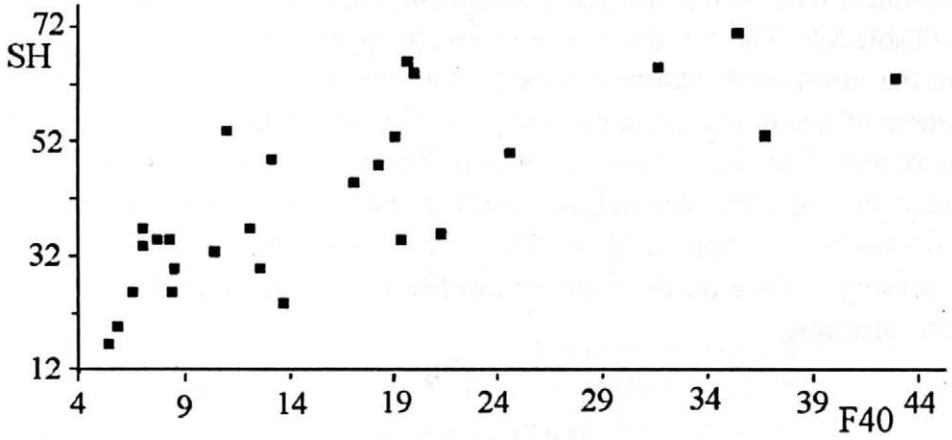
2. Relation between the force needed for the 20% and for the 40% compression



DC = the disintegration during chewing (% of the graphical scale); SH = the sensory hardness (% of the graphical scale)

3. Relation between the sensory hardness and the resistance towards disintegration of chewing

High variability was found in the Instron measurements of hardness (Table IV) if single seeds were measured. Therefore, the analysis had to be repeated 19 up to 33 times. The reproducibility of sensory assessment was better (Table V) as the morsel contained many individual lentil seeds. Nevertheless, the analysis was based on 16–18 responses in order to eliminate the individual variability in response.



SH = the sensory hardness (% of the graphical scale); F40 = the force needed for the 40% compression [N]

4. Relation between the sensory hardness and the force needed for the 40% compression

The mechanical measurements (Table II) indicate the resistance at the compression levels of 20% and 40%, respectively. Forces necessary to obtain the compression are relatively high, as 4.0 kg/g is considered as the limit between readily cooked and poor cooked lentils, when the shear force after Kramer was used (Bhatty, Sinkard, 1989; Bhatty et al., 1983, 1984). The conditions of determination were of course not identical. We were interested in differences measured at values corresponding to real forces occurring in the mouth cavity during consumption of cooked lentils.

Both the soaking time and the cooking time were important. The cooking time was linearly correlated with weight increase ($r = 0.863$), with decreased sensory hardness ($r = -0.856$) and the disintegration at chewing ($r = -0.852$). The linear correlations were found between the weight increase and mechanical properties (as determined with use of Instron), as they were $r = -0.759$ and $r = -0.831$ for the 20% and 40% compressions, respectively. The relation between the weight increase and the mechanical hardness is shown for the force at the 40% compression (Fig. 1). The regression slightly deviated from the linearity. Values obtained at the 40% compression were in no simple relation with those obtained at the 20% compression (Fig. 2), even when the correlation coefficient was very high ($r = 0.912$).

Values from the two methods of sensory texture assessment were closer to linearity ($r = 0.992$), as shown in Fig. 3. Values obtained by the estimation of hardness were in linear regression to values obtained by chewing the sample (Table VI). The sensory hardness was in approximately linear regression with the mechanical hardness measured at the 20% compression, while the easiness of disintegration at chewing was in a linear correlation with the values obtained at the 40% compression. The regression between the force needed for the 40% compression and the sensory hardness showed deviations from the linearity (Fig. 4). The results show the possibility to express the sensory texture on the basis of mechanical measurement, using the Instron apparatus.

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Vliv máčení a vaření na mechanické vlastnosti a senzorickou texturu čočky

Kanadská zelená čočka byla zkoumána z hlediska změn během namáčení a vaření. Byly zvoleny doby máčení 1, 2 a 3 hodiny a vaření 15, 30 a 45 min. Při vaření byl stanovován také vliv koncentrace chloridu sodného (0, 1 a 2 %).

Byl zjišťován přírůstek hmotnosti, která závisela na době máčení i na době vaření, ale nezávisela na obsahu soli ve vodě při vaření. Podle očekávání neměly podmínky máčení a vaření vliv na barvu a vůni uvařené čočky, ale překvapivě se neprojevily ani na chuti, u níž nebyl průkazný ani vliv obsahu soli. Při stanovení mechanické pevnosti na přístroji Instron byla stanovena síla potřebná ke stlačení jediného zrna o 20 a 40 %. Vzhledem k velké variabilitě pevnosti jednotlivých zrn bylo nutné analýzu opakovat až 33krát, aby se získaly použitelné výsledky.

Aby se eliminoval vliv individuální variability hodnotitelů, byla senzorická analýza opakována 12–18krát. Mechanická pevnost, vyjádřená jako síla potřebná pro stlačení o 20 %, nebyla v jednoduchém vztahu se silou potřebnou pro stlačení o 40 %. Obě tyto síly značně závisely na podmínkách máčení a vaření, ale závislost na změně hmotnosti nebyla zcela lineární a byla zatížena značnou variabilitou. Senzorická textura byla hodnocena s použití nestrukturovaných grafických stupnic. Byla hodnocena jednak tvrdost, jednak rozpadavost sousta při žvýkání. U první hodnoty byly pozorovány odchylky od linearity při stanovení závislosti na přírůstku hmotnosti, kdežto u rozpadavosti při žvýkání byla závislost skoro úplně lineární. Tvrdost stanovená senzoricky byla v lineárním vztahu k síle potřebné pro stlačení o 20 %, nikoli však při stlačení o 40 %. Rozpadavost při žvýkání byla naopak v lineárním vztahu se silou potřebnou pro stlačení o 40 %, nikoli však se silou potřebnou pro stlačení o 20 %. Přes značnou variabilitu se ukázalo, že existují průkazné vztahy mezi mechanickými vlastnostmi zjištěnými na přístroji Instron a texturou zjištěnou senzorickou analýzou.

