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OCCURRENCE AND RISK ASSESSMENT OF ETHYL CARBAMATE IN ALCOHOLIC BEVERAGES

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Abstract: The presented study is focused on the monitoring of ethyl carbamate (EC) levels in several types of distillates available from Czech production. Experiments aimed at possible prevention of ethyl carbamate formation in cherry brandy (representing product with typical high concentration of this toxin) were carried out. The mean exposure of humans to ethyl carbamate via the intake of alcoholic beverages was considered, too.

ethyl carbamate; alcoholic beverages; distillates; EC occurrence; EC formation; EC risk assessment

Ethyl carbamate (EC) is the ethyl ester of carbamic acid.

Chemical formula:	$NH_2COOCH_2CH_3$
Relative molecular weight:	89.1
The Chemical Abstract (CAS) number:	51-79-6

Synonymous chemical names are ethyl urethane, urethane, urethan etc.

General Occurrence of EC in Food

The potential human carcinogen ethyl carbamate is commonly present in low concentrations in almost all fermented foods and drinks. Tea, cider, orange and grape juices, yoghurt and cheese usually contain very low levels, ranging from non-detectable amounts up to 6 ng/g, whereas bread and wine vinegar usually have measurable EC concentrations within a range of 1 to 15 ng/g, soya sauces up to 95 ng/g. Alcoholic beverages, such as table wine, dessert wine and distillates, are commodities showing high levels of EC, typically in a range 10–20 000 ng/g. The highest EC concentrations were clearly found in fruit brandies derived from plums, apricots and cherries (so called 'stone' fruits), compared to other distilled spirits the levels of this

"natural toxin" could be 1-2 orders of magnitude higher (Ough, 1976; Zimmerli, Schlatter, 1991).

The maximum permissible levels of EC in alcoholic beverages have been recently established in some countries, as examples of hygienic limits accepted in Canada and USA are summarised in Table I. At present in the Czech Republic there is not any restrictive limit for EC content in alcoholic beverages, nevertheless, determinations of EC levels in fruit brandies and other spirits intended for export are carried out (on the exporters' request) by Czech Agriculture and Food Inspection.

I. Regulatory limits for EC (ng/g) in alcoholic beverages in Canada and USA

Alcoholic beverages	Canada	USA
Table wines	30	15
Fortified wines	100	60
Distilled spirits	150	160 (125)[a]
Fruit brandies and liqueurs	400	160

[a]EC level for domestic (American) whiskey

Formation of EC in Food

Ethyl carbamate found in fermented food, including alcoholic beverages, is believed to be formed under natural conditions via several different pathways. Mains of those are mentioned below:

1. Reaction of diethyl pyrocarbonate (DEPC) and ammonia

$$O(COOC_2H_5)_2 + NH_3 \rightarrow H_2NCOOC_2H_5 + CO_2 + C_2H_5OH \qquad (1)$$

The initial interest in ethyl carbamate was stimulated by the findings of Lfroth and Gejvall (1971), who reported that diethyl pyrocarbonate (DEPC) as an antimicrobial food additive to alcoholic and non-alcoholic beverages could be a source of EC formation. The added DEPC reacted with endogenously produced ammonia to yield EC (1). Due to this fact, DEPC and its use as a preservative was forbidden in the USA in 1971 and one year later by the Joint FAO/WHO Expert Committee.

2. Reaction of carbamoyl phosphate and ethanol

$$H_2NCOOPO_3H_2 + C_2H_5OH \rightarrow H_2NCOOC_2H_5 + H_3PO_4 \qquad (2)$$

The EC formation from carbamoyl phosphate (2) was demonstrated by Ough (1976). Carbamoyl phosphate is formed in yeast cell enzymatically from ATP, ammonia and carbon dioxide or by the breakdown of certain amino acids such as citrulline, arginine and ornitine. However, Ough et al. (1988) have suggested that carbamoyl phosphate is not significant source of EC in wine.

3. Reaction of urea, its derivates and/or biogenic precursors

$$H_2NCONHR + C_2H_5OH \rightarrow H_2NCOOC_2H_5 + H_2NR \qquad (3)$$

EC can also be formed by route that involves urea as precursor (3). Urea, however, is a natural metabolite in the nucleic acid and amino acid pathways, and it must be assumed to be present, albeit in small amounts, in most fermentation processes. It seems that EC in wine is formed predominantly by the reaction of ethanol and urea, whereas the formation from cyanide is unambiguously the major route to EC in stone fruit brandies (4, 5) (Battaglia et al., 1990).

4. Reaction of products originating from cyanide precursor

$HCN + [O] \rightarrow HOCN$ [the initial step – oxidation of cyanide to cyanate] (4)

$$HOCN + C_2H_5OH \rightarrow H_2NCOOC_2H_5 \qquad (5)$$

Cyanide or cyanogenic glycosides like amygdalin can be found in a wide variety of plant materials used in fermentation. It is a well known fact that bitter almonds, linseed and seeds of stone fruits show relatively high concentrations of such compounds. The presence of cyanogens in many raw materials could also explain the almost ubiquitous occurrence of EC in fermented food (cyanide liberates during fermentation). However, this pathway (4, 5) has not yet been experimentally demonstrated *in situ* (Battaglia et al.,

1990). It has been mentioned that fermentation of cyanide-containing plant material is not absolutely necessary to get EC, it seems sufficient that such material comes into contact with ethanol (Wucherpfennig et al., 1987).

Determination of EC

Good analytical methodology is essential for monitoring the presence of EC in alcoholic beverages and other food products. Many methods have appeared in the last two decades. Gas chromatography (GC) has been one of the most frequented choice for determination of EC at trace levels (ng/g). Detection was formerly by either flame ionization (FID) or alkali flame ionization (AFID). In the recent improvements in GC resolution using polar capillary columns coupled with improved detectors such as Hall electrolytic conductivity (HECD) or nitrogen phosphorus thermionic (NPD) detectors has enabled some simplification in sample preparation process for alcoholic beverages (without EC isolation by solvent extraction, derivatization etc.). The use of mass selective detectors or complete mass spectrometers as routine GC detectors instead of only for conformation has also enabled simplified sample preparation techniques for EC while maintaining selectivity with excellent sensitivity (Lawrence et al., 1990). In the case of distilled spirits direct sample injection into capillary GC systems with mass spectrometric detection appears to be adequate even for routine analysis.

MATERIAL AND METHODS

Samples of Alcoholic Beverages (Distillates of Czech Origin)

Fermented mashes were distilled twice in copper pot still and high wines (raw distillates with characteristic aroma) were stored in wooden casks (whisky, grape brandy) and enamel or stainless vessels (fruit brandies) for period from 1 to 4 years. Average ethanol concentrations in samples were in the range of 65–75% (v/v). Distillates directly after maturation without any adjustment manufactured for sale of alcoholic beverages were used as samples for analysis.

Analytical Method

Gas chromatography coupled with mass spectrometry (GC/MS) operated in selected ion monitoring (SIM) mode was employed for determination of

EC. Analyses were carried out on capillary column HP 20M (30 m length x x 0.25 mm ID x 0.25 μm film thickness). Settings of Hewlett Packard 5890 Series II gas chromatograph equipped with mass selective detector HP 5972 were as follows:

- oven temperature regime: 70 °C for 1 min, then gradients of 30 °C/min to 130 °C, 5 °C/min to 170 °C and 30 °C/min up to 220 °C, held for 2 min.
- temperature of injector: 250 °C
- splitless period: 1.5 min
- carrier gas: helium
- linear velocity: 34.4 cm/s
- detector temperature: 280 °C
- monitored ions (*m/z*): 62, 74 and 89, the *m/z* 62 was chosen as a quantification ion 1 μl injections of standard solutions of EC and those of tested distillates were carried out by autosampler HP 7673 GC/SFC. EC concentrations were quantified on HP ChemStation by an external calibration method.

For direct sample injections the detection limit of GC/MS was 1 ng/ml.

RESULTS AND DISCUSSION

Monitoring of EC in Distillates

In the first phase of experimental work the EC levels in various types of distillates from local production were determined (Table II).

Concentrations of EC found in selected samples of distillates are comparable to results published by other investigators (Battaglia et al., 1990; Zimmerli, Schlatter, 1991). The high EC levels were estimated in so-called stone fruit brandies that were produced from plums, apricots and cherries.

Maturation and ageing of distillates do not have any significant effect on EC contents. The EC source would appear to be the fruit as a raw material rather than method of production (fermentation process, distillation, maturation, etc.). The highest concentrations of EC were found in plum brandies. This could be explained by the fact that during transport of harvested plums into the fermentation tank by a mono-pump the part of stones were damaged, afterwards amygdalin (one of cyanogenic glycosides) can be easily released and extracted from the kernel into the mash. On the other hand relatively low

II. EC levels in various raw distillates of Czech origin

Beverage	Maturation (year)	EC level per absolute alcohol (ng/ml)
Grape brandy	3	450
	4	3300[a]
Malt whisky	3	700
	4	740
Apple brandy	3	600
Pear brandy	3	560
Plum brandy	1	14 000
	2	13 000
	3	9 000
	4	10 000
Apricot brandy	1	6 300
	2	590[b]
	3	400[b]
	4	470[b]
Cherry brandy	1	3 800
	2	4 200
	3	4 200
	4	5 200

[a] from wines of secondary quality made from grape pomace
[b] from stewed fruit

EC content was obtained in distillates from stewed apricots comparing to distillates made directly from fresh fruit.

Possibilities of EC Content Reduction in Alcoholic Beverages

The major reaction pathway for the EC formation in stone fruit brandies is related to cyanide (equation 4 and 5), which is originated from precursors – cyanogenic glycosides (e.g. amygdalin). Although the indicated EC levels in fermented mash and in fresh distillates were found relatively very low (not exceeding ~100 ng/ml) its concentration rapidly increased by several orders after exposure to light during storage and manipulation. Therefore methods resulting in reduction of EC levels in distillates have not been focused to

direct EC removing but to withdrawal of EC precursors. The main problem arise from the difficulty in altering the fermentation or distillation process to remove these substances while not affecting taste or appearance of the final product.

Our experiments were carried out in industrial scale with cherry brandy which has been a good export article on the western markets (Germany, Switzerland, ...).

The first method employed interaction of copper (I) chloride (Cyanurex™, T. Goldschmidt AG, Mannheim, Germany) which forms with cyanates and other EC precursors precipitates and/or complexes, and in this way to decrease their level in a distillate. Cyanurex™ should be applied into mash or low wine (distillate after the 1st step of distillation) according to cyanide concentration easy assayed by Cyan-EC-Test kid (Merck). This method is relatively effective (Table III) and useful namely for small distilleries. It should be noted that an incorrect dosing of Cyanurex™ can affect the quality of raw distillate in a negative way, going to the loss of typical fruit aroma.

III. Methods for reduction of EC levels in cherry brandy

Process	Note	EC level per absolute alcohol (ng/ml)
Cyanurex™	no addition	8 200
	dose 10 g/m^3	5 500
	dose 20 g/m^3	4 100
Copper part inserted in pot still lyme	vapour does not pass	3 200
	vapour passes through	400

In the second method the contact of alcoholic vapours with large copper surface placed in the upper part of traditional pot still significantly reduces EC levels and subsequent light-induced formation of EC in stone fruit distillates. During the 2nd step of distillation the alcoholic vapours passed through narrow channels formed between pleated copper plates (apparatus made by firm Arnold Holstein, Markdorf, Germany). The high efficiency of this method was demonstrated in this case where nearly 85% of EC were re-

moved (Table III) and on the other hand the characteristic organoleptic properties of distillates are not so affected and remain preserved.

As it was mentioned above most of EC in stone fruit brandies is formed after distillation. Due to presence of cyanides and other cyanogenic compounds (natural constituents of stone fruits and the main precursors of EC in fruit brandies) post-distillation EC formation may be markedly accelerated by light or heat (Battaglia et al., 1988). This photochemical process has been time-depending and maximal plateau values of EC have been reached in the course of a relatively short time interval, which has not exceeded 2 or 5 days for clear glass bottles exposed to full or overcast daylight (Lawrence et al., 1990). Therefore all samples were stored in colourless clear glass bottles exposed to daily light for more than one week before EC measurements. The determined EC levels corresponded to the maximum feasible EC concentrations in distillates. This manipulation with samples has simulated common situation at the bottling, storage, transport and sale of alcoholic spirits where the consumer mostly drinks these spirits after light exposure, it means with the highest EC content.

Risk Assessment

Regarding potential negative health effect of EC, exposure of humans to this toxin via diet should be estimated. For this purpose mean consumption habits must be known. Considering the recent compilation "Market basket in the Czech Republic" by Ruprich et al. (1993) and taking into account literature data about average levels of EC in foodstuffs, conclusion can be drawn that the most important source of EC in diet is alcoholic beverages. Their average consumption in the Czech Republic together with calculated EC intake is given in Table IV.

As can be seen a total intake of 2.2, resp. 83.5 mg EC per year is equivalent to a daily intake of 6, resp. 230 μg EC. For a person of 70 kg this corresponds to daily dose of 70 ng/kg body weight (b.w.) for "normal" spirits or 3 300 ng/kg b.w. for stone fruit brandies.

Although the toxicological evaluation of EC has not been finished yet, tolerable daily intake (TDI) (mice) of 20 ng/kg b.w. was estimated by the methods using the lowest observed effect level (LOEL) together with safety factor of 5 000 (Uggla, Busk, 1992). Comparing the above data with this value, it is obvious that even in the case of "low EC content spirits" TDI of

IV. Risk assessment of EC for main groups of alcoholic beverages

Alcoholic beverages	Consumption per head and year[a] (l)	Typical EC levels (ng/ml)	Total intake of EC per year (μg)
Spirits (40%)	8.3	200[b] (10 000)[c]	1 700[b](83 000)[c]
Wine	14.8	10	150
Beer	147	2	300
Total EC intake			2 150[b] (83 450)[c]

[a] Statistic data from 1992

[b] Alcoholic spirits such as vodka, gin, rum, etc.

[c] Stone fruit brandies

EC is rather exceeded. Supposing the exclusive intake of stone fruit brandies, calculations indicate a significant risk, TDI is exceeded by two orders of magnitude for such consumer.

CONCLUSIONS

Distilled spirits contain relatively higher levels of EC, namely stone fruit brandies. To reduce the EC content to as low as possible level (it means technically feasible concentration), considerable research is under way to gain an understanding of the mechanism of EC formation in distilled spirits as well as other alcoholic beverages. Due to the significant differences of EC levels found in various alcoholic beverages one can suggest different mechanisms and precursors of EC formation in every single type of distillate such as stone fruit brandies, other fruit brandies, whisky, grape brandy, etc. The reduction of EC in alcoholic beverages should be achieved by optimisation of the whole technological regime (including receipt of raw material, upstream manipulation, fermentation, distillation, maturation, bottling, etc.) having consideration for removing operations that could lead to formation of potential precursors of EC.

EC is a natural and almost ubiquitous food carcinogen, however it has not still been well-know in what way we can reduce its level found in non-alcoholic food. In this connection, we could speculate to what degree EC can be formed directly endogenously in the body. For persons with a high alcohol consumption the lifetime risk can be increased at least by factor of approximately 4–5, due to exposure to EC. In addition, exaggerated consumption of

alcoholic beverages of stone fruit origin might even more contribute to the individual cancer risk as those in general contain high EC concentration.

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References

BATTAGLIA, R. – CONACHER, H. B. S. – PAGE, B. D.: Ethyl carbamate (urethane) in alcoholic beverages and food: a review. Food Add. Contamin., *7*, 1990: 477–496.

LAWRENCE, J. F. – PAGE, B. D. – CONACHER, H. B. S.: The formation and determination of ethyl carbamate in alcoholic beverages. In: NRIAGU J. O. et al. (Eds.): Agents from Food Contamination from Environmental Sources. John Wiley & Sons, Inc. 1990: 457–471.

LFROTH, G. – GEJVALL, T.: Diethyl pyrocarbonate: formation of uretan in treated beverages. Science, *174*, 1971: 1248–1250.

OUGH, C. S.: Ethyl carbamate in fermented beverages and foods – part I and II. J. Agric. Food Chem., *24*, 1976: 323–328 and 328–331.

OUGH, C. S. – CROWELL, E. A. – GUTLOVE, B. R.: Carbamyl compound reactions with ethanol. Am. J. Entol. Vitic., *39*, 1988: 239–242.

RUPRICH, J. (Ed.) – ČERNOEVIČOVÁ, M. – KOPŘIVA, V. – OSTRÝ, V. – RESOVÁ, D. – ŘEHUŘKOVÁ, I. – WALTEROVÁ, D.: Food basket in Czech Republic 1993. Centre of Hygiene of Food Chain in Brno and National Institute of Public Health in Prague 1993.

UGLA, A. – BUSK, L.: Ethyl carbamate (urethane) in alcoholic beverages and foodstuffs – a Nordic View. Nordiske Sem. Arbejds rapporter, *570*, 1992: 1–59.

WUCHERPFENNIG, K. – CLAUSS, E. – KONJA, G.: Beitrag zur Entstehung des Ethylcarbamats in alkoholischen Getrenkenaus der Basis der Sauerkirsche 'Maraska'. Dtsch. Lebensm. Rdsch., *83*, 1987: 344–349.

ZIMMERLI, B. – SCHLATTER, J.: Ethyl carbamate: analytical methodology, occurrence, formation, biological activity and risk assessment. Mutation Res., *259*, 1991: 325–350.