čočka; máčení; vaření; tvrdost; textura

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DETERMINATION OF 1,4-DIHYDROPYRIDINE ANTIOXIDANTS IN EDIBLE OILS*

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Abstract: The 1,4-dihydropyridine antioxidants diludine (2,6-dimethyl-3,5--diethoxy-1,4-dihydropyridine) and cyclohexyldiludine (2,6-dimethyl-3,5-di--menthyl-1,4-dihydropyridine) were determined in edible oils. They were extracted with 90% aqueous methanol (diludine) or 95% aqueous methanol (cyclohexyldiludine) from refined rapeseed oil with fair yield. The two 1,4-dihydropyridines can be determined in the extract with use of reversed-phase high-performance liquid chromatography (HPLC) with the UV detection at 230 nm or with higher sensitivity at 370 nm or still several times higher sensitivity with an electrochemical (EC) detector at 0.9 V using a graphite cell. Ascorbyl palmitate and tocopherols give no response while tert. butyl hydroxyanisol (BHA) and di-tert. butyl hydroxy toluene (BHT) are co-eluted. The repeatability is satisfactory.

diludine; cyclohexyldiludine; antioxidants; rapeseed oil; HPLC; electrochemical detection; 1,4-dihydropyridines

A series of 1,4-dihydropyridine derivatives, related to nicotinic acid, were synthetized, possessing pronounced hydrogen-donating properties (Duburs et al., 1970). They were found active in biological membranes (Dubur et al., 1975), acting both as free radical inhibitors (Tirzit, Dubur, 1972) and as singlet oxygen deactivators (Tirzit et al., 1981). They inhibited the autoxidation of cholesterol (Tirzit et al., 1978). They were efficient as synergists of α-tocopherol in methyl oleate (Tirzit, Dubur, 1977; Tirzit et al., 1983). The antioxidant activities of different 3,5-dicarbonyl derivatives of 2,6-dimethyl-1,4-dihydropyridines were compared in methyl oleate at the concentrations of 0.75 and 3.0 mmol/l under free access of oxygen (Tirzitis et al., 1988).

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Diludine (2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine) is used as an antioxidant in pharmaceutical preparations and for the stabilization of carotene. We have observed (Kouřimská et al., 1993; Farníková et al., 1995) efficient inhibition of polyunsaturated oils with diludine and cyclohexyldiludine. The latter derivative, better soluble in edible oils, was found satisfactorily active (Farníková et al., 1995) in rapeseed and sunflowerseed oils.

For the application of 1,4-dihydropyridine derivatives in foods and pharmaceutical preparations, it is necessary to develop a suitable analytical method. Therefore, we tested the application of HPLC provided with a UV or an EC detector.

MATERIAL AND METHODS

The 1,4-dihydropyridine derivatives (diludine and cyclohexyldiludine) were synthetized in the Institute of Organic Syntheses, Riga, Latvia. Ascorbyl palmitate, BHA, BHT (Koch-Light), and α-tocopherol (Slovakofarma, Hlohovec, Slovakia) were used. Commercially refined rapeseed oil had the acid (AV = 0.05) and peroxide (PV = 1.0 mval/kg) values conform with the requirement, and had good sensory value (acceptability rating: 60% of the graphical scale).

Conditions of the HPLC analysis: The apparatus HP 1050 (Hewlett-Packard), provided with an isocratic pump and the automatic sampler were used; column: HP 200 mm x 4.6 mm, packed with Hypersil ODS (C_{18}) – 5 μm; detectors: UV detector, measuring at 230 nm and 370 nm; electrochemical detector (EC) HP 1094A, provided with a graphite electrode, operation potential 0.9 V – range 0.2–1.2 V; mobile phase for the UV detector: methanol–water 900 : 100; mobile phase for the electrochemical detector: methanol–water 900 : 100, containing sodium acetate (50 mmol/l) and sodium chloride (5 mmol/l); flow rate: 1 ml/min; room temperature; injected sample: 10 μl.

RESULTS AND DISCUSSION

Extraction of Antioxidants

The antioxidants were dissolved in fresh refined rapeseed oil to produce 0.02% solutions. Water, aqueous methanol and anhydrous methanol were

used as extraction solvents. The sample (10 g) was dissolved in 60 ml of light petroleum (boiling interval: 40–60 °C), and extracted three times: twice with 20ml portions, and the once using 10 ml of solvent. The lower layer was filtered through a paper filter into a 50 ml volumetric flask, which was then filled up with methanol. The content was mixed, and used for the injection. The calibration curve was prepared using 0–80 mg diludine and cyclohexyldiludine, respectively, in 1 l of methanol. The concentration/response relations were highly correlated: $r = 0.9835$ for diludine and $r = 0.9944$ for cyclohexyldiludine. The effect of solvent on the yield of 1,4-dihydropyridines is shown in Table I. The yield obtained with 90% and 95% methanol for diludine and cyclohexyldiludine, respectively, were considered as optimal.

I. Effect of the methanol concentration in aqueous methanol on the yield of 1,4-dihydropyridines (3 extractions)

Methanol content [%]	Yield [%] of diludine	Yield [%] of cyclohexyldiludine
0	0	0
10	0	0
20	0	0
30	0	0
40	3.98	0
50	25.15	0
60	46.07	0
70	59.27	12.06
80	66.65	37.04
90	76.94	66.13
95	55.03	80.86

The yield is improved by repeating the extraction five times with 20ml portions of the solvent, filtering the combined extracts into a 100ml volumetric flask, and injecting into the HPLC. The calibration curve was prepared with 0–50 mg/l in 90 % aqueous methanol in the case of diludine (correlation coefficient $r = 0.9993$), and in the same range in 95% methanol in the case of cyclohexyldiludine (correlation coefficient $r = 0.9999$). The average yield of diludine was 95.57%, that of cyciohexyldiludine was 85.48%.

In the case of anhydrous methanol as solvent, the lower phase contained light petroleum, interfering with the subsequent chromatographic determination. Therefore, the solvent was evaporated from the filtrate, the dry residue was dissolved in methanol, and filled up to 50 ml. The extract obtained with anhydrous methanol was heavily contaminated with oil, and therefore, the use of 100% methanol is not recommended.

The extraction of control rapeseed oil, containing only natural antioxidants, mainly α-tocopherol and γ-tocopherol, showed that no EC-active detectable substances were co-eluted.

HPLC with UV Detection

Both 1,4-dihydropyridines showed two absorption maxima in the UV region: (1) at 230 nm (A = 2.9 for diludine and A = 2.2 for cyclohexyldiludine, respectively) and (2) at 370 nm (A = 1.6 for diludine and A = 1.2 for cyclohexyldiludine, respectively); the values were measured in methanolic solutions (50 mg/l) of diludine and cyclohexyldiludine, respectively. The detection limits are given in Table II.

II. Detection limits of the HPLC determination of 1,4-dihydropyridines

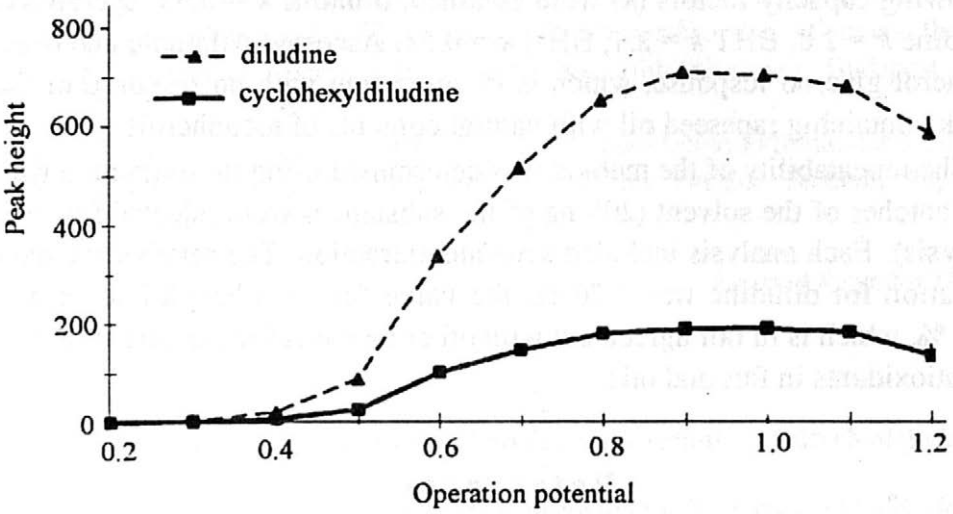
Substance tested	UV at 230 nm [g]	UV at 370 nm [ng]	EC at 0.9 V [ng]
Diludine	0.16	1.35	0.30
Cyclohexyldiludine	0.46	3.20	1.00

HPLC with EC Detection

The mobile phase starts showing the decomposition above the potential of 0.53 V, but the decomposition in not yet fast enough even at 0.9 V, allowing the measurement at the operation potential of 0.9 V. The peak height depended on the operation potential (Fig. 1), the optimum value being V = 0.90 V.

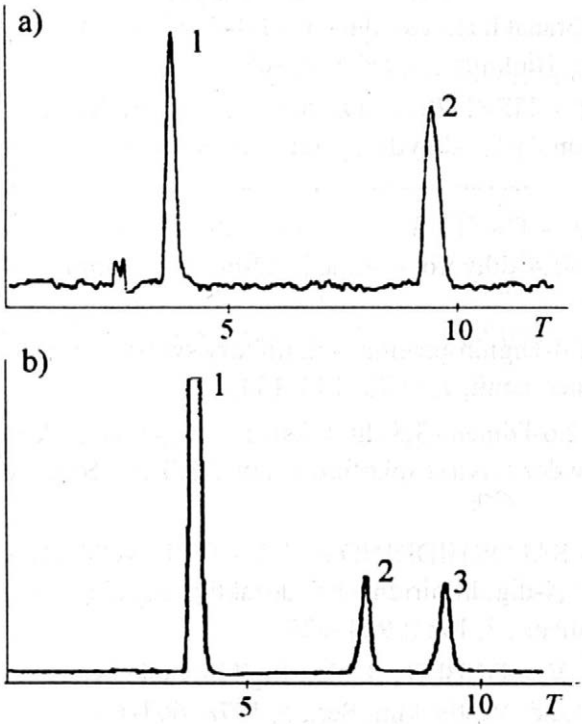
The detection limits are given in Table II, showing sensitivities higher at 370 nm than at 230 nm with the UV detector, however, the EC detector is more specific and several times more sensitive even than the UV measurement at 370 nm.

An example of the separation is given in Fig. 2a (the retention times being Rt = 3.7 min and Rt = 9.5 min for diludine and cyclohexyldiludine, respec-



1. Effect of operation potential on peak height in the case of the HPLC with EC detection

tively). In presence of other antioxidants, BHA and BHT passed into the extract. While BHT is well separated from the two diludines (Rt = 7.5 min), BHA (Rt = 3.5 min) is eluted with diludine in the same peak (Fig. 2b). The



a = in absence of other antioxidants
1 – diludine
2 – cyclohexyldiludine

b = in presence of BHT, BHA, AP and tocopherols
1 – diludine and BHA
2 – BHT
3 – cyclohexyldiludine

2. Separation of dihydropyridines

following capacity factors (k) were obtained: diludine $k = 0.53$, cyclohexyl diludine $k = 2.8$, BHT $k = 2.1$, BHA $k = 0.51$. Ascorbyl palmitate and α-tocopherol give no response, which is in agreement with no response of the blank containing rapeseed oil with natural contents of tocopherols.

The repeatability of the method was determined using the extraction with five batches of the solvent (200 ng of the substances were injected for each analysis). Each analysis included separate extraction. The relative standard deviation for diludine was 1.76 %, the value for cyclohexyldiludine was 3.33 %, which is in fair agreement with other methods for the determination of antioxidants in fats and oils.

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Stanovení 1,4-dihydropyridinových antioxidantů v jedlých olejích

Pro stanovení 1,4-dihydropyridinových antioxidantů diludinu a cyklohexyldiludinu v řepkovém oleji je k extrakci pro první antioxidant vhodný 90% vodný metanol a pro druhý 95% vodný metanol. Extrakt pak může být rozdělen s použitím HPLC na obrácených fázích s UV detektorem při 230 nm nebo s větší citlivostí při 370 nm. Citlivost se ještě několikrát zlepší použitím elektrochemického detektoru s grafitovou elektrodou při 0,9 V, a to s uspokojivou opakovatelností. Za těchto podmínek nedávají askorbylpalmitát ani tokoferoly žádnou odezvu, zatímco BHA a BHT se extrahují a stanoví.

diludin; cyklohexyldiludin; 1,4-dihydropyridiny; řepkový olej; antioxidanty; HPLC s elektrochemickou detekcí

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Potrav. Vědy, *14*, 1996 (1) : 63–70

CHANGES OF PHOSPHOLIPASE D ACTIVITY DURING RAPE SEED DEVELOPMENT AND PROCESSING*

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Abstract: Phospholipase D (PLD, E.C.3.1.4.4) activity was measured during the development of winter rape seed var. Lirajet in relation to geographical location of plant cultivation. These experiments were performed within the harvest of the year 1994. Significant differences in the PLD activities were found for the green and brown seeds when compared to the fully matured seeds. The subcellular distribution of PLD during the ripening process was also investigated. Finally PLD activity variations were measured within the oil processing in six subsequent steps of rape seed oil production.

rape seeds; phospholipase D; oil seed maturation; localization; plant oil processing

Oilseeds are ancient crops having many uses as food and fodder and resources for a diverse industrial applications covering production of plastics, fuels, detergents, lubricants and cosmetics. Rape seed represents one of the most important oil seeds in EC and Central Europe where almost 1/3 of the total world crop is harvested (30 millions of tons in 1991). The world production increased by more than one third at the last decade (Murphy, 1993). On a worldwide production basis rapeseed ranks fifth among vegetable oils (after soybean, sunflowerseed, peanuts and cottonseed). In the Czech Republic, rape seed is surely the oil seed number one (production of about 1/2 milions of tons per year).

Rape seed oil contains according to the recent studies (Hougen et al., 1985) 0.8–3.5% of lecithin, the main components of which are phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol. All mentioned

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phospholipids are potential substrates for phospholipase D, a lipolytic enzyme hydrolysing the phosphorus ester linkage between 1,2-diacylglycero--*sn*-3 phosphate and the hydrophilic alcohol substituent. The product of this enzyme reaction is phosphatidic acid which affects in an undesirable way industrial oil processing and flavor of final product. In soybeans (Schalfield, 1985) phosphatidic acid has been found in larger amounts in developing seeds. The content of phosphatidic acid increased in soybean phospholipids stored at high temperature and humidity. It seems to be evident that the content of phosphatidic acid in oilseed itself and during the processing as well, is dependent on phospholipase D activity.