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Výskyt a zhodnocení zdravotního rizika („risk assessment") ethyl karbamátu v alkoholických nápojích

Údaje o obsahu ethyl karbamátu (EC) v alkoholických nápojích vyráběných v České republice doposud nebyly v odborném tisku souborně publikovány, proto první část této studie je zaměřena na monitorování obsahu EC v některých typech vybraných destilátů domácí produkce. Ethyl karbamát jako potencialní karcinogen je běžně přítomen v nízkých koncentracích ve většině fermentovaných potravin a nápojů (např. čaj, citrusuvé džusy, jogurt, sýr, chléb, vinný ocet, sójová omáčka, pivo). Poněkud vyšší obsah EC byl zjištěn u alkoholických nápojů jako jsou vína, dezertní vína a lihoviny. Nejexponovanější skupinou podle obsahu EC jsou destiláty, a to zejména destiláty z peckového ovoce (švestky, meruňky, třešně, višně atd.), u kterých se koncentrace EC pohybovaly ve velmi širokém rozmezí 400 až 14 000 ng/ml absolutního alkoholu. Hygienické limity přípustných koncentrací EC v alkoholických nápojích jsou zavedeny v řadě vyspělých států (např. Kanada, USA, SRN a Švýcarsko), v ČR zatím nikoliv. Nicméně analýzy EC jsou běžně prováděny Centrální laboratoří ČZPI v Praze a dalšími institucemi (např. VŠCHT Praha) na vyžádání výrobců u destilátů exportovaných do těchto států.

Druhá část práce se zabývá možnostmi snížení obsahu EC v třešňovém destilátu, který je výhodným exportním artiklem a pro který je bohužel typický právě zvýšený obsah EC. V destilátech z peckového ovoce je převážná část EC tvořena po destilaci fotochemickými reakcemi z prekursorů EC, kterými jsou kyanogenní glykosidy, např. amygdalin. Proto metody, které vedou ke snížení obsahu EC, se zaměřují na odstranění či snížení koncentrace těchto prekursorů EC ve zralé zápaře nebo v průběhu destilace. Jednou z možností, jak snížit množství EC přítomných v konečném destilátu, je použití komerčního preparátu Cyanurex™ (výrobce T. Goldschmidt AG, Mannheim, SRN), který využívá reakce chloridu měďného s deriváty kyanidů, nebo zařazení speciálního nástavce firmy Arnold Holstein (Markdorf, SRN) před deflegmátor do parního dómu klasického destilačního zařízení, ve kterém dochází k intenzivnímu kontaktu lihových par s velkým povrchem speciálně uspořádané měděné výplně. Použití tohoto nástavce vedlo ke snížení obsahu EC až o 85 % proti stavu, kdy byl v destilačním systému vyřazen.

V práci se autoři také zabývají hodnocením zdravotního rizika EC konzumovaných ve formě alkoholických nápojů. Publikované výsledky vycházejí ze statistických údajů průměrné spotřeby jednotlivých druhů alkoholických nápojů na obyvatele a z údajů tzv. Spotřebního koše potravin v ČR a ukazují na poměrně vysoké zatížení lidského organismu EC pocházející právě z lihovin a zejména z ovocných destilátů, u kterých tolerovaná denní expozice – TDI (stanovený zdravotní limit)

– byla překračována o více než dva řády. Vzhledem k zabezpečení ochrany zdraví obyvatelstva je otázka snižování EC v lihovinách velice aktuální.

ethyl karbamát; alkoholické nápoje; destiláty; výskyt EC; tvorba EC; hodnocení zdravotního rizika EC; „risk assessment" EC

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PURIFICATION OF THIAMINASE I FOR ANALYTICAL PURPOSES

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Abstract: Extracellular thiaminase I was purified by successive fraction saturation with ammonium sulphate followed by dialysis and FPLC gel chromatography on Superose 12. Final lyophilized product was stable for 1 month at 28 °C and for more than 4 months at –15 °C.

thiaminase; thiamine determination

Bacterial thiaminase I (i.e. thiamine: base 2-methyl-4-amino-pyrimidin-5--methenyl transferase EC 2.5.1.2) has been successfully used for the simplification of the determination of thiamine in both foods and clinical material (Ruml, 1984; Ruml et al., 1988; Mochida et al., 1984). Fermentation production of thiaminase I uses *Bacillus thiaminolyticus* and relatively rich medium which is the main source of ballast material in the obtained solution of extracellular thiaminase I. Moreover, *B. thiaminolyticus* produces also extracellular proteinases that contribute to relatively low stability of this enzyme in centrifuged fermented medium. For these reasons, we tried to eliminate the effect of proteinases and to purify thiaminase I for analytical purposes. Wittliff and Airth (1968) isolated thiaminase I from centrifuged fermented medium by partial saturation with ammonium sulphate (75%, 50% and 70% successively) followed by gel chromatography on Sephadex G-100, dialysis and ion-exchange chromatography on DEAE--Sephadex. They reached very high purity of the enzyme but the final yields were about 10%.

MATERIALS AND METHODS

Microorganism and its cultivation

Bacillus thiaminolyticus DBM 1068 was cultivated as described previously (Ruml, Šilhánková, in press).

Thiaminase Precipitation

Fermented medium was centrifuged (40 min, 3000 g) and supernatant was saturated in two steps (50% and 85%) with ammonium sulphate. The first precipitate was discarded and the second was resuspended in distilled water and dialysed 24 h at 4 °C against distilled water.

Gel Chromatography on Sephadex G-100

4 ml samples were applied to a Sephadex G-100 column (16 x 80 mm) equilibrated with 0.05 M Tris-HCl buffer (0.05 M, pH 6.5). Proteins were eluted with the same buffer at the flow-rate of 12 ml/h.

FPLC Gel Chromatography

0.2 ml samples were applied to Superose 12 column (Pharmacia-LKB, Bromma, Sweden) equilibrated with Tris-HCl buffer (0.05 M, pH 8.2). Proteins were eluted with the same buffer at the flow rate of 30 ml/h.

FPLC Anion Exchange Chromatography

0.2 ml samples were applied to a Mono Q column (Pharmacia-LKB, Bromma, Sweden) equilibrated with 0.05 M Tris-HCl buffer pH 8.2. Bound proteins were eluted with the linear gradient of 0–0.5M NaCl under the pressure of 2.1 MPa at the flow rate 60 ml/h.

Determination of Proteolytic Activity

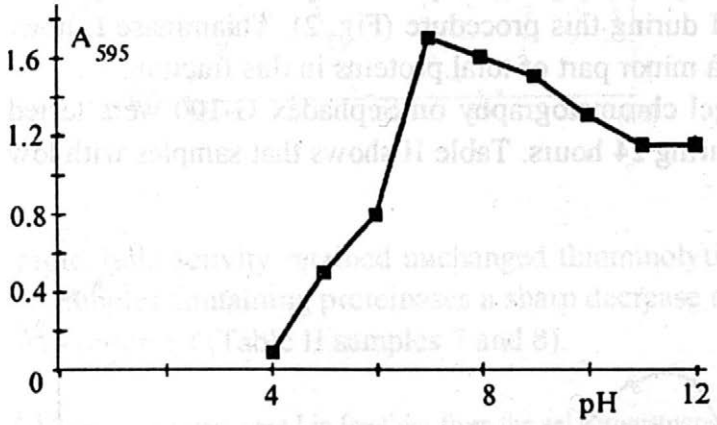
The presence of proteinases was detected as a lysed zones on the plates with 15% gelatine with adjusted pH. The proteolytic activity was estimated using Hide Powder Azure (Calbiochem, USA). The samples were incubated 15 min at 40 °C with the swollen substrate in Tris-HCl buffer (0.05 M, pH 8.2). The amount of deliberated dye corresponding to proteolytic activity of the sample was measured at 595 nm after centrifugation (3 000 g, 10 min).

Determination of Thiaminolytic Activity

The mixtures containing thiamine (7.4 μM) and pyridine (0.04 M) in 0.05 M citrate-phosphate buffer of pH 6.5 were incubated with thiaminase at 40 °C for 10 min. The reaction was stopped by the addition of NaOH to final 3% concentration and remained thiamine was determined by thiochrome method.

RESULTS AND DISCUSSION

In fermented centrifuged medium, the activity of thiaminase I ranged from 0.5 to 1.3 U/ml after 74h fermentation. This means that 2 µl of this medium destroy more than 3 nmols of thiamine within 10 min and are, therefore, sufficient for the preparation of blanc for the determination of thiamine by thiochrome method. Crude thiaminase I was however very unstable due to the presence of extracellular proteinases produced by *B. thiaminolyticus*. The dependence of proteolytic activity of fermented medium on pH (Fig. 1) shows that the pH optimum of present proteinases is approximately 7. At the pH 5.8–6.8, which is optimum for thiaminase I, the activity of proteinases still represents more than 50% of their maximum activity. Much lower decrease of proteinase activity in alkaline region (Fig. 1) suggests the presence of alkaline proteinases.



1. The effect of pH on proteolytic activity

As proteinases are known to be inhibited by EDTA, we tested the effect of EDTA in the centrifuged fermented medium. 1 mM EDTA (final concentration) was shown to be optimal. It led to the lowering of A_{595} from the original value of 0.210 to 0.080, but further additions of EDTA (5–20 mM) led only to slight lowerings (to 0.070–0.065). For these reasons, we tried to purify thiaminase I. Centrifuged fermented medium was brought to partial ammonium sulphate saturation (Table I). Most contaminating proteins precipitated at 50% saturation. 85% saturation of the supernatant from the 50% saturation led to a 17-fold increase of specific activity in the dialysed precipitate (Table I). Total recovered activity was 83%.

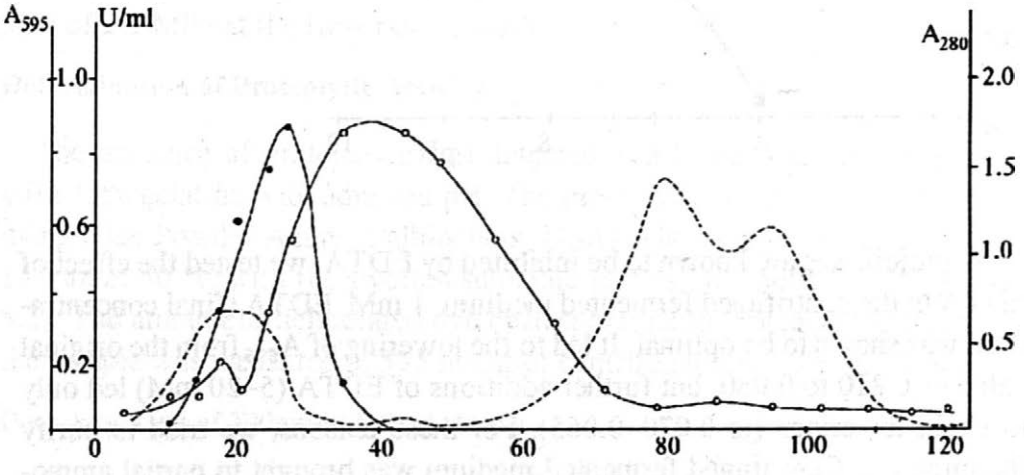
I. Purification steps during precipitation in urea

Saturation [%]	Thiaminase activity			Proteolytic activity	
	total [U/ml]	[U/mg]	[U[	[U/ml]	[U/mg]
0	0.412	0.090	536	0.300	0.658
50 (supernatant)	0.360	0.139	468	0.020	0.069
50 (precipitate)	5.120	0.203	52	1.200	0.477
85 (supernatant)	0.032	0.019	29	0.001	0.006
85 (precipitate)	8.080	0.411	121	1.060	0.539
85 dial. precip.	6.190	1.510	445		

The activity of thiaminase I was estimated using thiochrome method. Proteolytic activity was estimated using chromogenic substrate as described in Materials and Methods

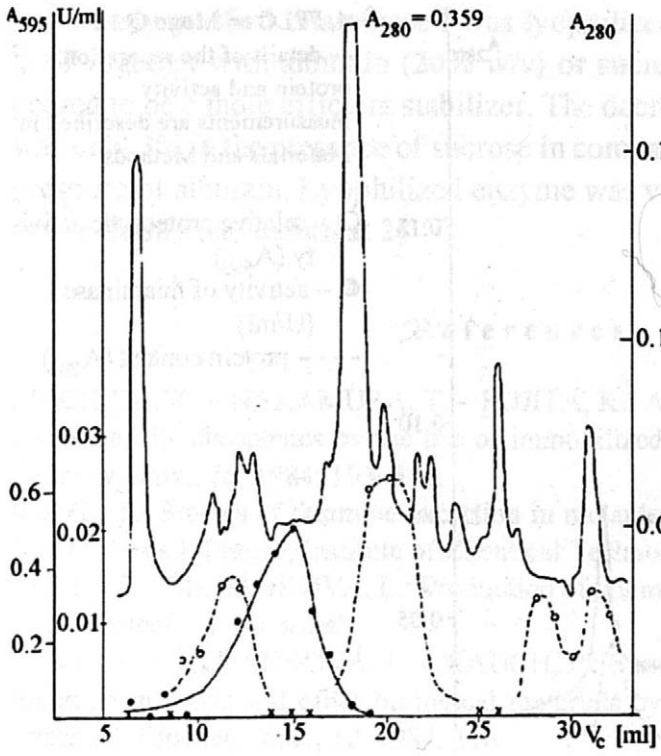
Gel chromatography on Sephadex G-100 was used as the further purification step. Most residual proteolytic activity was separated from fractions containing thiaminase I during this procedure (Fig. 2). Thiaminase I, however, represented only a minor part of total proteins in this fraction.

Fractions from the gel chromatography on Sephadex G-100 were tested for stability at 28 °C during 24 hours. Table II shows that samples with low



○ – relative proteolytic activity (A_{595}); ● – activity of thiaminase I (U/ml); - - - - protein content (A_{280})

2. Gel chromatography on Sephadex G-100 – details of the separation, protein and activity measurements are described in Materials and Methods



3. FPLC on Superose 12 – details of the separation, protein and activity measurements are described in Materials and Methods

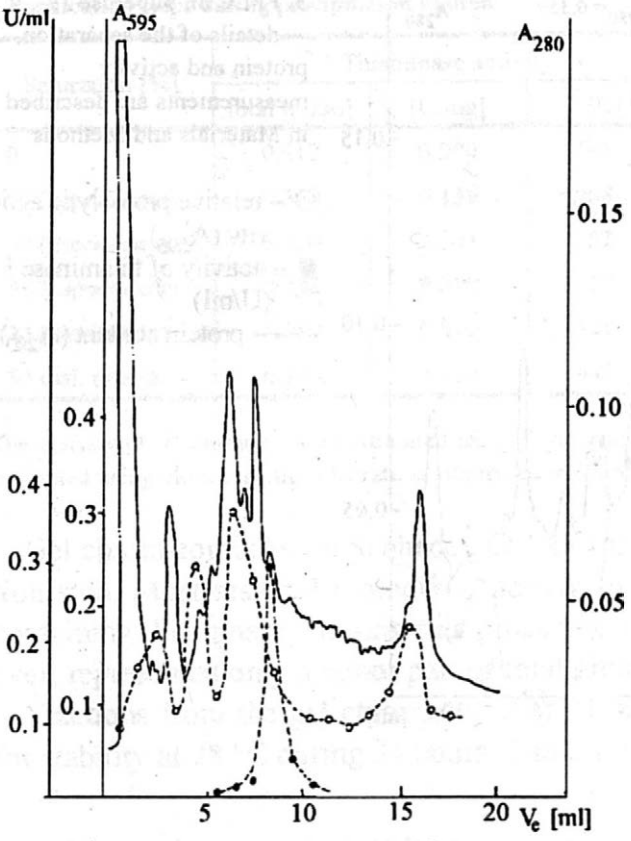
○ – relative proteolytic activity (A_{595})
● – activity of thiaminase I (U/ml)
- - - - protein content (A_{280})

proteolytic activity retained unchanged thiaminolytic activity for 24 hours. In samples containing proteinases a sharp decrease of thiaminolytic activity was observed (Table II samples 7 and 8).

II. Stability of thiaminase I in fractions from the gel chromatography on Sephadex G-100

Fraction	Relative proteolytic activity	Tiaminase I activity [U/ml]	
		0 h	24 h
3	0.114	0.09	0.08
4	0.205	0.23	0.23
5	0.135	0.46	0.49
7	0.540	0.86	0.44
8	0.840	0.14	0.02

Thiaminase I was incubated 24h at 28 °C in the presence of dithiothreitol (0.1 mM), the residual activity of thiaminase I was estimated using thiochrome method as described in Materials and Methods



4. FPLC on Mono Q 12 – details of the separation, protein and activity measurements are described in Materials and Methods

O – relative proteolytic activity (A_{595})
● – activity of thiaminase I (U/ml)
- - - - protein content (A_{280})

Better separation of thiaminase I from proteinases and other proteins was achieved using FPLC with Superose 12 column (Fig. 3). Most ballast proteins originated from the growth medium, particullarly from yeast extract. FPLC ion-exchange chromatography with Mono Q column (Fig. 4) gave again four peaks with proteolytic activity as with Superose 12, but the peak with thiaminase activity was much broader here and had much higher content of proteinases (Fig. 3 and 4).