MATERIALS AND METHODS

Plant Material

Winter rape seed, variety Lirajet, harvested in 1994 at six localities (Červený Újezd, Staňkov, Svitavy, Jaroměřice, Kujavy, Vysoké u Příbrami) and in 1995 at the locality Červený Újezd was collected in different stages of maturity. Seeds were stored at –85 °C. Samples from oil processing were taken on 25th of May and 25th and 26th of October 1995 in Setuza a. s.

Assay of PLD Activity

Substrate emulsion: 40 mg of L-α-phosphatidylcholine, type X-E from dried egg yolk (Sigma) were dissolved in 0.05 ml of ethanol and the solvent was evaporated under nitrogen atmosphere. Then 0.33 ml of 1.83M $CaCl_2$ solution and 0.67 ml of 0.4M Tris acetate buffer pH 6.0, containing 13mM sodium dodecylsulfate (SDS), were added. The mixture obtained was emulsified on vortex for 2 min.

Incubation: The reaction mixture containing 0.1 ml of 0.2M MES (2-N--Morpholinoethanesulfonic acid) buffer pH 6.0, 0.1 ml of substrate emulsion, 0.02 ml of 0.173M SDS and 0.2 ml of the sample was incubated at 37 °C for 10 min. Enzyme reaction was terminated by adding 0.2 ml of 50mM ethylenetetraacetic acid (EDTA) in 1M Tris-HCl buffer pH 8.0 and subsequent boiling for 5 min.

Determination of choline: The concentration of choline released by PLD action was determined using choline biosensor (Vrbová et al., 1993): 0.1 ml of the incubation mixture was added to the stirred reaction vessel

(30 °C), containing 1.4 ml of 0.1M Tris-HCl buffer pH 8.0. The amount of choline was measured with the inserted sensor and calculated from a calibration curve constructed from the responses measured for the standard solutions of choline chloride. The enzyme activity was calculated in international units U (1 U was defined as 1 μmol of choline produced per minute).

Preparation of Extracts

5 g of rape seeds in different stages of maturity or samples from the individual steps of oil processing were ground three times for 20 s (on the coffee grinder), suspended in 25 ml of 0.05M Tris-HCl buffer pH 7.4 containing 1mM EDTA and 1mM DTT (dithiothreitol) and shaken for 45 min at 4 °C. The mixture was filtered through a cheese-cloth and centrifuged at 17 600 g at 4 °C for 30 min. The floating fat cake was removed and the supernatant was used for the activity measurement.

Preparation of Microsomal Fraction

5 g of rape seeds in different stages of maturity were ground as described above and suspended in 25 ml of 0.05M Tris-HCl buffer pH 7.4 containing 0.5M sucrose, 1mM DTT and 1mM EDTA. After shaking for 30 min at 4 °C the homogenate was filtered through cheese-cloth and centrifuged at 11 300 g for 20 min. Pellets and floating fat cake were removed. Supernatant (crude extract) obtained was centrifuged at 105 000 g for 60 min. All the operations were carried out at 4 °C. Supernatant was referring to cytosolic fraction. Sedimented microsomes were resuspended in 1 ml of Tris-HCl buffer pH 7.4 mentioned above.

Determination of Proteins

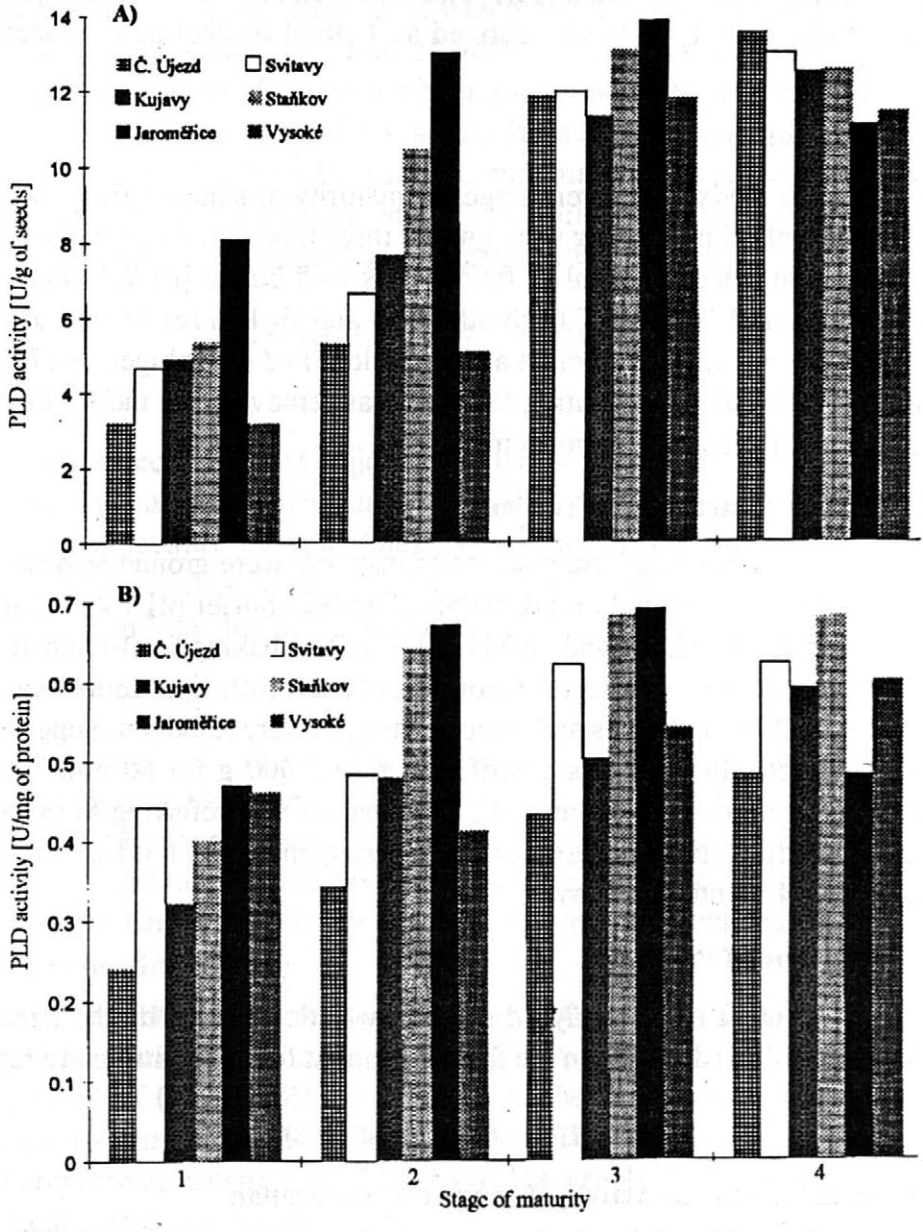
Protein content in the analysed samples was determined by the Bradford method (Bradford, 1975) in the format adapted for microtitre plate reader.

RESULTS AND DISCUSSION

Determination of PLD Activity during Seed Maturation

Samples of rape seed variety Lirajet were gradually harvested during seed development at six different localities in the Czech Republic. The first samples of green seeds were collected at the beginning of July, two weeks before

anticipated "full combine harvester maturity", the last samples at the beginning of August, one week after the harvest of fully matured seeds. The activity of PLD was measured in the seed extracts and calculated either per g of



1 – harvested 14 days before full maturity; 2 – harvested 7 days before full maturity; 3 – harvested in the full maturity; 4 – harvested 7 days after full maturity

1. Changes of the PLD activity during seed maturation (Lirajet, 1994)

seeds or per mg of protein (specific activity). Results of these measurements are presented in Fig. 1 (A, B). The effect of distinct planting area on the amount of extracted activity was more apparent for the first two stages of seed maturity (green and brown seeds).

PLD activity determined in the green non-matured seeds was found in the range from 3.0–5.5 U/g of seeds or 0.2–0.5 U/mg of protein. Brown, non-matured seeds, collected in the middle of July, showed the activity (per g of seeds) 1.5 times higher than the green ones. PLD activity of fully matured, black seeds increased to 11–14 U/g of seeds, and shows approximately three times higher activity than found in green seeds. In accordance with our expectation, due to the higher production of proteins during the seed development, the increase of specific activity was not significant.

Subcellular Distribution of PLD Activity in Different Stages of Seed Maturity

Owing to the proven activity fluctuations, more detailed study of the changes in the distribution of soluble (cytosolic) and bound (microsomal) form of rape seed PLD has been provided (Lirajet, Č. Újezd, 1995) in different stages of seed maturity. Results summarized in Table I show a significant increase of the total activity within the process of ripening which is caused by the activity rise in soluble fraction (more than 10 times higher in black seeds). Total activity of microsomal fraction remains at the same level. Specific activity of the soluble PLD in green and brown seeds is almost the same and increases rapidly in the fully matured black seeds. In contrast the specific activity of microsomal fraction remained almost unchanged within

I. Subcellular distribution of rape seed PLD activity during the seed development

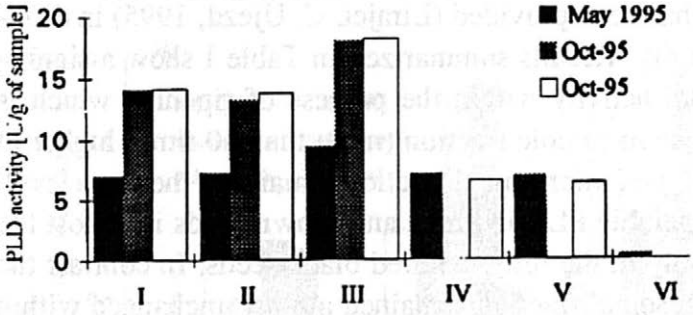
Fraction of PLD	14 days before full maturity			7 days before full maturity			Matured seeds		
	total prot. [mg]	total activity [U]	spec. activity [U/mg]	total prot. [mg]	total activity [U]	spec. activity [U/mg]	total prot. [mg]	total activity [U]	spec. activity [U/mg]
Crude extract	29.0	7.9	0.3	69.9	18.5	0.3	87.8	95.0	1.1
Cytosolic PLD	21.8	7.1	0.3	55.2	18.1	0.3	72.4	79.2	1.1
Microsomal PLD	1.6	0.5	0.3	1.9	0.5	0.3	2.7	0.6	0.2

the whole maturation process. The ratio of the total activity of cytosolic and microsomal PLD for matured seeds of the variety Arabella (Valentová et al., 1994) corresponds to that found for var. Lirajet.

Monitoring of PLD Activity in Different Steps of Oil Processing

The activity of PLD was also monitored during the industrial oil processing at six stages of the process. The first sampling was performed in May 1995. It means that the oil was produced from the rape seeds stored for about nine months in the storage tank. The PLD activity in the processed seeds depends on the time of storage. During the nine month storage of seeds at 20 °C the PLD activity drops to 50% of the value measured after three month storage (Fig. 2). The second set of samples was taken in October 1995 in the two following days (i.e. three months after harvest).

PLD activity in the "old" seeds remained during processing at the same



2. Changes of the PLD activity during oil processing

I. the seed entering the process (fat content 42%, water 7%, 15–25 °C)
II. the seeds after steaming (fat content 42%, water 7.5%, temperature increases by10–15 °C)
III. the seeds are grinded – flakes (fat content 42%, water 7.5%, approx. 35 °C)
IV. the flakes after conditioning (fat content 42%, water 7.5%, temperature 85–90 °C)
V. press cake (fat content 20%, water 7%, high pressure within hundreds of magnitude of atp.)
VI. crush after extraction of the press cake by isohexane (fat content 2.8%, water 12% temperature finally increases to 110–130 °C)

level up to the fifth step (treatment with high temperature and pressure), while in the seeds stored about three months the activity dropped to a half in one case after the third step of processing. The absolute value, however, corresponds well with those activities measured for the "old" seeds. In the second case, no activity was found after the third processing step. When

comparing with the results reported recently (Valentová et al., 1994) the PLD protein in its "natural" environment is more stable under the conditions during processing. Partially purified enzyme is totaly inactivated after 10 min at 60 °C. PLD activity is even increased by 25% at the third step (flakes) of the process and in following steps in some samples the enzyme is not inactivated even by the temperature higher than 90 °C, most probably due to unequal heat distribution. In these cases the activity decreased drastically only when treated with isohexane.

Conclusions

The screening of PLD activity in rape seed var. Lirajet grown at different localities in the Czech Republic showed activity variations for non-matured seeds (green and brown), while fully matured seeds (black) exhibited practically the same activity values independently on the place of harvest.

Total PLD activity in the extracts increased more than 10 times during the seed maturation. The main action was found in the soluble form (cytosolic PLD) for all maturation stages. It still remains to be determined if the activity increase is due to the activation of PLD protein or the increased production of this protein.

Monitoring of the PLD activity within the oil processing showed that the enzyme keeps his ability to produce phosphatidic acid at least up to the third or fourth step of the industrial process. It means that the amount of phosphatidic acid within the oil production is increasing up to the enzyme inactivation.

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Změny aktivity fosfolipasy D během zrání a zpracování semen řepky

Řepka je v celosvětovém měřítku jednou z nejdůležitějších olejnin, její produkce se v posledním desetiletí zvýšila o třetinu. V České republice je její roční produkce okolo půl milionu tun.

Řepkový olej obsahuje asi 0,8–3,5 % lecithinu, jehož hlavními složkami jsou fosfatidylcholin, fosfatidylethanolamin a fosfatidylinositol. Všechny tři jmenované fosfolipidy jsou potenciálními substráty fosfolipasy D (PLD, E.C.3.1.4.4), která katalysuje hydrolysu fosfoesterové vazby mezi 1,2-diacylglycero-*sn*-3 fosfátem a hydrofilním substituentem. Jedním z produktů této enzymové reakce je kyselina fosfatidová, jejíž přítomnost ovlivňuje senzorické vlastnosti finálních produktů a působí určité technologické problémy při zpracování olejů.