Thiaminase I was stabilized by the addition of 10mM dithiothreitol (DTT), 10mM cystein, 1mM EDTA, sucrose (10%) or albumin (20%). To prevent microbial contamination sodium azide (0.02%) was added to all the samples. The addition of EDTA and albumin appeared to be the best combination which resulted in unchanged stability during five weeks at 4 °C. Results obtained in this experiment suggested that the addition of 1 mM EDTA had a slight stimulatory effect. DTT and particularly cystein had practically no effect probably because of their rapid oxidation. Neither the addition of sucrose (10% final concentration) was efficient.

Partially purified thiaminase I was lyophilized in the presence of 10 mM DTT together with albumin (20% w/v) or sucrose (10% w/v). Sucrose appeared to be a more efficient stabilizer. The decrease of thiaminase I activity was only 3% in the presence of sucrose in comparison to 12% decrease in the presence of albumin. Lyophilized enzyme was very stable. It remained fully active during one month at 28 °C.

References

MOCHIDA, K. – NAKAMURA, T. – FUJITA, K.: Automatic determination of thiamine and its phosphates by the use of immobilized thiaminase I. Bull. Fac. Agr. Shimane Univ., *18*, 1984: 193–196.

RUML, T.: Studies of thiamine excretion in mutants of *Saccharomyces cerevisiae*. [PhD. Thesis.] Prague, Institute of Chemical Technology 1984.

RUML, T. – ŠILHÁNKOVÁ, L.: Production of thiaminase I for analytical purposes. Acta. Biotechnol. (in press).

RUML, T. – ŠILHÁNKOVÁ, L. – RAUCH, P.: Simplification of the estimation of thiamine in foods and other biological materials by means of thiaminase I. Bull. Czechosl. Biochem. Soc., *12*, 1984: 116.

RUML, T. – ŠILHÁNKOVÁ, L. – RAUCH, P.: Pat. ČSSR 239033. 1988

WITTLIFF, J. L. – AIRTH, R. L.: The extracellular thiaminase of *Bacillus thiaminolyticus* I. Purification and physicochemical properties. Biochemistry, *7*, 1968: 736–744.

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Purifikace thiaminasy I pro analytické použití

Thiaminasa I byla purifikována frakčním srážením síranem amonným, po němž následovala dialýza a FPLC na Superose 12. Byl získán lyofilizovaný produkt, který byl stabilní jeden měsíc při teplotě 28 °C a čtyři měsíce při –15 °C.

thiaminasa; stanovení thiaminu

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DETERMINATION OF D-GLUCOSE IN SOFT DRINKS USING OPTICAL BIOSENSOR*

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Abstract: An optical glucose biosensor has been tested on five samples of different soft drinks. It has been shown that this biosensor is comparable in accuracy, specificity and duration of the analysis with the amperometric glucose biosensor as well as the classical spectrophotometric enzyme test. The biosensor's operational stability was higher than 200 analyses within two months. Its characteristics are discussed here with respect to its application in food industry.

biosensor; glucose oxidase; glucose; absorption; fibre optic biosensor; soft drink

Glucose is the most frequently occurring saccharide in nature. It is a component of many polysaccharides (starch, glycogen, cellulose) and oligosaccharides (sucrose, lactose, maltose); free glucose is the most important nutrition for living organisms. Therefore, its analysis has a key position in food industry, biotechnology and clinical analysis. In food industry the determination of glucose is applied in supervising the production and foodstuff quality control (fruit juices, wines, confectionery, milk products, honey) (Dremel et al., 1989). In clinical practice glucose concentration in blood and urine is the most valuable indicator of the current metabolic state of a patient suffering from diabetes or some other metabolic diseases (Schaffar, Wolfbeis, 1990).

For glucose determination classical spectrophotometric methods for reducing sugars are frequently used, e.g. Somogyi's method, Luff-Schoorl's method (Davídek et al., 1977). Their main disadvantage is low specificity and tediousness of the determination. For resolution of saccharides in mixture the gas chromatography could be applied, which is extraordinary demanding with respect to complications with volatile sample preparation, requirements on staff's experience and high equipment cost.

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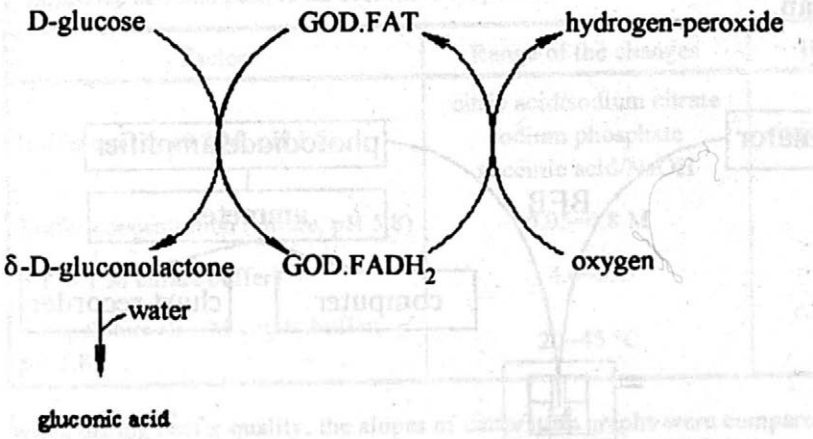
An effort invested into increasing the specificity of the glucose analysis has led to preparation and expansion of standardised enzyme tests (from Lachema Brno, Sigma Chemical Company, Boehringer Mannheim, etc.); as the enzyme preparations are relatively expensive, the price per analysis is high here. For this reason, biosensors with immobilized enzymes are becoming widely used. Immobilization enables to utilise an enzyme repeatedly, making the analysis more simple and considerably cheaper. In addition, biosensors are also the only systems that allow continuous determination of glucose, e.g. on-line monitoring of glucose as a raw material in fermentations or control of glucose level in blood of diabetic patients (Trettnak et al., 1988). The most frequently applied biosensors utilise glucose oxidase (GOD, Fig. 1) and consumed oxygen or produced hydrogen-peroxide are detected amperometrically. Extensively differentiated requirements for glucose determination could bring about certain difficulties in some of their applications; therefore, further methods and principles have been developed.

Fibre optic biosensors (Yun-Xiang Ci et al., 1992; Narayanaswamy, Sevilla, 1988; Meadows, Schultz, 1993) are one of the novel analytical approaches that appeared in the late 70's when optical fibres started to be applied in communications. The relatively simple construction, versatility and displacement of an electric signal by an optical one make these biosensors an interesting and promising alternative to the already introduced analytical schemes, as shown in this paper.

MATERIALS AND METHODS

Material

Glucose oxidase (EC 1.1.3.4, β-D-glucose:oxygen 1-oxidoreductase, from *Aspergillus niger*, GOD, first preparation: 250 U/mg solid containing 10 U of catalase per mg solid from Biozyme Laboratories Ltd., Blaenavon, Great Britain; second preparation: 273 U/mg solid containing 5 U of catalase per mg solid from Serva Feinbiochemica GmbH & Co., Heidelberg, Germany) was immobilized on a nylon net. Catalase (EC 1.11.1.6, hydrogen-peroxide:hydrogen-peroxide oxidoreductase, from beef liver, CAT, 2 000 U/mg of suspension, Reanal, Budapest, Hungary) was applied in the form of undiluted crystalline suspension. Other chemicals of analytical grade were purchased from Lachema Brno, CZ,



1. Scheme of glucose oxidase reaction

Sigma Chemical Company, St. Louis, USA or Fluka Chemie AG, Buchs, Switzerland. Reference studies were performed with Oxochrom GLUCOSE (bio-test for enzyme determination of D-glucose) from Lachema Brno, CZ.

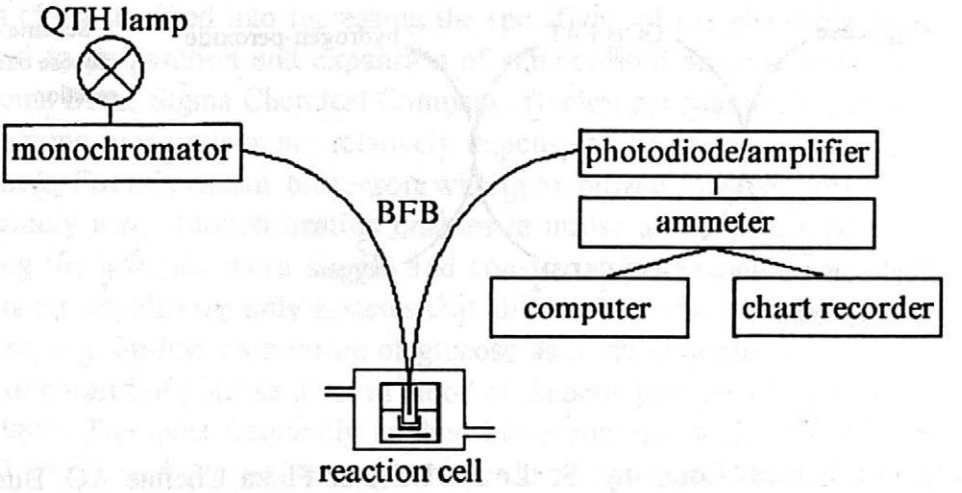
Analysis with Fibre Optic Biosensor

The analysis was performed in batch system (Fig. 2). Upon the common end of a bifurcated optical fibre bundle a nylon net with immobilized GOD (Vrbová, Marek, 1990) was fixed. The bundle was then immersed into thermostated (30 °C) and stirred reaction cell with 1 ml of 0.1M citrate buffer (pH 5.8); after 100 µl of sample was added, the yellow enzyme layer (GOD.FAD) decolorized (GOD.$FADH_2$) and this analytical information was processed by the electrooptical system. One leg of the bundle brought monochromatic light of 490 nm to the enzyme layer; the second leg led away light reflected from the layer to photodiode. Change of photodiode signal in time was registered by computer and/or chart recorder (Fig. 3).

Reference Methods

Amperometric Biosensor (Vrbová et al., 1990): Immobilized GOD was fixed on Clark-type oxygen sensor. Detected consumption of dissolved oxygen was proportional to glucose concentration in the sample.

Spectrophotometric Enzyme Test (Roháček et al., 1987): This method also utilised the reaction catalysed by GOD (Fig. 3). In the presence of GOD and peroxidase in solution, hydrogen-peroxide produced was determined by oxidative coupling with substituted phenol and 4-aminophenazone. Absorption of the dye produced at 492 nm was proportional to the glucose concentration in the sample.

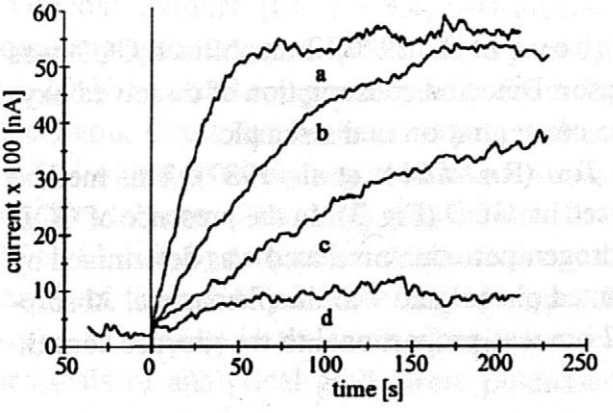


2. Arrangement of the analytical system: quartz tungsten-halogen lamp, ARC model TS-428, 250W, Acton Research Corporation, USA; monochromator MonoSpec 18, Thermo Jarrel Ash Corporation, USA; BFB – bifurcated fibre bundle from Optokon Jihlava, CZ; "home-made" glass thermostated and stirred reaction cell; photodiode, model 71925, Oriel, Oriel Corporation, USA; ammeter, model 7072, Oriel, Oriel Corporation, USA; PC 386; chart recorder, model 4200, Laboratorní přístroje, Prague, CZ

RESULTS AND DISCUSSION

Characterisation of the Sensor Prepared

The influence of several factors on the biosensor response has been tested at a constant glucose concentration (Table I); these experiments are, in detail, described elsewhere (Chudobová et al., 1995). All further experiments were performed using 0.1M citric buffer pH 5.8 at 30 °C. Under these



3. Typical shapes of the time-dependencies of photocurrent:
a) 80 mM glucose, b) 30 mM glucose, c) 20 mM glucose, d) 10 mM glucose

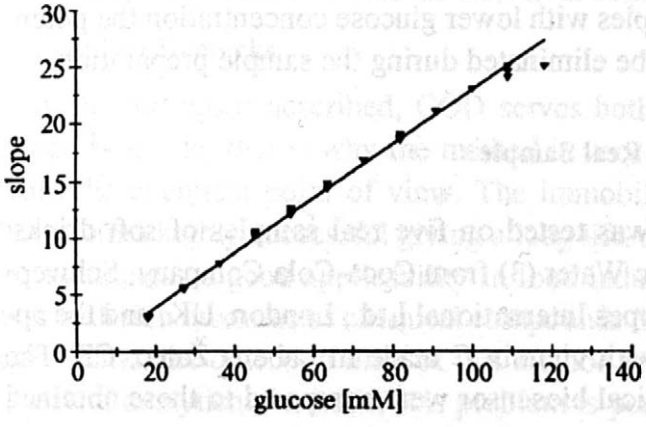
I. Influence of some factors on biosensor response

Factor	Range of the changes	Highest response
Buffer quality (0.2 M, pH 5.8)	citric acid/sodium citrate sodium phosphate succinic acid/NaOH	citric acid/sodium citrate
Buffer concentration (citrate, pH 5.8)	0.05–0.8 M	0.2 M
pH (0.1 M citrate buffer)	4.0–8.0	5.8
Temperature (0.1 M citrate buffer, pH 5.8)	20–45 °C	37 °C

When testing buffer quality, the slopes of calibration graphs were compared; in all other experiments 100 µl of 60 mM glucose were applied into the reaction cell

conditions the stability of the immobilized enzyme layer (GOD from Biozyme) has been very good, no change in activity has been achieved after 200 analyses, and the storage stability has been longer than 30 months.

The maximum slope – glucose concentration dependence is depicted in Fig. 4. Analytical range from 20 to 110 mM glucose in the sample has been achieved. Lower value is also the lowest objectively detectable concentration. Response time of the sensor ranges from several seconds for high glucose concentrations to 5 minutes, which are necessary for the analysis of concentrations close to detection limit. Regeneration of the biosensor lasts from 2 to 15 minutes and its length is strongly dependent on the glucose concentration used in the previous experiment.



4. The dependence of biosensor response on glucose concentration.
The biosensor response represents the maximum slope of the time-dependence of the photocurrent (Fig. 3). Each measured concentration was analysed three times

The Biosensor and Oxygen

Oxygen is one of the reasons for the relatively high detection limit of glucose determination as confirmed by our further experiments. Oxygen from the 0.1M citrate (pH 5.8) stock buffer solution was displaced by nitrogen (bubbling). One ml of deoxygenated buffer was pipetted into the reaction vessel and above the surface of the liquid nitrogen was introduced. Detection limit of this arrangement has been shifted from 20 mM down to 5 mM of glucose in the sample.

Catalase is an enzyme which is able to decompose hydrogen-peroxide produced in GOD reaction. Therefore, it is frequently coimmobilized with GOD and in this way protection of GOD layer against hydrogen-peroxide is achieved. On the other hand, catalase produces oxygen that increases the value of detection limit. As catalase could appear in real samples and commercial preparations of GOD, its influence on the analysis has been studied. The addition of 20 000 U of catalase into reaction mixture affected the lower part of the calibration curve only and raised detection limit by approximately 35%.

As catalase returning oxygen back to the reaction helps to reoxidise prosthetic group of the GOD it also shifts the steady state between yellow and leucoform of the enzyme in undesirable direction for the analysis and causes increase of detection limit. During the tests with catalase in the reaction mixture both calibration graphs, with and without catalase, were comparable for the glucose concentrations higher than 30 mM where the GOD.FAD reduction was the only process influencing the analysis. Therefore, addition or coimmobilization of catalase would be advantageous for the determination of higher glucose concentrations, where catalase will bring longer life-time of the enzyme layer; in samples with lower glucose concentration the potential catalase activity should be eliminated during the sample preparation.

Determination of Glucose in Real Sample

The biosensor prepared was tested on five real samples of soft drinks: Sprite (1), Lift (2) and Tonic Water (3) from Coca-Cola Company, Schweppes Lemon (4) from Schweppes International Ltd., London, UK, and the apple beverage (5) enriched with vitamin C made in Labena Žatec, CZ. The results reached with the optical biosensor were compared to those obtained

II. Calibration procedures

	Range of the calibration, glucose [mM]	Calibration equation	Standard error of the slope	Number of data points	*T*-value	$T_{krit;0.05.}$
Optical biosensor	20–100	$y = 0.264c$	0.0023	24	0.129	2.07
Enzyme electrode	1.25–10.00	$y = 2.43c$	0.027	15	0.308	2.16
Spectro-photometric enzyme test	1.0–20.0	$y = 0.047c$	0.0004	6	0.069	2.78

The hypothesis, whether Y — intercept of the calibration straight-line is 0 ($\alpha = 5\%$), is analysed by T-test. The answer is positive if T-value < $T_{krit;0.05}$

with an amperometric biosensor (Vrbová et al., 1990) and a commercial enzyme test (Roháček et al., 1987). All three methods required preparation of calibration graph (Table II). From the equations of calibration graphs the concentrations of glucose in the samples were evaluated (Table III).