Aktivita fosfolipasy D byla sledována v průběhu zrání ozimé řepky odrůdy Lirajet v závislosti na geografických podmínkách pěstování. Určité rozdíly v naměřených aktivitách byly nalezeny pro nezralá semena. U zralých semen byly hodnoty aktivit srovnatelné. Dále byl sledován poměr cytosolické a mikrosomální formy PLD u semen o různém stupni zralosti. Tento poměr se u zralých semen výrazně zvyšuje.

Změny fosfolipasové aktivity byly také sledovány v průběhu výroby řepkového oleje. Lze konstatovat, že fosfolipasa D zůstává aktivní v průběhu procesu po poměrně dlouhou dobu. K její úplné inaktivaci dochází většinou až v závěrečných fázích výroby.

řepka; fosfolipasa D; zrání olejnatých semen; lokalizace; průmyslová výroba řepkového oleje

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PŘEHLEDY

BAREVNÉ ZMĚNY BĚHEM ZPRACOVÁNÍ BRAMBOR*

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Během technologického zpracování nebo kulinární úpravy konzumních brambor dochází k barevným změnám, které mohou být do určité míry žádoucí, protože jsou pro daný způsob úpravy charakteristické a spotřebitel je očekává. Většinou však tyto barevné změny mohou negativně ovlivňovat kvalitu a prodejnost výrobku (Rybáček, 1988). Vedle dalších vlastností, jako je obsah sušiny a škrobu, je tendence k barevným změnám velmi důležitá pro výběr hlíz k určitému způsobu zpracování. Barva dužniny bramborových hlíz před tím, než se projeví barevné změny, je dána obsahem karotenoidních barviv, podle kterého je možné rozlišit „bělomasé" a „žlutomasé" odrůdy brambor. Během zpracování se uplatní barevné změny vyvolané tvorbou intenzivně zbarvených produktů. Po oloupání, dělení apod. to jsou reakce enzymového hnědnutí. Při sušení a zejména smažení se uplatňují reakce neenzymového hnědnutí a poslední skupinou barevných změn je tzv. černání po uvaření („post cooking blackening").

Enzymové hnědnutí

Tmavnutí vyvolané enzymovým hnědnutím nastává po mechanickém poškození buněk čerstvých brambor při loupání, krájení, strouhání apod. Porušení buněčných struktur umožní kontakt přítomných enzymů polyfenoloxidas (PPO) se substrátem – s fenoly a vzdušným kyslíkem. Zpočátku se hlízy barví červenohnědě, později vzniká tmavohnědé až černohnědé zbarvení. Enzymová je pouze první část reakcí, hydroxylace monofenolů (např. tvorba 3,4-dihydroxyfenylalaninu z tyrosinu) a oxidace difenolů na chinony. Další reakce zahrnující široký komplex změn, kterého se účastní i další složky, jsou neenzymové (Kadam et al., 1991b). Konečným produktem reakcí jsou polymerní pigmenty označované jako melanin.

Hlavním substrátem polyfenoloxidasy brambor jsou kyselina chlorogenová a tyrosin, přičemž z modelových pokusů je zřejmé, že zejména oxidace tyrosinu vede

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ke vzniku intenzivněji zbarvených produktů (Mapson et al., 1973). Podobně jako v případě dalších technologicky významných plodin náchylných k enzymové oxidaci není ani u brambor zcela jasné, který faktor – aktivita přítomných enzymů nebo obsah a složení fenolů – je pro barevné změny významnější. Některé práce uvádějí jako limitující obsah tyrosinu (Gray, Hughes, 1978), podle jiných je intenzita barevných změn dána aktivitou přítomné PPO, celkovým obsahem fenolů, obsahem bazických aminokyselin, sušiny, kyseliny chlorogenové (Amberger, Schaller, 1975). Tendence k enzymovému hnědnutí je ovlivňována i dalšími faktory, jako jsou klimatické podmínky, složení půdy, podmínky kultivace apod. Jak uvádějí Mapson et al. (1973), vyšší srážky vedou k vyšším sklonům k hnědnutí. Přídavek draselných hnojiv vyvolal snížení obsahu tyrosinu, aktivity PPO a vedl k redukci enzymového hnědnutí (Mondy et al., 1967). Vyšší přísun dusíku zvyšoval tendence hlíz k hnědnutí (Mondy, Koch, 1978). Jiné práce (Amberger, Schaller, 1975) však souvislost mezi hnojení a enzymovým hnědnutím nepotvrdily. Matheiz, Belitz (1977) a Amberger, Schaller (1975) spojují sklony k enzymovému hnědnutí zejména s odrůdou brambor, přičemž do jisté míry se mohou uplatnit také další vlivy, jako jsou klimatické podmínky, hnojení a další.

Dispozice k hnědnutí závisí také na způsobu manipulace s hlízami a jejich vystavení mechanickému namáhání, které může vést ke zranění hlíz i bez viditelných známek na povrchu a tím k vnitřním barevným změnám, které se nevyvinou bezprostředně po nárazu, ale dosahují maxima po třech dnech. Enzymové hnědnutí je často řazeno mezi obranné mechanismy rostlinných pletiv, poškození hlíz v určitém fyziologickém stavu může vyvolat zvýšení intenzity respirace v okolí poranění, zvýšení aktivity polyfenoloxidas i nárůst obsahu fenolů a tím se zvýší sklony hlíz k barevným změnám (Voldřich et al., 1995).

Obranou proti reakcím enzymového hnědnutí může být také správná manipulace s hlízami a technologie zpracování. K určitému snížení intenzity průběhu barevných změn vede oloupání – odstranění peridermu, vrstvy pod slupkou, která je vzhledem k nejvyššímu obsahu fenolových látek a enzymů k hnědnutí nejcitlivější (Clark et al., 1957). Opláchnutí narušených buněk z povrchu oloupaných, dělených brambor a uložení hlíz pod vodu (zamezení přístupu vzduchu) významně omezí barevné změny (Burton, 1989). Časová prodleva mezi oloupáním a tepelným zpracováním by měla být co nejkratší, enzymová oxidace může proběhnout bez zjevných známek a následující inaktivace enzymů barevným změnám nezabrání. Snížení teploty na 0–5 °C zpomalí rychlost šednutí, která je nejvyšší kolem 40 °C a téměř zanedbatelná při –20 °C. Účinným způsobem inhibice barevných změn je termoinaktivace polyfenoloxidas, které jsou poměrně termolabilní a jsou inaktivovány při teplotách kolem 80 °C. Chemické způsoby prevence hnědnutí zahrnují zejména použití siřičitanů (oxidu siřičitého, pyrosiřičitanů), thiolů, kyseliny

askorbové a směsí kyselin. Oxid siřičitý a siřičitany mají komplexní účinek (redukují chinony, tvoří s chinony stabilní nebarevné produkty, inhibují enzym), jsou ve srovnání s ostatními činidly nejúčinnější a jsou zatím s výhradami tolerovány (při dodržení limitních koncentrací ve výrobcích) (Voldřich et al., 1993). Z dalších látek nachází uplatnění zejména směsi kyselin (citronové, fosforečné), jejichž účinek spočívá v okyselení a chelatačních vlastnostech, ve srovnání se siřičitany je jejich účinek nižší. Kyselina askorbová redukuje oxidované chinony zpět na fenoly, proto působí pouze dočasně (Muneta, 1981; Dimpf, Somogyi, 1975).