As sample 5 was used almost undiluted in the case of fibre optic biosensor, the interference due to its chromaticity was expected. However, since the immobilized enzyme layer was in intimate contact with the common end of optical fibre bundle, light reflected from the layer (and the net) could be only slightly influenced by colour alternations in the bulk solution.

The correlation coefficient 0.9996 was achieved for comparison of both biosensors – the optical one and the amperometric one. Correlation coefficient for the optical biosensor and spectrophotometric method was found at 0.9992. It can be concluded from both table, and correlation coefficients, that the optical biosensor accords well with both standard methods.

Concluding Remarks

In the biosensor described, GOD serves both as an indicator and transducer molecule; that is why the method is very simple and straightforward from the chemical point of view. The immobilization technique used has been found highly successful giving a long life-time to the sensor; its stability facilitates its good applicability in food industry where it comes in contact with rich mixtures of chemical compounds forming real samples. On the other hand, the detection limit of this method is quite high compared to the classical analytical methods; this problem is possible to overcome working

III. Determination of glucose concentrations in five soft drinks by fibre optic biosensor (A), amperometric biosensor (B) and spectrophotometric enzyme test (C)

Method		Sample				
		1	2	3	4	5
A	dilution	–	1:3	–	1:3	1:1
	conc. 1	67.1	241.0	16.7	145.5	77.3
	conc. 2	67.1	245.5	16.7	153.0	82.6
	conc. 3	67.8	253.0	17.4	154.6	81.1
	conc. 4	70.1	230.3	16.3	145.5	82.6
	c [mM]	68.0	242.4	16.8	149.7	80.9
	s	1.4	9.5	0.5	4.9	2.5
B	dilution	1:9	1:39	1:9	1:39	1:9
	conc. 1	67.1	237.0	15.2	144.9	74.1
	conc. 2	66.7	242.0	16.5	144.9	74.9
	conc. 3	65.8	238.7	17.3	141.6	75.3
	conc. 4	66.3	237.0	16.0	144.9	74.1
	conc. 5	67.1	235.4	16.5	139.9	74.5
	c [mM]	66.6	238.4	16.3	143.2	74.6
	s	0.5	2.5	0.7	2.3	0.5
C	dilution	1:9	1:19	1:1	1:19	1:7
	c [mM]	66.2	242.4	16.7	142.6	76.3

dilution = sample : buffer solution; conc. = concentration calculated from experimental data by using calibration equation from Table II; *c* = average of concentration; *s* = standard deviation of concentration

in the deoxygenated buffer, using GOD layers with the lowest catalase activity (see above) or applying the flow system analysis.

The equipment used for the method could be considerably simplified compared to our system (Fig. 2): cheaper plastic fibres as well as a simple tungsten lamp in combination with a narrow band-pass filter (instead of a monochromator) could be applied; these changes would significantly decrease both equipment cost and size. Our experiments have confirmed that the photodiode is sufficiently sensitive in this application and that it is not

necessary to use a more expensive and fragile photomultiplier here. In combination with other general advantages of optical biosensors this system could compete well with already existing and commercially exploited analytical schemes.

References

CHUDOBOVÁ, I. – VRBOVÁ, E. – KODÍČEK, M.: Fibre optic biosensor for determination of D-glucose based on absorption changes of glucose oxidase. 1995 (in press).

DAVÍDEK, J. a kol.: Laboratorní příručka analýzy potravin. SNTL, Praha 1977: 227–252.

DREMEL, B. A. A. – SCHAFFAR, B. P. H – SCHMID, R. D.: Determination of glucose in wine and fruits based on fibre-optic biosensor and flow-injection analysis. Anal. Chim. Acta, *225*, 1989: 293–301.

MEADOWS, D. L. – SCHULTZ, J. S.: Design, manufacture and characterization of an optical fiber glucose affinity sensor based on an homogeneous fluorescence energy transfer assay system. Anal. Chim. Acta, *280*, 1993: 21–30.

NARAYNASWAMY, R. – SEVILLA, F.: An optical fibre probe for the determination of glucose based on immobilized glucose dehydrogenase. Anal. Lett., *21*, 1988: 1165–1175.

ROHÁČEK, J. – CHROMÝ, V. – KOLÁŘOVÁ, J.: Kinetické stanovení glukosy soupravou Bio-La-Test Oxochrom GLUKOSA. Biochem. Clin. Bohemoslov. *16*, 1987: 183–188.

SCHAFFAR, B. P. H. – WOLFBEIS, O. S.: A fast responding fibre glucose biosensor based on an oxygen optrode. Biosens. Bioelectron., *5*, 1990: 137–148.

TRETTNAK, W. – LEINER, M. J. P. – WOLFBEIS, O. S.: Fibre optic glucose biosensor with an oxygen optrode as the transducer. Analyst, *113*, 1988: 1519–1523.

VRBOVÁ, E. – MAREK, M.: Application of the Ugi reaction for the preparation of an enzyme electrodes, Anal. Chim. Acta *239*, 1990: 263–268.

VRBOVÁ, E. – MAREK, M. – BŘEZINA, P.: Stanovení D-glukosy, sacharosy a laktosy pomocí enzymové elektrody na D-glukosu. Potrav. Vědy, *8*, 1990 (3): 161–168.

YUN–XIANG CI – JIAN-KE TIE – FENG-JI YAO – ZHAO-LAN LIU – SA LIN – WEI-OING ZHENG: Catalytic behaviour of iron (II) – oxime complexes in the chemiluminescence reaction of luminol with hydrogen peroxide. Anal. Chim. Acta, *277*, 1993: 67–72.

BIOZYME LABORATORIES, Ltd.: Glucose oxidase – product information. Great Britain.

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Použití optického biosenzoru pro stanovení D-glukosy v nealkoholických nápojích

Nově připravený optický glukosový biosenzor byl testován na pěti vzorcích různých nealkoholických nápojů. Přesnost, specifita a délka trvání analýzy tohoto biosenzoru je srovnatelná s amperometrickým biosenzorem i se spektrofotomerickým enzymovým testem. Po provedení 200 analýz během dvou měsíců se odezva biosenzoru výrazněji nezměnila. Chování a vlastnosti biosenzoru jsou zde diskutovány s ohledem na jeho potenciální aplikaci v potravinářském průmyslu.

biosenzor; glukosaoxidasa; glukosa; absorpce; optický vláknový biosenzor; nealkoholický nápoj

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Potrav. Vědy, *13*, 1995 (3) : 199–212

A MATURATION OF RYE-WHEAT DOUGHS BY SOUR DOUGH OR BY BAKER'S YEAST

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Abstract: The characteristic feature of the contemporary breadmaking technology is an application of many different improvers. One of the possibilities how to shorten production time of mixed rye-wheat bread production is to use a complex improver based on dried or liquid sour dough concentrate. Dough is leavened by yeast instead of matured sour dough. In our work, different doughs were made using the natural sour dough from rye flour as well as using three types of sour dough concentrates. The maturation of these doughs was compared with RHEOFERMENTOMETER F2 (Chopin, France). Dough volume, CO_2 volume and breaking point of gas retention were measured to testify the maturation of doughs.

rye-wheat bread; sour dough concentrates; Rheofermentometer

The products from mixed rye-wheat flour have a dominant position in our traditional bakery assortment.

The production of rye-wheat doughs differs from wheat dough production by several specific characteristics:

a. Special low-viscous suspension of sound rye flour can be prepared and left to ferment spontaneously to make rye sour. It can be used for leavening the dough instead of yeast. However, in industrial production a part of matured sour is usually retained as a starter from which new sour is obtained for the next production cycle. Its purpose is to introduce the microflora consisting of lactic acid bacteria and yeasts.
b. Due to different dough structure, mixing of rye dough should be less intensive and shorter than mixing of wheat dough as a gluten network does not develop in the same way. In rye-wheat dough with a prevailing part of wheat flour mixing is more similar to wheat dough.
c. Rye doughs are less elastic, more flowing and more sticky than wheat doughs.

d. Yields of rye doughs and products are usually higher than that of wheat products.

Two methods are used for rye-wheat dough processing and leavening:
– the sour dough, using a sour as leavening agent,
– the straight dough, using baker's yeast for leavening.

A contemporary breadmaking technology can choose from a wide range of improving ingredients (enzymes, emulsifiers, oxidizing and reducing agents, hydrocolloids, whey or milk proteins, etc.). The aims of the improvers application is to optimize dough properties and to improve bakery products quality. The main effects on the characters of dough quality can be described as follows:
– rheological dough properties (increasing or decreasing of dough stability, adjustment of dough extensibility – gluten complexing to improve handling and machinability of doughs, optimizing gas retention),
– release of fermentable sugars to increase the fermentation rate,
– water absorption (rather increasing is desirable),
– shortened dough development processes (dough mixing time reduction) (Příhoda et al., 1993).

Consequently, improvers affect the quality parameters of final products – loaf volume and symmetry, crust color, crumb firmness, smoothness of break and shread, texture, extension of shelf-life. The beneficial effect of improvers on flavor, aroma or nutritional value is also important.

The nature of improving effect of improvers in rye-wheat dough can be rather different. The complex improvers are used together with baker's yeast for a method of straight dough (all ingredients are mixed together without any preferment step) and it helps to give the rye bread taste and aroma similar or close to the bread processed by the sour method. It is based mostly on sour dough concentrate in various "easy to add" forms (powder, liquid, paste) and contains primarily thickening agent, lactic, acetic, citric acids and natural colorants.

Originally, the complex improvers enabled to produce the "rye character" products in the small-scale bakeries where it is not space and device enough for the fermentation of natural sour dough. Recently they have also been used in large-scale and industrial bakeries to replace the continuous systems of sour dough processing and in this way to save plant productiom time, energy and space.

The advantages of their use are especially:

- shortened production time,
- simplification of the production process,
- flexible production planning and possibility to react quickly to consumer demands,
- due to the standard quality, higher shelf-life, and easier manipulation in comparison to the natural sour dough, they can serve as a supply for usual start of production, for a fluent continuation in the case of the operation breakdown, or they can balance deviations in the quality of natural sour dough that occur e.g. as a consequence of high summer temperatures or not enough qualified workers.

The purpose of this work was to compare the maturation of dough prepared in two different ways, i.e. using either natural sour dough or sour dough concentrates + yeast.

MATERIAL AND METHODS

Material

The commercially available flour (wheat and rye) and vital baker's yeast were used. The natural sour dough was obtained from standard production at the bakery: Michelské pekárny, a.s. To evaluate the influence of different sour dough concentrates, four types of them were tested as chosen from the bakery improvers that are offered on the Czech market:

* KWAS (Lactoprot Bohemia)
* Backsauer R 22 (Enzyma)
* BOEROL + BROTSTABIL (Kontinua)
* Backaromasauer-BAS + QUELLSTAR (Backaldrin)

The components – Brotstabil and Quellstar – are aimed to the increase of water absorption and bread volume.

Methods

The maturation of the dough has been testified by Rheofermentometer (Chopin, France).

Description of the Rheofermentometer

The apparatus is composed of two parts – the gasometer unit and the plotter x, y, which presents information furnished by the micro-processor in a graphical way.

Measuring principle

The development of wheat or rye products during baking depends both on the quantity of CO_2 included in the aqueous phase of the dough and on the rheological properties of the dough itself. One of the important characteristics is an ability of the protein network to deform under the gas pressure until the proteins are thermally denatured and the starch is gelatinized. The progress of the dough development and above all the period during which the protein structure (a steric network for the dough) resists the stress of gas is an index which corresponds to the tolerance of doughs in use.

The Rheofermentometer can measure simultaneously:

* the development of the rheological qualities of the dough during rising by monitoring the growth in volume of the dough which is submitted to the constraint generated by a variable weight from 230 to 2 330 g;
* the gaseous (CO_2) release potential of a flour by measuring the speed of formation of CO_2 in the risen dough.

The measurement is the combination of values which provide indications concerning the quality of the dough:

I. Recording curve of the variations in volume of the dough under constraint:

T1 – Maximum rising time of the dough expressed in hours and minutes. It is closely tied to the "speed" of the yeast and its activity.

Hm – Maximum rising "volume" of the dough under constraint, expressed as height in mm. It relates to the volume of the bread.

T2 – Relative stabilization time located 10% lower than Hm height. It is the indicator of the tolerance of the dough.

h – Rising "volume" of the dough in mm at the end of the test (T : 3 hours for a complete test or *T* : *x* for a test, the stop has of which been forced).

D – percentage of relative drop in rising time from T1,

where: $D = \frac{Hm - h}{Hm} . 100$

T1 and *D* indicate the optimum time for processing the dough.

II. Recording curve of the flow of gas released:

H'm – maximum height of the gaseous release curve,
T'1 – time necessary to get *H'm*,
Tx – time of dough porosity appearance,
Total volume of gaseous release in ml,
CO_2 volume loss – CO_2 volume in ml released by the dough during its fermentation,
Retention volume – CO_2 volume in ml still kept in the dough at the end of the test,
Retention coefficient – percentage calculated between the volume kept in the dough and the total volume of gas produced during the test x 100.

Procedure and measurement conditions

Doughs were prepared using a basic formula recommended by the supplier. Mixture (wheat and rye flour, sodium chloride, water, natural sour dough or complex improver and yeast) was kneaded in a Farinograph (ISO 5530) mixing bowl (two mixing blades with different speed at a ratio 2 : 3, 63 r.p.m. at slower one) at 30 °C for 8 min. Doughs prepared less intensively were mixed in a Stöllner "Kitchen Aid" mixer with less intensive kneading (88 r.p.m. of mixing hook). The mixer works on a planetary mixing principle. During operation, the dough hook moves at a controlled stir speed around the stationary stainless steel bowl, at the same time turning in the opposite direction on its own axis.

The loaf (315 g) was placed in the gasometric unit in the movable basket. The characteristics of the dough maturation were determined subsequently according to the standard CHOPIN procedure on the Rheofermentometr (Chopin, 1992).

Since dough tested should be as much as possible similar to that produced in plant process, where not too tough doughs are made, we mixed dough on farinographic consistency 380 ± 20 Brabender Units. The maturation of dough was then measured under the lowest possible pressure at Rheofermentometer.

RESULTS AND DISCUSSION

Measurements on Rheofermentometer have been evaluated by several ways:

I. Fermentation of dough with either yeast + improver or natural sour dough was testified with the doughs containing different parts of rye flour (40%, 50%, 60%).

II. Doughs containing 40% rye flour and 60% wheat flour with either natural sour dough or yeast + improver were measured after pre-fermentation out of gasometric unit for 15, 30, 45, and 60 minutes. The reason was to simulate a situation in a bakery when dough can be overproofed due to some delay in process (as technical problems, etc.).

III. Doughs with either natural sour dough or yeast + improver were mixed more intensively in a Farinograph bowl or less intensively in a Stöllner mixer to compare the two types of mixing.

The values measured are shown in tables that were formed to express the effect of different improvers, ratio of rye/wheat flour in dough and different intensity of mixing. The aim was to determine conditions under which improvers tested were most effective and gave us best baking results.

I. The effect of different ratio of rye/wheat flour (Table I and II)

Results obtained with the rye/wheat flour ratio 40/60

CO_2 release during dough fermentation is faster with original sour dough than with sour dough concentrate (time *T1* on the curve registered by Rheofermentometer was 1 hour for original sour dough and approximately 2 or 3 hours for the concentrates). On the other hand, total volume of CO_2 was higher with the concentrates than with original sour dough. CO_2 release in dough with concentrates was more regular. Using the concentrate KWAS, greatest total volume of CO_2 released was obtained. It was in concordance with the increase of dough volume.

Results obtained with the rye/wheat flour ratio 60/40

Dough volumes did not differ significantly when original or concentrated sour doughs (KV or KW or R 22) were used. With the complex improvers Boerol + Brotstabil higher dough volumes were recorded. Maximum height of dough was obtained from fastest to slowest with R 22 – KW – KV and Boerol + Brotstabil.

I. Development of the dough with different ratio of rye/wheat flour

Sample	*Hm* [mm]	*h* [mm]	*T1* [h min]	*T2* [h min]	Stabilisation coefficient [%]
KV 60	34.5	34.4	3 04	–	0.2
KV 50	26.5	11.5	1 54	2 40	56.6
KV 40	32.7	25.6	2 16	3 03	21.7
KW 60	35.4	33.6	2 54	–	5.0
KW 50	42.0	39.7	2 45	–	5.4
KW 40	53.2	45.8	2 27	3 00	13.9
KW 30	55.2	49.5	2 45	–	10.3
KW 0	55.8	47.4	2 52	3 04	15.0
R 60	35.0	29.5	2 36	–	15.7
R 50	40.3	38.0	2 54	–	5.6
R 40	46.3	46.3	3 06	–	0
B+B 60	45.0	44.7	3 03	–	0.6
B+B 40	43.4	43.4	3 06	–	0
Q+B 40	41.4	40.0	2 57	–	3.3

Total CO_2 release was greatest with Boerol + Brotstabil, then in decreasing order with KW, R 22. Original sour dough showed the lowest volume but greatest gas retention.

I. The effect of rye/wheat flour ratio on the application of sour dough and different concentrates and improvers

Original sour dough

CO_2 release was very similar in the dough with 40 or 50% of rye flour, in dough with 60% of rye flour CO_2 was released more slowly (*T1* more than 3 hours in comparison with less than 2 hours for 50% rye flour). Dough with 50% rye flour showed fastest decrease of gas volume and unsatisfactory stability of dough. CO_2 release in dough with 60% rye flour was lowest and slowest, but dough volume was highest.