Neenzymové hnědnutí

Při tepelném zpracování brambor (smažení, pečení, sušení) probíhají dva typy reakcí neezymového hnědnutí vedoucí k pigmentaci, tj. karamelizace a Maillardova reakce (Kadam et al., 1991). Vlastní degradace cukrů (karamelizace) se vzhledem k energetické náročnosti uplatňuje méně, významnější pro barevné změny je Maillardova reakce a komplex navazujících změn. Při obvyklých teplotách smažení (165–170 °C) probíhá hnědnutí cukrů pouze v přítomnosti aminokyselin, výraznější hnědnutí je při teplotách kolem 100 °C při sušení bramborové kaše a v případě dlouhodobého skladování výrobků z brambor za normální teploty (Burton, 1989). V modelových směsích je rychlost Maillardovy reakce dána koncentrací aminosloučeniny (Gray, Hughes, 1978), ale v bramborách jsou α-aminosloučeniny zřídkakdy limitujícím faktorem, proto intenzita barevných změn závisí zejména na obsahu redukujících cukrů (Kadam et al., 1991a), s výjimkou smažení za vyšších teplot (220 °C), při němž ve větší míře degraduje také sacharosa a vývoj barvy je závislý na obsahu všech sacharidů (glukosy, fruktosy a sacharosy). Vliv na barevné změny mají také další látky přítomné v hlízách, kromě aminosloučenin je to zejména kyselina askorbová, která se účastní barevných změn. Vliv dalších faktorů je zřejmý také z uváděných korelací mezi obsahem redukujících cukrů a intenzitou barevných změn, které se pohybují od 0,32 do 0,9 (Kadam et al., 1991). Kromě složení je průběh změn závislý také na způsobu zpracování. Reakce je urychlována teplotou, např. čím vyšší je teplota smažení, tím rychlejší a intenzivnější je tvorba zabarvení produktů. Podobně se projeví také podmínky sušení a doba a teplota skladování v případě sušené bramborové kaše.

Z praktického hlediska jsou reakce neenzymového hnědnutí významné zejména pro smažené výrobky. Prevence barevných změn spočívá hlavně ve výběru vhodných surovin pro zpracování. Např. doporučený maximální obsah redukujících cukrů v bramborách pro přípravu lupínků by neměl překročit 0,2 %, naopak pro dosažení očekávané barvy by neměl být nižší než 0,1 %. V případě hranolků je maximální doporučený obsah redukujících cukrů nižší – pod 0,8 % (Kadam et al., 1991b). V této souvislosti jsou významné změny obsahu cukrů v hlízách během

skladování, zejména kumulace redukujících cukrů v hlízách skladovaných za nižších teplot. Obsah takto vytvořených cukrů je možné snížit prodýcháním při zvýšení skladovací teploty (na 15–20 °C) několik týdnů před zpracováním. Tendence kumulovat cukry, resp. je prodýchat je od odrůdy k odrůdě různá (Poppr et al., 1995). Kromě výběru hlíz s nízkým obsahem cukrů, případně prodýchání nakumulovaných sacharidů patří mezi metody inhibice barevných změn: vymytí cukrů a dalších prekursorů hnědnutí před smažením horkou vodou (uvedený postup však vede k vyšší absorbci tuků při smažení), horkým roztokem chloridu sodného, zablokování karbonylových skupin ošetřením siřičitany, oxidace glukosy glukosaoxidasou (Kadam et al., 1991b). Popsány byly také další způsoby: ponoření syrových brambor před smažením do roztoků kyseliny chlorovodíkové, směsí kyselin citronové a fosforečné a další bez vysvětlení mechanismu účinku (zřejmě inhibují enzymové změny během přípravy plátků nebo hranolků. Barevné změny je možné omezit také optimalizací podmínek smažení – teplotou lázně, ukončením smažení na výsledném obsahu vody mezi 6 a 10 % (Kadam et al., 1991b).

Černání (tmavnutí) po uvaření

Tmavnutí po uvaření se projevuje u vařených brambor, zejména v případě vaření citlivých odrůd brambor ve vodě nebo v páře. Občas se černání projeví také u předsmažených nebo jinak tepelně upravených mražených hranolků, u pasterovaných brambor a sušených brambor (bramborové kaše) (Gray, Hughes, 1978). Příčinou barevných změn je tvorba komplexů kyseliny chlorogenové se železem během vaření a jeho oxidace během chladnutí, při které vznikají zbarvené komplexy kyseliny a trojmocného železa (Huhges, Swain, 1962). Podle podmínek mohou být různé: monofenolát při pH 5,5 (zelený), difenolát při pH 6,5 (šedomodrý) a trifenolát při pH 7,5 (hnědý). V bramborách převládá šedomodrý difenolát. Zbarvení se projeví zejména v peridermálních vrstvách těsně pod slupkou s nejvyšším obsahem kyseliny chlorogenové. Intenzita zbarvení souvisí také s další distribucí kyseliny chlorogenové v hlíze; její koncentrace je nejvyšší v bazální části, nižší ve vrcholové části a nejnižší uprostřed hlízy. Schopnost tvořit zbarvené komplexy se železem mají i další orto-fenoly, kromě kyseliny chlorogenové to v bramborách jsou kyselina kávová, esculetin a další, ale vzhledem k obsahu kyseliny chlorogenové je její vliv na vývoj barvy převládající. Sklony k černání závisejí i na obsahu kyseliny citronové, jablečné a fosfátů a dalších chelatačních látek v hlízách, které mají schopnost vázat železo do komplexů (Hughes, Swain, 1962; Kadam et al., 1991b). Zjednodušeně intenzita černání je přímo úměrná koncentraci železa, kyseliny chlorogenové a pH a nepřímo úměrná koncentraci kyseliny citronové a fosforečné. Nejlépe pak vychází korelace mezi poměrem kyseliny chlorogenové/citronové a tmavnutím (Mondy et al., 1991).

Vedle komplexů orto-fenolů a železa může k barevným změnám po uvaření přispívat také tvorba oxidovatelných komplexů kyseliny askorbové se železem. Nachově zbarvený komplex kyseliny askorbové s dvojmocným železem, který oxidací tmavne, popsal Muneta (1981).

Podobně jako enzymové hnědnutí jsou sklony k černání po uvaření ovlivňovány řadou dalších faktorů: odrůdou, půdou, hnojením a sezónou. Při zjišťování vzájemného působení ukazatelů na tmavnutí došli autoři často k protichůdným výsledkům. Vyšší sklony k tmavnutí po uvaření byly pozorovány ve vlhkých a studených letech, při vyšším poměru dusíku a draslíku. Jak uvádějí Hughes a Evans (1967), brambory pěstované v půdách s vyšším obsahem organického materiálu a nižším obsahem draslíku tmavly více než brambory pěstované v půdách s nižším obsahem organických látek a vyšším obsahem draslíku. Sklony k tmavnutí se zvyšují se skladováním brambor (Míča et al., 1984).