II. Gaseous release in the dough with different ratio of rye/wheat flour

Sample	*H'm* [mm]	*T'1* [h min]	*Tx* [h min]	Total volume [ml]	CO_2 loss [ml]	Retention volume [ml]	Retention coefficient [%]
KV 60	27.8	2 03	–	591	17	574	97.1
KV 50	36.2	1 03	–	694	15	678	97.8
KV 40	33.2	1 15	–	673	21	651	96.9
KW 60	49.5	2 21	2 01	1 093	53	1 039	95.2
KW 50	52.4	2 33	2 19	1 079	39	1 039	96.4
KW 40	56.0	2 21	2 19	1 200	40	1 159	96.7
KW 30	50.8	2 21	2 13	1 106	63	1 042	94.4
KW 0	51.4	2 33	2 25	1 107	43	1 064	96.2
R 60	43.7	2 39	2 07	948	52	896	94.6
R 50	43.4	2 45	2 31	852	28	823	96.7
R 40	43.5	2 51	2 49	795	15	779	98.1
B+B 60	62.1	3 03	1 43	1 333	82	1 250	93.9
B+B 40	38.3	3 03	–	682	10	672	98.6
Q+B 40	48.4	3 03	2 37	860	22	836	97.4

Sour dough concentrate KWAS

Very small or no difference was found out between doughs with small parts of rye flour (ratio rye/wheat 30/70 and 0/100). With increasing part of rye flour the maximum height of dough volume decreases, with more rye flour than 50/50 the decrease is accelerated.

Time *T1* showed decrease with increasing part of rye flour from 0/100 to 40/60 only. For higher parts of rye flour from 50/50 *T1* turned to increase. It corresponded to gas volume that was highest at rye flour ratio 40/60.

The retention of gas was similar for all of the samples.

Sour dough concentrate R 22

The rising of dough was regular, time *T1* regularly shortened with increasing part of rye flour in dough. CO_2 release fastened and retention decreased regularly with increasing ratio of rye flour, but differences were not too dis-

tinctive. While with KWAS maximum gas volume was reached with rye flour ratio 40/60, with R 22 it was at the ratio 60/40.

Improver Boerol + Brotstabil

The height of dough volume was almost the same after 3-hour rising with the rye flour ratio 40/60 and 60/40. The relase of CO_2 was almost twice faster with the rye flour ratio 60/40 than 40/60 but the dough volume, as it was already mentioned, did not fully correspond to it.

II. The effect of pre-fermentation and final proofing time (Table III and IV)

Different pre-fermentation times were tested. As an optimum pre-fermentation time for original sour dough as well as for sour dough concentrate KWAS was found out 30 minutes.

III. Development of the dough (after pre-fermentation 60 min)

Sample	*Hm* [mm]	*h* [mm]	*T1* [h min]	*T2* [h] min]	Stabilisation coefficient [%]
KV	27.9	26.2	2 42	–	6.0
KW	45.7	29.2	2 04	2 15	36.1
R	42.8	34.4	2 13	2 55	19.6
Q+B	38.2	33.1	2 31	–	13.3

For the measurement of final proofing time effect 60 minutes of pre-fermentation were used to find out a possible effect of overproof.

IV. Gaseous release (after pre-fermentation 60 min)

Sample	*H'm* [mm]	*T'1* [h min]	*Tx* [h min]	Total volume [ml]	Retention volume [ml]	Retention coefficient [%]
KV	22.8	0 27	–	355	350	98.7
KW	54.6	1 57	1 55	1 418	1 327	93.6
R	48.2	1 51	1 49	1 253	1 140	91.2
Q+B	49.6	2 33	1 49	1 226	1 124	91.8

Original sour dough

Gas release was quite unsufficient, time *T'1* was recorded after 30 minutes, but then the speed of release dropped. Dough volume was only approximately a half of that with improvers.

Sour dough concentrates

Shortest time for the maximum dough volume was found out for KWAS, R 22, Quellstar + BAS. But best stability of dough volume was obtained with Quellstar + BAS. CO_2 release and retention were greatest with KWAS; R 22 and Quellstar + BAS were similar to each other. Time *T'1* and *Tx* was very similar to KWAS and R 22.

III. The effect of different mixing of the dough (Table V and VI)

Sour dough concentrate KWAS

Doughs with rye flour ratio 40/60 showed a small difference in dough volume for doughs mixed at Farinograph and Stöllner mixer. CO_2 release was slower and more regular in dough mixed in farinograph bowl. Dough mixed at Stöllner mixer showed faster gas release and greater volume, but lower gas retention. Doughs with rye flour ratio 60/40 showed relatively low CO_2 volume. Dough volume rising was also much slower at the dough mixed at Stöllner mixer.

It can be concluded that with lower intensity of mixing the differences between doughs of different formula (esp. rye-wheat flour) are emphasized.

Sour dough concentrate R 22

Dough volume differed for fermentation time longer than 30 minutes only. After 30 minutes mixing dough mixed at Stöllner mixer showed a faster volume increase and maximum volume was reached in shorter time. The differences in CO_2 release were more distinctive than the differences in dough volume. Higher gas volume, but lower retention was found out for Stöllner mixer. For doughs with rye flour ratio 60/40 hardly any significant differences were obtained.

In general, for R 22, the differences in dough volume as well as in gas release were more distinctive when less intensive dough mixing was used.

For less intensive mixing, higher gas volume was obtained with decreasing rye flour ratio. Opposite conclussion can be done for more intensive mixing at Farinograph bowl.

V. Development of the dough mixed at different intensity

Sample	*Hm* [mm]	*h* [mm]	*T1* [h min]	*T2* [h min]	Stabilisation coefficient [%]
KV 60 F	34.5	34.4	3 04	–	0.2
KV 40 F	32.7	25.6	2 16	3 03	21.7
KV 60 S	35.8	35.8	3 06	–	0
KV 40 S	39.0	39.0	3 06	–	0
KW 60 F	35.4	33.6	2 54	–	5.0
KW 40 F	53,2	45.8	2 27	3 00	13.9
KW 60 S	24.4	24.4	3 06	–	0
KW 40 S	43.2	42.3	2 30	–	2.0
R 60 F	35.3	29.5	2 36	–	15.7
R 40 F	46.3	46.3	3 06	–	0
R 60 S	37.3	34.7	2 45	–	6.9
R 40 S	43.0	41.1	2 48	–	4.4
B+B 60 F	45.0	44.7	3 03	–	0.6
B+B 40 F	43.4	43.4	3 06	–	0
B+B 60 S	42.3	42.2	3 03	–	0.2
B+B 40 S	50.0	48.1	2 45	—	3.7

Improver Boerol + Brotstabil

For the dough with rye flour ratio 60/40 hardly any significant difference was obtained in dependence on mixing intensity. For the dough with the ratio 40/60 total dough volume was almost identical, but rising speed was more regular for dough mixed more intensively. In general for more intensively mixed dough the greater dependence on the rye flour ratio was found out for CO_2 release.

VI. Gaseous release in dough mixed at different intensity

Sample	*H'm* [mm]	*T'1* [h min]	*Tx* [h min]	Total volume [ml]	CO_2 loss [ml]	Retention volume [ml]	Retention coefficient [%]
KV 60 F	27.8	2 03	–	591	17	574	97.1
KV 40 F	33.2	1 15	–	673	21	651	96.9
KV 60 S	32.8	1 15	–	630	3	626	99.5
KV 40 S	33.7	1 27	–	645	11	633	98.3
KW 60 F	49.5	2 21	2 01	1 093	53	1 039	95.2
KW 40 F	56.0	2 21	2 19	1 200	40	1 159	96.7
KW 60 S	33.9	3 03	–	601	17	583	97.2
KW 40 S	64.9	1 39	1 25	1 523	178	1 344	88.4
R 60 F	43.7	2 39	2 07	948	52	896	94.6
R 40 F	43.5	2 51	2 49	795	15	779	98.1
R 60 S	49.6	3 03	2 01	1 035	55	980	94.8
R 40 S	57.8	2 15	1 43	1 324	100	1 224	92.5
B+B 60 F	62.1	3 03	1 43	1333	82	1 250	93.9
B+B 40 F	38.3	3 03	—	682	10	672	98.6
B+B 60 S	64.6	2 45	1 49	1 391	67	1 324	95.3
B+B 40 S	62.2	2 45	1 37	1 535	131	1 403	91.6

Conclusions

Rheofermentometer was used for the testing of mixed rye-wheat doughs. It proved to be a suitable instrument for doughs based on natural rye sour dough or yeast leavened doughs, when lower constraint weight was applied.

For definitive evaluation of improvers baking quality and utility these measurements should be completed with test baking.

1. When natural sour dough was compared with sour dough concentrates and yeast, better improving effect of sour dough concentrates was proved for doughs with lower parts of rye flour (up to ratio 40% rye). The formation

release of CO_2 was more regular and higher dough volume was obtained, but the time necessary to reach maximum volume was in most cases longer in dependence on different improvers used.

2. When the effect of overproof was studied, a stability of dough volume and gas retention very significantly differed in natural sour dough and concentrates. The order of decrease of dough volume did not correspond to the order of decrease of gas retention for the same sour doughs and concentrates.
3. Addition of KWAS and R 22 that are based on actual sour dough concentrates emphasized the differences in maturing dough characteristics when less intensive mixing and lower part of rye flour was used.

Natural sour dough and improver Boerol + Brotstabil showed larger differences when more intensive mixing was used.

Indexes

KV	natural sour dough
KW	KWAS
R	R 22
B+B	Boerol + Brotstabil
Q+B	BAS + Quellstar
0–30–40–50–60	part of rye flour in total flour quantity (%)
F	the dough mixed at Farinograph
S	the dough mixed at Stöllner mixer

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References

PŘÍHODA, J. – HOLAS, J. – KRATOCHVÍL, J.: Rye flour, wholemeal breads and rye breads. Advances in baking technology. London, Blackie Academic and Professional 1993.

ISO 5530: Method for Using the Brabender Farinograph. Geneve, International Organization for Standardization 1988.

Chopin (Groupe Tripette and Renaud): Rheofermentometer F2. France 1992.

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Sledovanie kysnutia rôznych druhov ražno-pšeničných ciest

Na našom spotrebiteľskom trhu zaujímajú výrobky z ražno-pšeničného cesta významné miesto. Súčasná ponuka zlepšujúcich prípravkov umožňuje ich výrobu nielen spôsobom klasickým, teda vyvádzaním ražného kvasu, ale i tzv. priamym vedením. Pri tomto postupe je cesto kyprené droždím a charakteristické znaky výrobku z kvasu (aroma, chuť) získava použitím kvasových koncentrátov. Kvasové koncentráty (sypké, pastovité alebo tekuté) sú v prevažnej miere tvorené organickými kyselinami (mliečna, octová, citrónová apod.), zahušťovacím prostriedkom, príp. doplnené prirodzeným farbivom. Môžu sa tiež pridávať spoločne s prípravkami zlepšujúcimi reologické vlastnosti cesta.

Sledovali sme kysnutie ražno-pšeničných ciest pripravených pomocou klasického kvasu i pomocou droždia a kvasového koncentrátu. Testovanie prebiehalo na prístroji Rheofermentometer F2 (firma Chopin, Francúzsko), ktorým je možné registrovať zvýšenie objemu cesta a stabilitu jeho lepkovej štruktúry, rýchlosť tvorby $C0_2$, jeho celkové množstvo, ako aj množstvo $C0_2$ cestom zadržané. Našou snahou bolo zistiť rozdiely v kysnutí medzi cestami v závislosti na množstve ražnej múky v receptúre, na dobe odležania cesta a na intenzite hnetenia.

Výsledky meraní ukázali, že pri použití droždia a kvasových koncentrátov sa vývin plynu dá charakterizovať ako rovnomernejší, vytvára sa kvalitnejšia lepková sieť a predlžuje sa doba úplného nakysnutia. Pozitívnejšie sa vplyv kvasových koncentrátov prejavuje pri nižších podieloch ražnej múky v ceste. Po hodinovom odležaní sa cestá s droždím a kvasovým koncentrátom a cestá s kvasom od seba výrazne líšili; cestá s kvasom vykazovali nižší objem i menšie vytvorené množstvo plynu, ale vyššiu stabilitu. Rozdiely medzi jednotlivými cestami (s rôznym podielom ražnej múky v ceste) sú výraznejšie pre prípravok KWAS a R 22 pri menej intenzívnom hnetení, pre klasický kvas a Boerol + Brotstabil pri hnetení intenzívnejšom.

ražno-pšeničný chlieb; kvasové koncentráty; Rheofermentometer

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QUALITY CHARACTERS OF WHEAT FLOUR GRANULATION FRACTIONS

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Abstract: In order to study an effect of flour granulation on its characters, 15 different commercial wheat flours T530 were fractionated on a laboratory sieve each to six fractions. Besides, seven of these flours were separated to two parts, on one sieve only. Moisture, ash content, Falling Number, protein content, wet gluten, Gluten Index, and sedimentation value were determined to characterise quality of any of flour fractions as well as original non-fractionated flours. The following relations were testified using regression analysis: 1. flour particle size versus analytical data, 2. between some of the couples of analytical characters. Significant and in some cases non-linear relations between particle size and some of the quality characters were proved.

flour particle size; wheat gluten properties; flour quality characters; gluten quality characters; gluten index

Processing quality of cereal flours as well as final quality of products is undoubtedly affected by particle size distribution. Due to the nature of flour-milling process not only particles of different size are obtained, but, moreover to this, not all particles obtained have identical composition.

The variation in the size of particles and in composing parts content depends on many parameters of grain (as hardness, shape, moisture, content of protein, minerals, etc.) and on processing parameters of milling.

A wide range of granules is usually obtained as a result of milling, ranging from smallest separated starch granules (approx. 7 micrometers) to complexes of protein and starch (the order 10^{-1} mm). Entire effect of flour granulation on biscuits quality was found out in a former work (Čudová, 1993). In order to study an effect of granulation on cake and biscuit dough properties, mechanical way of separation of flour particles was used but the changes in quality of flour fractions were not studied.

In comparison to this, the effect of protein content and flour quality on biscuits was studied more frequently and was proved in other works (Aboud et al., 1985; Gaines et al., 1988).

The correlation of particle size vs. flour quality and chemical composition was proved for the flours in other countries (Sadkiewicz, 1985). Since the quality characters of those flours in most cases differ from standard Czech bakery flour T530, the relations between particle size and content or properties of composing parts were studied in this work.

MATERIALS AND METHODS

Wheat flours of the type T530 were tested only. In total 15 samples were obtained by a standard milling scheme in commercial mills.

Particle Size Analysis

The automatic sifter AP-2 (Czech production) was used. The samples were separated either to six fractions on metal sieves with the openings 160, 125, 90, 70, and 50 μm or to two fractions using the sieves 125 or 90 μm. The samples were sifted for 20 minutes in the case of separation to six fractions or 12 minutes for two fractions.

12 repeated siftings, every with new 100 g flour, was done with every sample. Since the quantity of some fractions was not sufficient for all necessary analyses, even after 12 siftings, some of analyses had to be omitted for a few flours.

Determination of Moisture

Method of drying for 90 minutes at 130 °C in electrically heated box with 5 g sample was used. The method is described in ČNS ISO Standard 712.

Determination of Falling Number

Standard method by ČNS ISO Standard 3093 was used with the instrument Falling Number 1400. This way starch damage and α-amylase activity can be evaluated.

Determination of Wet Gluten Content and Gluten Index

Wet gluten content and Gluten Index were determined with the gluten washer Glutomatic 2200 and Centrifuge 2015 according to the ICC draft Method 155.

The fraction indicated as B and C had to be washed out in such a way that wheat meal is washed, for the other fractions the way recommended for flour washing was applied.

Determination of Protein

Usual Kjeldahl method was used with Kjeltec (produced by Tecator, Sweden). Mixture of 3.5 g of K_2SO_4 and 0.4 g $CuSO_4$ in tablets was used, as a catalyzer, and hydrochloric acid was used for titration. The results were calculated using a coefficient value 5.7

Determination of Sedimentation Value

Reagents

Acetic acid solution, concentration 2 %; Bromphenol blue solution prepared from 4 mg of Bromphenol blue diluted in 1 litre destilled water.

The operation

Flour (3.2 g) is put into the volumetric cylinder with stopper, content 100 ml. Bromphenol blue (50 ml) is added and immediately measurement of time is started, the cylinder is closed and shaking with cylinder starts. The cylinder is turned to horizontal position and hand-shaked to right and left 12 times during 5 seconds with total amplitude 18 cm between the edge positions. The mixture should be fully suspended prior to determination will continue. Cylinder is turned to vertical position. After 15 seconds of resting cylinder is fully turned up the bottom 18 times during 30 seconds and after 90 seconds of another rest period, 25 ml of acetic acid solution is added. Cylinder is turned up the bottom again 4 times. Next resting and mixing periods follow: 105 s rest; 30 s 18-times full turning bottom up; 90 s rest; 15 s 9-times full turning bottom up; 5 minutes rest for sedimentation in vertical position. After this rest the volume of sediment is check out. Content of protein, wet gluten, and ash were recalculated to dry matter, sedimentation value to 14% moisture.

RESULTS AND DISCUSION

Separation of Flour to two Fractions

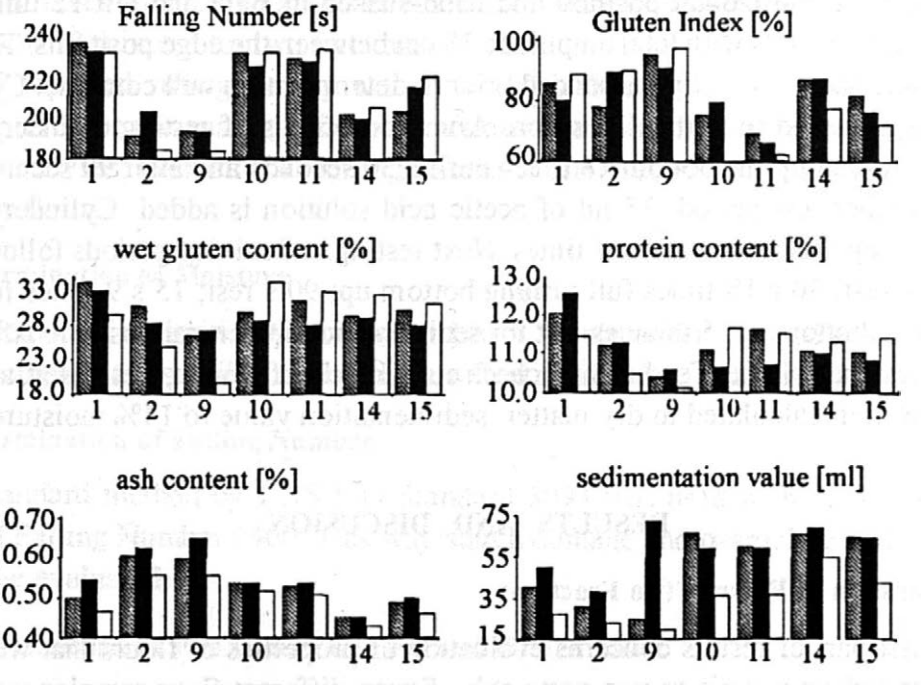
First part of results concerns evaluation of properties of flours that were separated on a sieve to two parts only. Seven different flour samples were used for this separation.

Due to the differences in granulation spectrum of flours, different sieves had to be used to get approximately equivalent parts over and under sieve. It affected some of the results, most of all the protein content, Falling Number and Gluten Index values.

When the flour was separated on the sieve with the opening 0.125 mm (Nrs. 1, 2, 9), higher content of proteins was found out in undersieve. The flour separated on the sieve 0.090 mm (Nrs. 10, 11, 14, 15) showed higher protein content in oversieve (Fig. 1). For both of the groups of flours separated on the sieve 0.125 mm or 0.090 mm, protein content of non-separated flour was between oversieve and undersieve with the only exception of sample No. 11.

We can conclude that apparently the protein content in flour type 530 is in prevailing part dependent on the fraction between 0.090 and 0.125 mm. This fraction is included in fraction D in next part of work with more detailed separation of fraction.

The same conclusion can be drawn for the results obtained with Falling Number (Fig. 1).



1. Quality characters of flour samples (numbers at horizontal axis) for non-separated flours (■) and parts of these flours after separation on one sieve (❑ – oversieve, ■ – undersieve)

Opposite conclusions can be drawn for the Gluten Index (GI). Higher values of GI were obtained at the oversieve of the sieve 0.125 and at the undersieve of the sieve 0.090 (Fig 1). However, apparent conclusion that could be deduced from these results, i.e. that lowest values of GI occur in particles between 0.090 and 0.125 mm, was not confirmed when flours separated to six fractions were analysed.

One-way general tendency of higher ash content in finer particles of flours was found in all cases. The ash content in undersieve was higher for the sieve 0.125 mm as well as for 0.090 mm. However, differences between ash content in oversieve, non-separated flour, and undersieve are not the same.

In the case of separation on the sieve 0.090 mm, differences between undersieve and non-separated flour are not significant, the same differences under the sieve 0.125 are more distinctive.

Separation of Flour to Six Fractions

Using the separation on five sieves, six fractions of different particle size were obtained. For every fraction an average particle size as an average of the two neighbouring sieves openings was calculated:

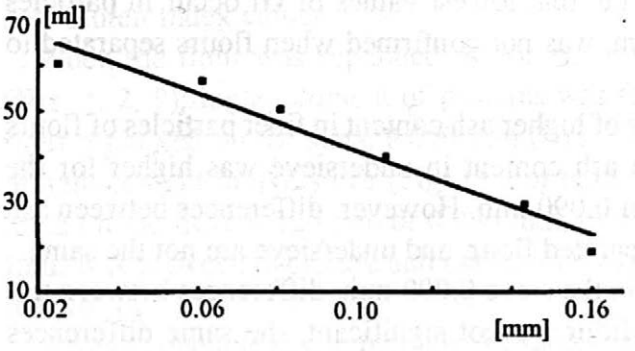
Fraction:	Average particle size (mm):
B	0.160
C	0.143
D	0.108
E	0.081
F	0.061
G	0.025

Analytical characteristics were determined for single fractions of commercial flours in order to determine relations either of analytical values to average particle size or mutual relations between different analytical characteristics. Relations between analytical characteristics and particle size were calculated from the averages of data obtained for each of six fractions of all 15 flours (with a few exceptions as was mentioned above). For sedimentation value, Gluten Index, and ash content linear relations to particle size were found out.

Sedimentation Value

The finest fraction G showed high sedimentation values in all cases but its part in flours has been very small and is not too important. The values for fraction F were close to values for fraction G and it seems that for particle

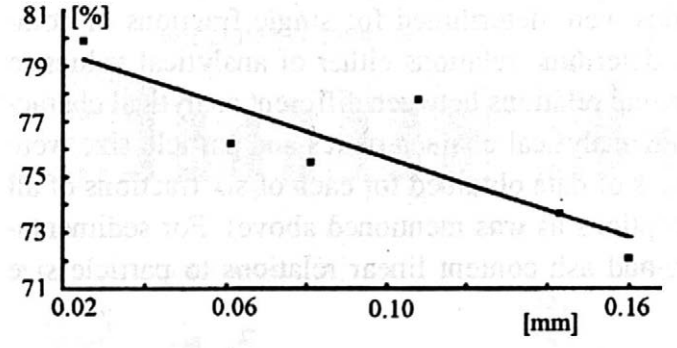
smaller than 0.06 –0.05 mm sedimentation value does not change so far with their decreasing size (Fig. 2).



2. Regression line for relation of sedimentation value (ml) to particle size (mm). The values are averages of all flour samples for single fractions
$r = -0.98$
$\alpha = 0.02.10^{-2}$

Gluten Index

We usually need not take into account that flours for the gluten washing and GI determination have not standard granulation. It seems rather surprising that correlation between GI and particle size was very strong. GI values of non-separated flours were higher in most cases than the values for the fractions of the same flours. However, it should be noticed that the maximum difference between lowest (fraction B) and highest (fraction G) values GI has been less than 8%. This range is rather small to confirm the validity of conclusion as more general (Fig. 3).

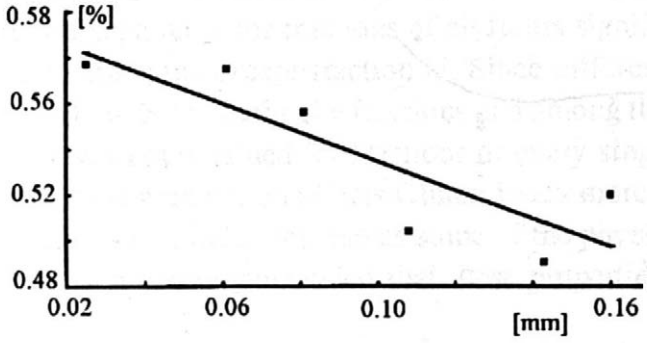


3. Regression line for relation of Gluten Index (%) to particle size (mm). The values are averages of all flour samples for single fractions
$r = -0.86$
$\alpha = 0.02$

Ash Content

In general, the ash content decreased with increasing particle size. But, probably due to different processing schemes in the commercial mills, the

differences between the fractions of one flour are varying very much in some flours. The ranges of ash content in fractions of single flours were from 0.04 to 0.13%. Regression line for the average of all flour samples is seen in Fig. 4.

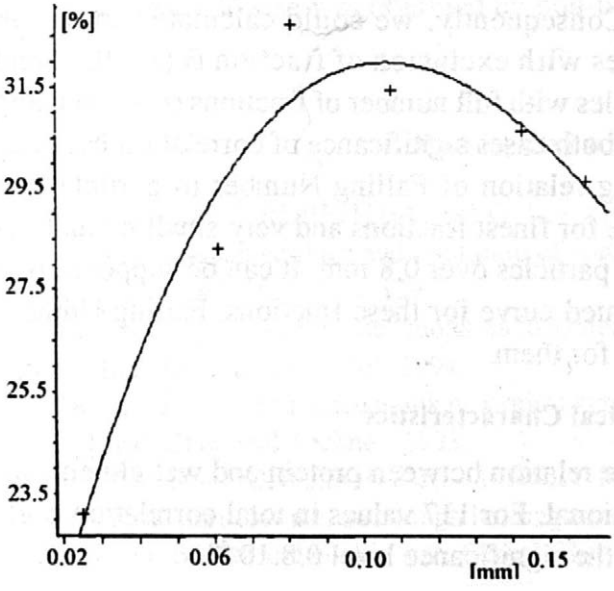


4. Regression line for relation of ash content (%) to particle size (mm). The values are averages of all flour samples for single fractions
$r = -0.87$
$\alpha = 0.02$

Protein and Wet Gluten Content

Best correlation between protein content or wet gluten content and particle size was found for non-linear power law regression (equation type $y = a.x^b$), for proteins: $r = 0.81$, $= 0.05$, for wet gluten: $r = 0.76$, $= 0.08$.

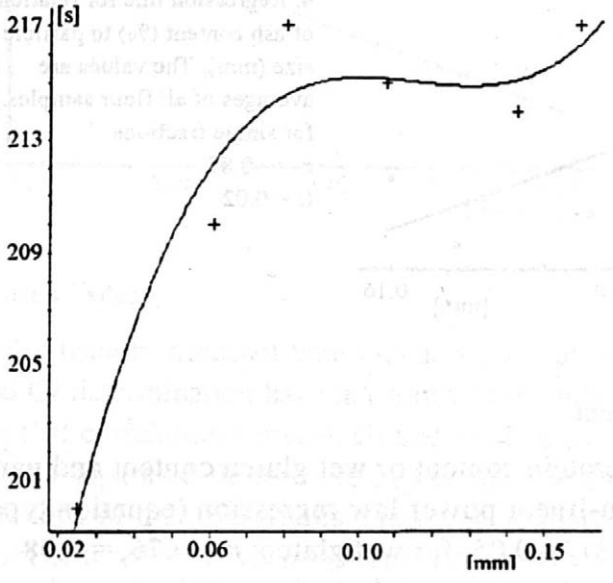
In order to study the relation between protein to the particle size polynomial function of 3rd order proved as best describing it (Fig. 5). Very similar diagram was obtained for the relation of wet gluten to particle size.



5. Relation of wet gluten content (%) to particle size (mm) approximated by polynomial function of 3rd order. The values are averages of all flour samples for single fractions

Falling Number

Relations of Falling Number (FN) values to particle size are not linear and the same expressions as for the protein content can be used to describe them (Fig. 6).



6. Relation of Falling Number (s) to particle size (mm) approximated by polynomial function of 3rd order. The values are averages of all flour samples for single fractions

For the determination of FN value in fraction B of flours only seven flour samples could be analysed. Consequently, we could calculate correlation either for all 15 flour samples with exclusion of fraction B ($r = 0.98$ and $\alpha = 0.003$) or for 7 flour samples with full number of fractions ($r = 0.906$ and $\alpha = 0.013$) It is seems that in both cases significance of correlation has been proved. The curve describing relation of Falling Number to particle size (Fig. 6) showed greatest slope for finest fractions and very small mutual dependence of variables for the particles over 0.8 mm. It can be supposed that with no regards to the calculated curve for these fractions, Falling Number does not change significantly for them.

The Relations between Analytical Characteristics

As it could be expected, the relation between protein and wet gluten content was proved as very functional. For 117 values in total correlation coefficient was obtained 0.920 at the significance level $0.8.10^{-46}$.

Another very significant relation was obtained for GI versus wet gluten content. For the population of 112 numbers correlation coefficient was 0.53 with the significance level $0.2.10^{-8}$. In general it has been supposed that GI is not significantly affected by wet gluten content.

When relation of GI to gluten content was tested separately in every flour sample, no significant correlation was found out. When similar relation was tested separately for fractions of all flours significant correlation was found in all fractions except fraction U. Since differences in gluten content were found out both among the fractions and among the flour samples but correlation was not obtained for fractions of every single flour, it can be supposed that flour granulation affects Gluten Index more than it does gluten content. Since Gluten Index represents some of the physical properties of gluten and dough, it can be concluded that these properties are also affected by flour granulation.

Conclusions

1. After the separation different quality characteristics were found out for the flours of various particle size. Most of all, the sedimentation value, protein and wet gluten content, and ash content were affected.
2. Linear correlation of sedimentation value, Gluten Index, ash content versus particle size was proved to be significant. All of these correlation coefficients were negative.
3. Best correlation of protein content, wet gluten content, and Falling Number versus particle size was obtained by non-linear regression.

References

ABBOUD A. M. – RUBENTHALER G. L. – HOSENEY R. C.: Effect of fat and sugar in sugar-snap cookies and evolution of tests to measure cookie flour quality. Cereal Chem., *62*, 1985: 124–129.

BROŽOVÁ, K.: Vliv granulace mouk na ukazatele jejich kvality). [Diploma work.] Praha, Inst. Chemical Technol. 1994.

ČUDOVÁ, J.: Vliv granulace mouk na kvalitu výrobků trvanlivého pečiva. [Diploma work.] Inst. Chemical Technol. 1993.

GAINES C. S. – DONELSON J. R. – FINNEY P. L.: The effect of damaged starch, chlorine gas, particle size, and dough holding time and temperature on cookie dough handling properties and cookie size. Cereal Chem., *65*, 1988: 384-389.

SADKIEWICZ, K.: Technological properties of flour and rheological properties of dough in dependence of disintegration degree of grain meal as well as determination of optimum particle size (in Polish). Zagadnienia Piekarstwa, *30*, 1985: 11–48.
ČSN ISO 3093. Stanovení čísla poklesu.
ČSN ISO 2171. Stanovení popela.
ČSN ISO 712. Stanovení obsahu vody.
ICC Draft Nr. 155 Determination of Wet Gluten Quantity and Quality (Gluten Index ac. to Perten) of Whole Wheat Meal and Wheat flour.

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Kvalitativní charakteristiky frakcí pšeničné mouky

Jedním z ukazatelů ovlivňujících kvalitu mouky a tím i kvalitu konečného výrobku je granulace. Při sledování kvality trvanlivého pečiva, upečeného ze dvou různých frakcí získaných rozdělením mouky T530 na sítě, byl zjištěn vliv granulace především na senzorické vlastnosti a rozměry sušenek. V uvedené práci nebyly sledovány kvalitativní ukazatele mouky po její frakcionaci (Čudová, 1993). Jiní autoři uvádějí vliv dalších kvalitativních ukazatelů, např. obsah bílkovin na kvalitu sušenek (Abboud et al., 1985; Gaines et al., 1988).

Cílem této práce bylo zjistit změny kvalitativních ukazatelů, ke kterým dojde po frakcionaci pšeničné mouky běžně používané v našem pekárenském průmyslu.

V práci bylo k měření použito 15 vzorků hladké pšeničné mouky T530 z různých mlýnů v České republice (Brožová, 1994). Mouky byly rozděleny pomocí pěti sít na šest frakcí se středními velikostmi částic 0,160 (B), 0,143 (C), 0,108 (D), 0,081 (E), 0,061 (F), 0,025 (G). Sedm vzorků z těchto mouk bylo ještě rozděleno jen na dvě frakce na sítě o velikosti oka 0,125 mm, případně 0,090 mm u mouk s nízkým podílem větších částic.

U netříděné mouky a takto získaných frakcí byly stanoveny následující ukazatele:
- vlhkost sušením při 130 °C po dobu 90 minut (ČSN ISO 712),
- obsah bílkovin podle Kjeldahla na přístroji Kjeltec,
- obsah mokrého lepku a lepkového indexu podle metody ICC draft č. 155,
- sedimentační hodnota podle Kaliny,
- číslo poklesu podle metody ČSN ISO 3093,
- obsah popela spalováním při 900 °C (ČSN ISO 2171).

Obsah bílkovin, lepku a popela je přepočten na sušinu, sedimentační hodnota na 14 % vlhkosti.

Analytické ukazatele byly vyhodnoceny ve vztahu k velikosti částic, která pro jednotlivé frakce byla vyjádřena jako průměr velikostí ok dvou sousedních sít. Pro výpočet korelací byly pro dané analytické ukazatele použity průměrné hodnoty jednotlivých frakcí.

Na obr. 1 je vidět, jak se mění hodnoty jednotlivých ukazatelů propadu a přepadu při použití síta o velikosti oka 0,125 mm (vzorky 1, 2 a 9), resp. 0,090 mm (vzorky 10, 11, 14 a 15). Z obrázku je patrné, že použití různého síta má vliv především na změnu obsahu bílkovin, na obsah lepku, lepkového indexu a číslo poklesu propadu a přepadu. Pro srovnání jsou uvedeny i hodnoty stanovené pro netříděnou mouku.