V případě tmavnutí po uvaření je jedinou možností prevence výběr odrůd s nejnižším sklonem k černání. Podmínky skladování a zpracování musí být takové, aby nedocházelo k poškození hlíz, které může vyvolat nárůst obsahu fenolů a tím také zvýšení rizika barevných změn.

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Colour Changes during the Processing of Potatoes

The processing quality of potatoes associated with structure, composition and culinary properties include pigmentation – the tendency to colour changes during the technological and/or culinary processing of tubers. The following ways of colour changes occur during the potato processing: enzymatic browning reactions, non-enzymatic browning reaction and after-cooking blackening. Enzymatic browning is based on the polyphenoloxidase (PPO) oxidation of phenols in native tissues (peeled, cut potatoes, peeled chilled potatoes, etc.). The intensity of colour changes depends mainly on tyrosine and o-diphenols (chlorogenic acid) concentration, PPO activity in tubers. Non-enzymatic browning is important for fries, and chips. The limiting factor affecting the colour intensity is the content of reducing sugars (and ascorbic acid, sometimes sucrose) in processed potatoes. The course of colour changes during the frying also depends on the temperature of oil bath and duration of processing. The after-cooking blackening takes place when tubers are cooked and cooled. During cooking complexes of chlorogenic acid and iron ions are formed, during subsequent oxidation grey-black products are formed. The formation of coloured products is affected by several factors, the most important is the ratio of chlorogenic and citric acid in tubers.

processing of potatoes; colour; enezymatic and non-enzymatic browing reaction; after-cooking blackening reaction

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FROM THE SCIENTIFIC LIVE

EURO FOOD CHEM VIII
"Current Status and Future Trends in Analytical Food Chemistry"

The latest event organized by the FECS-WPFC took place in Vienna, September 18.–20., 1995. Almost three hundred and fifty participants from thirty one different countries attended the meeting held at the Technical University, in the Austrian capital. A highly efficient and motivated team of students from the Institut für Biochemie und Lebensmittelchemie in Graz and from the Institut für Analytische Chemie in Vienna helped with organization details and ensured a smooth-running programme throughout.

Guided by the FECS-WPFC Secretary, Professor Werner Pfannhauser, the conference staged thirty seven lectures and one hundred eighty poster presentations and all delegates received the proceedings and a list of participants on registration. This was all the more remarkable given the organizers' policy of accepting last minute poster communications until August.

The three volumes of the proceedings *(ISBN: 3-900554-17X) provide an informative and authoritative statement of analytical food chemistry as we approach the end of the century, each volume contains a table of contents and author and subject (keyword) indexes. Volume 1 contains the plenary lectures and oral communications, Volume 2 the poster presentations addressing *Sampling and Sample preservation* and *Chromatography and other coupled techniques*, whilst the final volume deals with poster presentations about *Sensor techniques, Spectroscopic methods and Bioaffinity methods* (Section 5), as well as the aforementioned *Last minute posters*.

The five sections listed above provided the themes underpinning the five sessions of the conference. All posters were displayed throughout the conference, with ample time for discussion during the morning and afternoon breaks and at lunch time. For the first time in the history of Euro Food Chem series, prizes were awarded by the scientific committee for the three best poster presentations. This proved a very difficult exercise for the panel, but eventually the first, second and third prizes were awarded by Dr. Reto Battaglia, chairman of the FECS-WPFC to paper on "Mobilization and analytical availability of drug residues in food", on the "Applicability of neutron activation analysis (NAA) in quantitative determination of some essential and toxic trace elements in food articles" and on "A biosensor for the rapid determination of flatulence causing oligosaccharides in pea".

* Copies of the proceedings are available and may be ordered directly from Prof. G. Sontag, Institut Analytische Chemie, Währinger Str. 38, 1090 Wien

The conference was of a high scientific and technological standard, providing ample opportunities for the interchange of scientific information and discussion of specific topics of interest to participants. Two of the fields which promise the most exciting developments appear to be chiral-sensitive separation methods and polymerase chain reaction (PCR) analytical techniques, although the need for increased attention to multivariate methods in the study of complex food-based matrices was also evident.

From our country these contributions were presented: Kassahun B. W., Velíšek J., Davídek J.: *Glucosinolates and their degradation products in cooked cabbage*; Voldřich M., Marek M., Kvasnička F.: *A biosensor for rapid determination of fluctulence causing oligosaccharides in pea* and Hochel K., Málková K., Demnerová K., Fukal L., Polstein M., Škvor J., Rauch P.: *ELISA for detection of Salmonella in Foodstuffs.*

The meeting was graciously opened by the Austrian Minister of Health and Consumer affairs, Dr. Christa Krammer, who paid tribute to the recently retired Chairman of the FECS-WPFC Prof. Peter Czedik-Eysenberg. Thereafter, Prof. Pfannhauser drew the participants' attention to the developments in analytical food Chemistry since Euro Food Chem I, held in Vienna in 1981. Later in the programme, the participants attended the reception in the Rathaus and the conference dinner was held at Laxenburg Castle.

The meeting provided an ideal opportunity for colleagues and friends of Prof. Czedik and Prof. Werner Baltes (Technical University, Berlin), the Chairman of the Scientific Committees of previous Euro Food Chem meetings for their unique contribution to the development of European food chemistry over the last two decades. It was therefore fitting that during the conference Prof. Czedik was presented with the Special Medal of the Food Chemistry Group of the UK's Royal Society of Chemistry for "outstanding services to European food chemists".

Prof. Jiří Davídek

Instructions for authors

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Full research manuscript should consist of the following sections: Title page, Abstract, Keywords, a short review of literature (without "Introduction" subtitle), Materials and Methods, Results, Discussion, References, Tables, Legends to figures. A title page must contain the title, the complete name(s) of the author(s), the name and address of the institution where the work was done, and the telephone and fax (e-mail) numbers of the corresponding author. The Abstract shall not exceed 120 words. It shall be written in full sentences and should comprise base numerical data including statistical data. As a rule, it should not give an exhaustive review of literature. In the chapter Materials and Methods, the description of experimental procedures should be sufficient to allow replication of trials. Organisms must be identified by scientific name. Abbreviations should be used if necessary. Full description of abbreviation should follow the first use of an abbreviation. The International System of Units (SI) and their abbreviations should be used. Results should be presented with clarity and precision. Discussion should interpret the results. It is possible to combine Results and Discussion in one section. References in the text to citations comprise the author's name and year of publication. If there are more than two authors, only the first one should be named in the text, followed by the phrase "et al.". References should include only publications quoted in the text. They should be listed in alphabetical order under the first author's name, citing all authors, full title of an article, abbreviation of the periodical, volume number, year, first and last page numbers.

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