Na obr. 2–6 je znázorněna závislost jednotlivých ukazatelů na velikosti částic. Pro sedimentační hodnotu, lepkový index a obsah popela byla zjištěna lineární závislost a negativní korelační koeficient. Pro obsah bílkovin, mokrého lepku a číslo poklesu bylo dosaženo významné korelace při použití mocninového vztahu. Pokud ovšem chceme tento vztah aproximovat funkcí, byla nalezena jako nejlépe vystihující funkce polynomu 3. řádu. Proložená křivka pro vztah mezi číslem poklesu a velikostí částic (obr. 6) má stoupající část křivky pro nejhrubší podíly, ale vzhledem k malému rozpětí hodnot pro poslední čtyři granulační frakce lze předpokládat, že číslo poklesu se u částic velikosti nad cca 0,8 mm významně nemění.

Podle našich výsledků se projevil i vliv velikosti částic na lepkový index s dosti významnou korelací. Zajímavé zjištění vyplynulo z porovnání hodnot lepkového indexu pro netříděné mouky a pro jejich jednotlivé frakce. Hodnoty lepkového indexu pro netříděné mouky byly pro většinu vzorků vyšší než hodnoty lepkového indexu jejich frakcí.

Z naměřených hodnot byly dále zjišťovány vztahy mezi některými analytickými ukazateli kvality mouk navzájem. Všeobecně jsou již dávno známy vysoké korelace mezi obsahem bílkovin a mokrého lepku, což se potvrdilo i v naší práci. Lepkový index se však dosud považuje za ukazatel nezávislý na obsahu lepku. Pokud byly počítány korelační koeficienty mezi lepkovým indexem a obsahem lepku pro soubory hodnot všech vzorků mouk vždy pro jednotlivou frakci, byly prokázány významné korelace s jedinou výjimkou frakce tvořené propadem při třídění mouk jen jedním sítem. Pokud byly počítány korelace z hodnot pro každou mouku samostatně, významné korelace zjištěny nebyly. Vzhledem k tomu, že rozdíly v obsahu lepku jsou jak mezi frakcemi téže mouky (obvyklé rozpětí kolem 10 %), tak mezi jednotlivými vzorky netříděných mouk (rozpětí cca 6 %, jen dva vzorky byly mimo o 2 a 3 %), lze předpokládat, že granulace mouky má na lepkový index významnější vliv než samotný obsah lepku.

Z uvedených výsledků je patrné, že frakcionací mouk se získají podíly s dosti odlišnými kvalitativními ukazateli. Studuje-li se vliv granulace mouk řízeně frakcionovaných sítovou analýzou na jiné kvalitativní parametry, je nutné znát i významné změny, které vykazují mouky v jednotlivých frakcích.

lepkový index; granulace mouk; vlastnosti pšeničného lepku; kvalitativní ukazatele mouk; kvalitativní ukazatele lepku

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ANTIOXIDANT ACTIVITY OF 2,6-DIMETHYL-3,5-BIS(MENTHYLOXYCARBONYL)-1,4-DIHYDROPYRIDINE IN EDIBLE OILS*

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Abstract: The antioxidative activity of 2,6-dimethyl-3,5-bis(menthyloxycarbonyl)-1,4-dihydropyridine (DMDP) was found moderate at the concentration of 0.01%, and intermediate at 0.02%, but the antioxidant was rather active at the concentration of 0.05% in both refined sunflower and rapeseed oils, when tested with use of Schaal oven test. Synergists such as 0.02% ascorbyl palmitate, 0.05% soybean lecithin, 0.05% phosphatidic acids or 0.05% their ammonium salts increased the stability of edible oils containing 0.02% DMDT substantially. The antioxidant thus would be suitable for the stabilization of polyunsaturated edible oils, which are otherwise difficult to stabilize.

cyclohexyl diludine; dihydropyridine antioxidant; oxidative stability; stabilization against oxidation; sunflower oil; rapeseed oil

The oxidative stability of edible oils depends on the content of polyenoic fatty acids and on the content of natural or added antioxidants. Most antioxidants are phenolic derivatives, but amino compounds are active as well. Ethoxyquin is a very well known synthetic antioxidant, which is, essentially, a 1,2-dihydropyridine derivative. It is very active in animal fats, but it is suitable for plant products as well (Müller, 1980). Methylethoxyquin is a similarly powerful antioxidant, suitable even for such unsaturated materials as fish oils (Thorisson et al., 1993). Their disadvantage is, however, rather high toxicity in animals.

A series of dihydronicotine amide analogues has been synthesized in Riga (Dubur et al., 1970), and their hydrogen-donating properties were determined. The derivative diludine from this series was found effective for the stabilization of carotene in grass meal and feed mixes (Dvinskaya et al.,

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1972). As the substance was found practically harmless (LD_{50} 2 500 mg/kg according to the Latvian Institute of Organic Syntheses, unpublished data) it was proposed for the stabilization of pharmaceutical preparations containing carotene. Both the preparation (Uldrikis et al., 1981) and the use (Brit. pat., 1978) were covered by patent applications. Diludine is manufactured by State Enterprise Olaine Chemico-Pharmaceutical Plant (Latvia).

Diludine (2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine) was found active as an antioxidant in sunflowerseed oil at the concentration of 0.03% (Malyarova, 1979). Its antioxidant activity was tested in methyl oleate, and it was observed that the activity increased with the increasing hydrocarbon-substitution in the side chain (Tirzitis et al., 1983). The activities of several antioxidants of the diludine series were tested in methyl oleate (Tirzitis et al., 1988). We have found (Kouřimská et al., 1993) diludine as a moderately active antioxidant in rapeseed, sunflower and olive oils (where natural antioxidants, such as tocopherols, are already present) at the concentration of 0.02%, which is used in the stabilization by phenolic antioxidants.

The mechanism of the antioxidative activity of diludine was investigated as well. It was found an efficient deactivator of singlet oxygen (Tirzit et al., 1981). Older papers on the antioxidant activities of dihydropyridine derivatives synthesized in Riga were reviewed (Lukevits, 1987).

The disadvantage of diludine is its low solubility in fats and oils. Therefore, we have tested the cyclohexyl derivative of diludine which has much better solubility in vegetable oils.

MATERIAL AND METHODS

DMDP was synthesized in the Latvian Institute of Organic Chemistry, Riga, Latvia (Uldrikis et al., 1981); ascorbyl palmitate was produced by E. Merck and Co. (Darmstadt, Germany); soybean lecithin was manufactured by SETUZA a. s. (Ústí nad Labem, CZ) and contained 56% phospholipids (acetone insolubles); phosphatidic acids were produced in VTX s.p. by phosphorylation of diacylglycerol fraction of commerical monoglyceride emulsifier, their ammonium salts were prepared by introducing ammoniac into the solution of phosphatidic acids. Refined rapeseed oil Vitol (28.0% linoleic acid, 10.2% linolenic acid) was produced by Kosmos a. s.,

Čáslav, and the refined sunflower oil Lukana (68.6% linoleic acid) by Milo a. s., Olomouc.

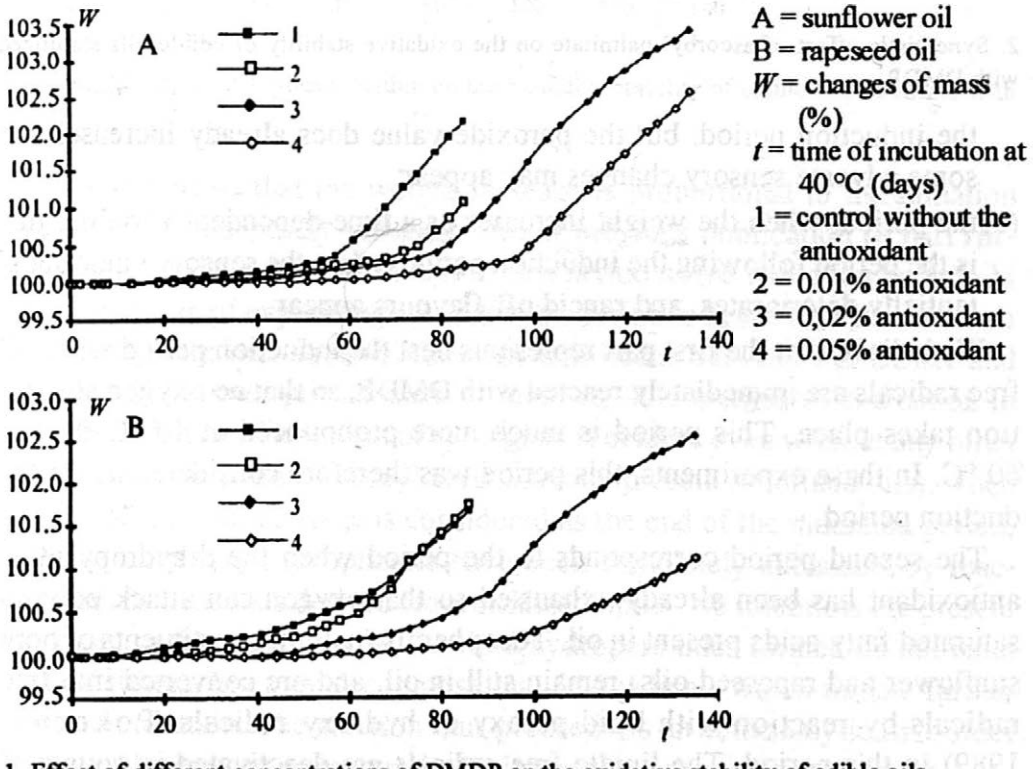
The antioxidative stability of oils and their mixtures with stabilizers was determined with use of Schaal test at 40 °C by weighing (Pokorný et al., 1985). The end of the induction period was determined graphically from the weight increase/time plots (details are given below in the text).

RESULTS AND DISCUSSION

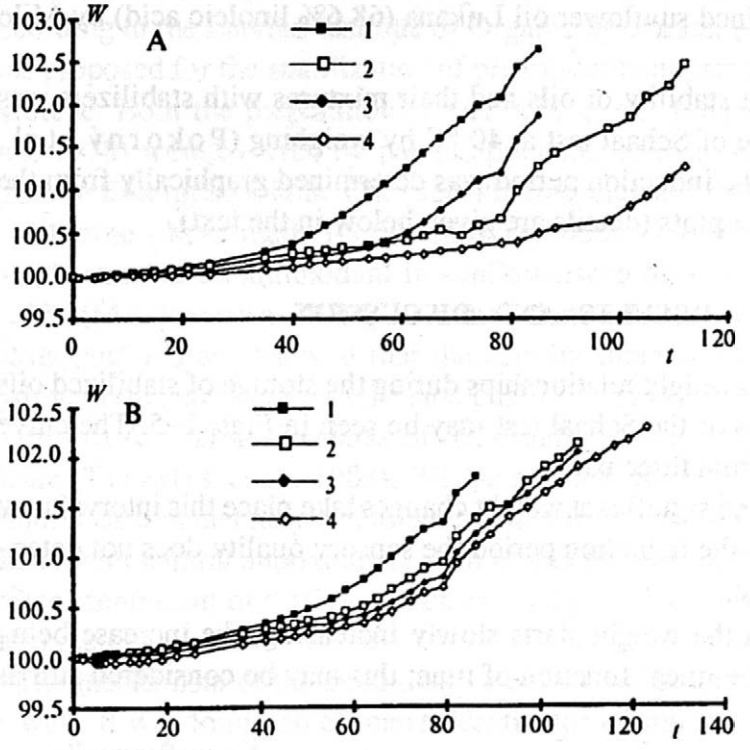
Examples of time-weight relationships during the storage of stabilized oils under the conditions of the Schaal test may be seen in Figs. 1–5. The curve consists generally from three parts:

(1) the period when no significant weight changes take place this interval may be considered as the induction period the sensory quality does not deteriorate at this stage;

(2) the period when the weight starts slowly increasing, the increase being approximately the linear function of time; this may be considered still as



1. Effect of different concentrations of DMDP on the oxidative stability of edible oils



A = sunflower oil
B = rapeseed oil
W = changes of mass (%)
t = time of incubation (days)
1 = control without the antioxidant
2 = oil containing 0.02% ascorbyl palmitate
3 = oil containing 0.02% cyclohexyl-diludine
4 = oil containing both 0.02% DMDP and 0.02% ascorbyl palmitate

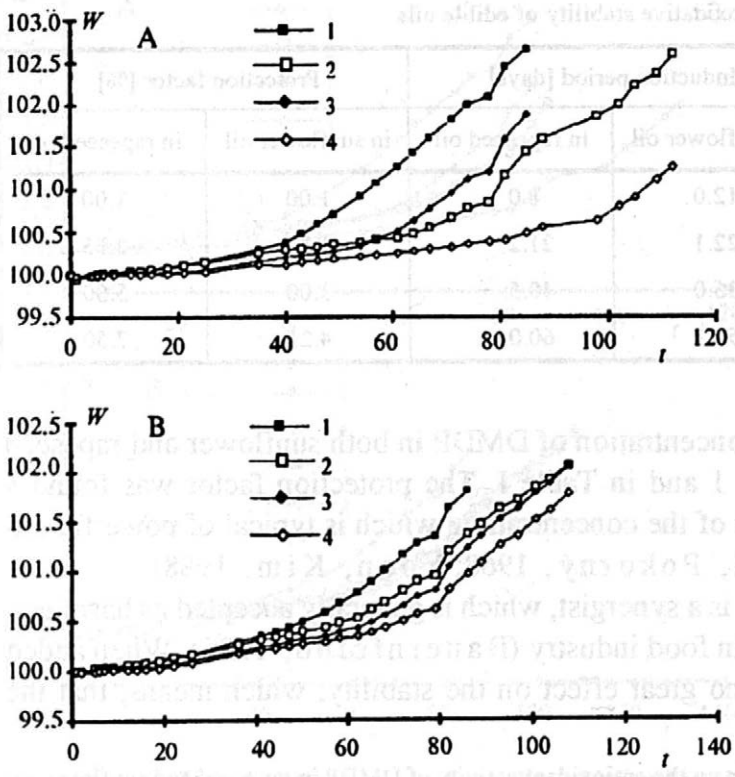
2. Synergistic effect of ascorbyl palmitate on the oxidative stability of edible oils stabilized with DMDP

the induction period, but the peroxide value does already increase, and some adverse sensory changes may appear;

(3) the period, when the weight increases as a time-dependent variable; this is the period following the induction period, when the sensory value substantially deteriorates, and rancid off-flavours appear.

We believe that the first part represents best the induction period when all free radicals are immediately reacted with DMDP, so that no oxygen absorption takes place. This period is much more pronounced at 40 °C than at 60 °C. In these experiments, this period was therefore considered as the induction period.

The second period corresponds to the period when the dihydropyridine antioxidant has been already exhausted so that oxygen can attack polyunsaturated fatty acids present in oil. Tocopherols (natural constituents of both sunflower and rapeseed oils) remain still in oil, and are converted into free radicals by reaction with lipid peroxy or hydroxy radicals (Pokorný, 1989) in this period. The lipidic free radicals are deactivated in course of



A = sunflower oil
B = rapeseed oil
W = changes of mass (%)
t = time of incubation (days)
1 = control without the antioxidant
2 = oil containing 0.05% soy lecithin
3 = oil containing 0.02% DMDP
4 = oil containing both 0.02% DMDP and 0.05% soy lecithin

3. Synergistic effect of soybean lecithin on the oxidative stability of edible oils stabilized with DMDP

these reactions so that the weight increase is proportional to the initiation rate, which is essentially constant. In our previous publication (Kouřimská et al., 1993), we used the end of this period as the induction period. At 60 °C, this way of expressing the induction period is better than that we used this time. Therefore, some differences will occur between our earlier and present conclusions, just because of more reliable method of evaluation in these experiments. The effect of synergists is evident even without any other antioxidants than natural tocopherols (already present in refined oils), when the end of this second stage is considered as the end of the induction period.

In the third stage, tocopherols have been completely exhausted by reaction with free radicals derived from oxidized lipids. No inhibitors are present any more in this stage so that the lipid hydroperoxides formed do not react with inhibitors, but they decompose into free radicals which initiate further oxidation reaction. The reaction thus proceeds as an autocatalytic first-order reaction (the oxygen concentration being nearly constant) in this stage.

I. Effect of DMDP on the oxidative stability of edible oils

Concentration of the antioxidant [g/kg]	Induction period [days]		Protection factor [%]	
	in sunflower oil	in rapeseed oil	in sunflower oil	in rapeseed oil
0	12.0	8.0	1.00	1.00
0.1	22.1	21.2	1.83	2.65
0.2	36.0	40.5	3.00	5.00
0.5	51.1	60.0	4.25	7.50

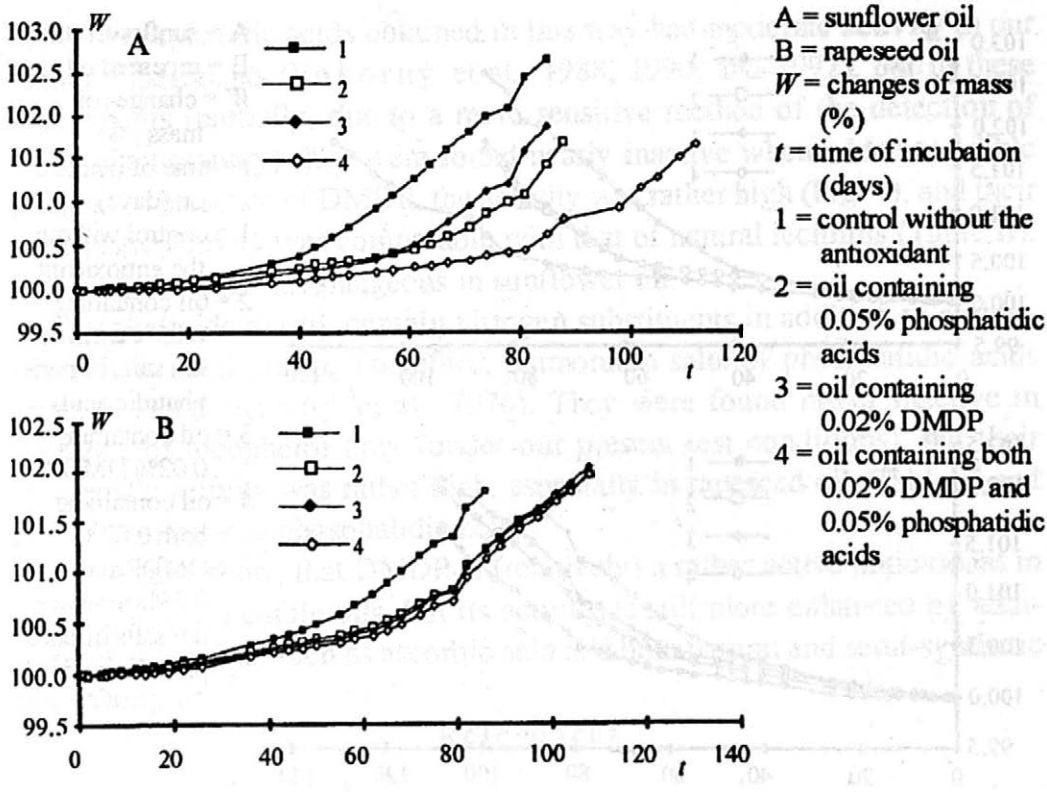
The effect of the concentration of DMDP in both sunflower and rapeseed oils is shown in Fig. 1 and in Table I. The protection factor was found a nearly linear function of the concentration, which is typical of powerful antioxidants (Pospíšil, Pokorný, 1968; Yoon, Kim, 1988).

Ascorbyl palmitate is a synergist, which is generally accepted as harmless, and it is widely used in food industry (Bauernfeind, 1985). When added to edible oils, it has no great effect on the stability, which means, that the

II. Effect of some synergists on the antioxidant activity of DMDP in rapeseed and sunflower oil

Oil	Synergist added	Induction period [days]		Protection factor [%]	
		with the antioxidant	without the antioxidant	without the antioxidant	with the antioxidant
Rapessed	Control	5.0	4.5	1.00	1.11
	0.02% AP	19.0	5.0	1.11	4.22
	0.05% LC	14.5	3.5	0.78	3.22
	0.05% PA	11.4	5.2	1.16	2.53
	0.05% PAm	19.0	5.0	1.11	4.22
Sun-flower	Control	12.5	4.0	1.00	3.12
	0.02 % AP	17.8	4.6	1.15	4.45
	0.05 % LC	18.3	4.7	1.18	4.58
	0.05 % PA	18.7	3.8	0.95	4.68
	0.5 % PAm	11.4	4.7	1.18	2.85

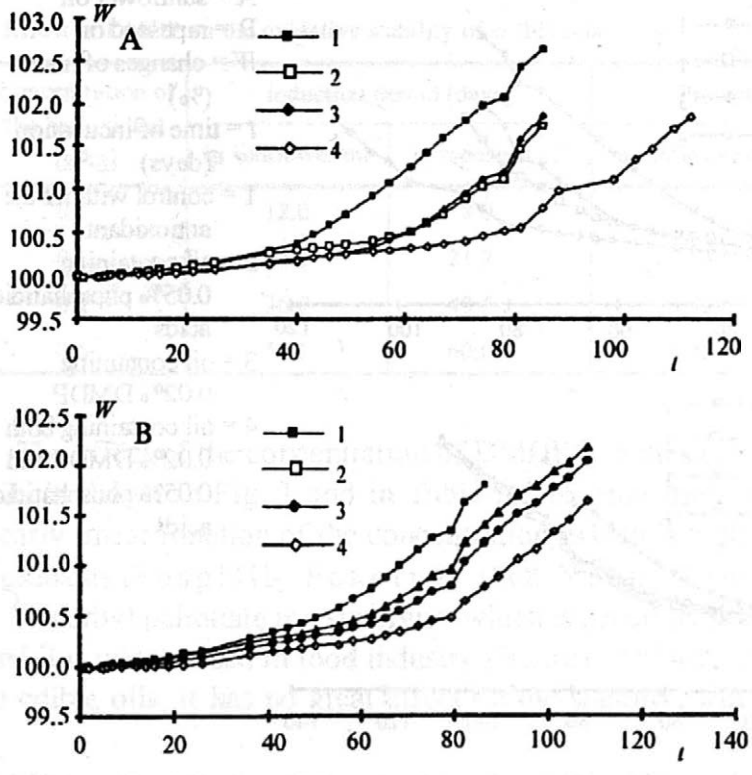
AP = ascorbyl palmitate, LC = soybean lecithin, PA = phosphatidic acids, PAm = ammonium salts of phosphatidic acids



4. Synergistic effect of phosphatidic acids on the oxidative stability of edible oils stabilized with DMDP

synergistic effect with tocopherols (natural antioxidants of both sunflower and rapeseed oils) is rather low, contrary to the literature (Bourgeois, Czornomaz, 1982). In presence of DMDP, the synergistic effect of ascorbyl palmitate was rather high (Fig. 2 and Tables II), which depends probably on higher reducing activity of DMDP in comparison with phenolic antioxidants, such as tocopherols. In our earlier experiments with diludine (Kouřimská et al., 1993), ascorbyl palmitate was found active as a synergist of natural tocopherols as well, but it was found less efficient than in combination with DMDP. The synergistic activity of ascorbyl palmitate was found higher in rapeseed oil, compared with sunflower oil. It is very fortunate, as rapeseed oil is particularly difficult to stabilize against the rancidity, because of its relatively high content of linolenic acid.

Natural phospholipid concentrates are active synergists of phenolic antioxidants as well (Nasner, 1985), especially because of their hydroperoxide--decomposing activity (Pokorný et al., 1981), besides their other inhibi-



A = sunflower oil
B = rapeseed oil
W = changes of mass (%)
t = time of incubation (days)
1 = control without the antioxidant
2 = oil containing 0.05% ammonium salts of phosphatidic acids
3 = oil containing 0.02% DMDP
4 = oil containing both 0.02 % DMDP and 0.05% ammonium salts of phosphatidic acids

5. Synergistic effect of ammonium salts of phosphatidic acids on the oxidative stability of edible oils stabilized with DMDP

tory activities (Pokorný, 1991). The effect of soybean lecithin in edible oils containing DMDP is evident in Fig. 3 and Table II. The effect is lower, but still substantial. It should be remembered, the lecithins are natural components of edible oils, so that they are generally considered as safe substances (GRAS status). From this standpoint, the use of lecithin is advantageous. We have found the level of 0.05% already active, i.e. the concentration which was not perceptible by sensory tests in refined oil (tested in our laboratory). The synergistic activity of lecithin was found higher in rapeseed oil than in sunflower oil, similarly like in the case of ascorbyl palmitate.

Soybean lecithin is available in only limited amounts on the market, especially in our country where soybeans are not grown, and where rapeseed and sunflowerseed are the main oilseeds for the plant-scale processing. Therefore, semisynthetic phospholipids are widely used. They are produced by phosphorylation of diglyceride concentrates, which are secondary products after the production of monoglyceridic emulsifiers (Sedláček, Ranný,

1990). Phosphatidic acids obtained in this way had moderate activity in our earlier experiments (Pokorný et al., 1988, 1990, and 1992), but in these experiments (partially, due to a more sensitive method of the detection of antioxidant activity) they were found nearly inactive when added to edible oils, but in presence of DMDP, the activity was rather high (Fig. 4), and their synergistic activity was comparable with that of natural lecithins (Table II). It was particularly advantageous in sunflower oil.

Phospholipids mostly contain nitrogen substituents in addition to a phosphate functional group. Therefore, ammonium salts of phosphatidic acids were prepared (Ranný et al., 1976). They were found rather inactive in presence of tocopherol only (under our present test conditions), but their synergistic activity was rather high, especially in rapeseed oil (Table II and Fig. 5), contrary to phosphatidic acids.

Our results show, that DMDP is (relatively) a rather active antioxidant in polyunsaturated edible oils, but its activity is still more enhanced by additions of synergists, such as ascorbic acid and both natural and semi-synthetic phospholipids.

References

BAUERNFEIND, J. C.: Antioxidant function of L-ascorbic acid in food technology. Int. J. Vitamin Nutr. Res. Suppl., *27*, 1985: 307–332.

BOURGEOIS, C. F. – CZORNOMAZ, A. M.: Utilization of ascorbyl palmitate, α-tocopherol and phospholipids as antioxidants in lard. Rev. fr. Corps gras, *29*, 1982: 111–116.

DUBUR, G. Ya. – ULDRIKIS, R. – KUMEROVA, A. O. – KUMSAR, K. K. – TIRZIT, G. D. – VELENA, A. H. – ZILBERS, Yu. A.: Hydrogen-donating properties of dihydronicotinamide analogues. Proc. Seventh Int. Symp. Chem. Nat. Prod., Zinatne, Riga, *1*, 1970: 651–652.

DVINSKAYA, L. M. – IVANOV, V. P. – MANUKHIN, B. D.: Use of diludine for carotene stabilization in grass meal. Khim. Sel'skom. Khozyai., *12*, 1972: 620–622.

KOUŘIMSKÁ, L. – POKORNÝ, J. – TIRZITIS, G.: The antioxidant activity of 2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine in edible oils. Nahrung, *37*, 1993: 91–93.

LUKEVITS, E. Ya: Institut organitcheskogo sinteza 1957–1987. Zinatne, Riga 1987: 163–167.

MALYAROVA, E. M.: Effect of UV iradiation on quality of vegetable oils. Izv. Vyssh. Ucheb. Zaved., Pishch. Tekhnol., *21*, 1979: 140–142.

MÜLLER, H.: Anwendung von Ethoxyquin zur Qualitätserhaltung pflanzlicher Produkte. Fette, Seifen, Anstrichm., *82*, 1980: 200–203.

NASNER, A.: Die antioxidativen Eigenschaften von Lecithin. Fette, Seifen, Anstrichm., *87*, 1985: 477–481.
POKORNÝ, J.: Stabilization of food. In: POSPÍŠIL, J. – KLEMCHUK, P. P. (Eds.): Oxidation Inhibition in Organic Materials. Vol. I, Boca Raton, CRC Press, 1989: 347–367.
POKORNÝ, J.: Natural antioxidants for food use. Trends Food Sci. Technol., *2*, 1991: 223–227.
POKORNÝ, J. – DAVÍDEK, J. – LIST, J. – RANNÝ, M. – SEDLÁČEK, J. – SOUČEK, J.: Jedlé oleje se zvýšenou stabilitou proti oxidačnímu žluknutí. Czech pat., 259,953 (1988).
POKORNÝ, J. – DAVÍDEK, J. – VIERECKLOVÁ, M. – RANNÝ, M. - SEDLÁČEK, J.: Effect of phophorylated acylglycerols on the oxidative stability of edible oils. Nahrung, *34*, 1990: 719–725.
POKORNÝ, J. – DOBIÁŠOVÁ, S. – DAVÍDEK, J.: Repeatability of the determination of oxidative stability of vegetable oils using the Schaal test. Sb. VŠCHT v Praze, *E 58*, 1985: 163–173.
POKORNÝ, J. – POSKOČILOVÁ, H. – DAVÍDEK, J.: Effect of phospholipids on the decomposition of lipid hydroperoxides. Nahrung, *25*. K, 1981: 29–31.
POKORNÝ, J. – RÉBLOVÁ, Z. – RANNÝ, M. – KÁŇOVÁ, J. –PÁNEK, J. – DAVÍDEK, J.: Natural lecithins and phosphorylated acylglycerols as inhibitors of autoxidation of fats and oils. Nahrung, *36*, 1992: 461–462
POSPÍŠIL, J. – POKORNÝ J.: Aktivität phenolischer Antioxidantien mit Voluminösen tertiaeren Alkylgruppen bei Verwendung von Schweineschmalz und Isopropylolät als Substrat. Fette, Seifen, Anstrichm., *70*, 1968: 442–445.
RANNÝ, M. – ŠILHÁNEK, J. – SEIFERT, R. – BRADÍKOVÁ, A. – ZBIROVSKÝ, M.: Synthetische Phosphoglyceride. 1. Tenside Detergents, *13*, 1976: 77–82.
SEDLÁČEK, J. – RANNÝ, M.: Fosforylace acylglycerolů oxidem fosforečným. Chem. Prům., *40*, 1990: 283–287.
THORISSON, S. – GUNSTONE, F. D. – HARDY, R.: 8-Methylethoxyquin: A possible replacement for ethoxyquin. J. am. Oil Chem. Soc., *70*, 1993: 931–933.
TIRZIT, G. D. – BYTEVA, I. M. – SALOCHIDDINOV, K. I. – GURINOVICH, G. P. – DUBUR, G. Ya.: Proizvodnye 1,4-digidropiridina kak dezaktivatory singletnogo kisloroda. Khim. Geterotsikl. Soed., *7*, 1981: 924–926.
TIRZITIS, G. D. - KIRULE, I. E. - DUBUR, G.: Antioxidant activity of some 1,4-dihydropyridine and 1,4-dihydroindeno[1,2-b]pyridine derivatives and their action as α-tocopherol synergists. In: Proc. 16th World Congr. ISF, Budapest 1983: 655–661.
TIRZITIS, G. – KIRULE, I. – DUBURS, G.: Antioxidationsaktivität der 3,5-dicarbonylderivate des 2,6-dimethyl-1,4-dihydropyridins. Fat Sci. Technol., *90*, 1988: 411–413.
ULDRIKIS, Y. – PREISA, I. – DUBUR, G. – ZIDERMANE, A. – BISENIEX, E. – TIRZIT, G.: 2,6-Dimethyl-1,4-dihydropyridine-3,5-dicarboxylic esters and method for preparing same. US Pat. 4,293,700 (1981).

YOON, S.-H.: Antioxidative effect of various antioxidants on the soybean oil. J. Korean Soc. Food Nutr., *17*, 1988: 1923.
Institute of Organic Syntheses, AN Latv. SSR: Method of stabilizing a vegetable oil or animal fat against oxidative spoilage and the stabilized preparations. Brit. Pat. 1 521 912 (1978).

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Antioxidační účinnost 2,6-dimethyl-3,5-bis-(menthyloxy-karbonyl)-1,4-dihydropyridinu (DMDP) v jedlých olejích

Řepkový a slunečnicový olej, u nás nejběžnější rostlinné jedlé oleje, se velmi obtížně stabilizují proti oxidačnímu žluknutí, protože obsah polyenových mastných kyselin je v těchto olejích vysoký. Pro tyto oleje se diludin osvědčil (Kouřimská et al., 1993), ovšem jeho nevýhodou je špatná rozpustnost v tucích a olejích. Proto byl vyzkoušen příbuzný DMDP, který se v nich rozpouští daleko lépe. Použili jsme metody podle Schaala – za indukční periodu jsme považovali takovou dobu skladování, během níž se znatelně nemění hmotnost směsi během skladování při 40 °C. V další etapě již hmotnost postupně narůstá, protože v této etapě je již diludin rozložen a postupně se rozkládají tokoferoly a synergisty. V koncentraci 0,01–0,02 % má jen mírnou účinnost (i když by pro tyto účely byla postačující), ale v koncentraci 0,05 % je neobyčejně účinný. Jeho účinnost se ještě podstatně zvýší přídavkem některých synergistů, např. 0,02% askorbylpalmitátu, který patří k běžným, i u nás používaných látkám. K přirozeným složkám tuků s mírným synergistickým účinkem patří rostlinné fosfolipidy a 0,05 % přírodního sójového lecithinu vykazovalo výrazný synergistický účinek za přítomnosti 0,02% DMDP. Podobný synergistický účinek měly také fosfatidové kyseliny (připravené fosforylací diglyceridické frakce monoglyceridových emulgátorů) a jejich amonné soli. Účinky inhibitorů byly různé ve slunečnicovém a v řepkovém oleji. Výsledky ukázaly možnosti použití DMDP ke stabilizaci rafinovaných rostlinných olejů.

DMDP; dihydropyridinový antioxidant; stabilizace proti oxidaci; oxidační stabilita; slunečnicový olej; řepkový olej

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A BIOSENSOR FOR RAPID DETERMINATION OF FLATULENCE CAUSING OLIGOSACCHARIDES IN PEA*

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Abstract: Occurrence of flatulence causing oligosaccharides (Raffinose Family Oligosaccharides – RFO – raffinose, stachyose, verbascose, ajugose) is one of the factors limiting the consumption of legumes in human nutrition. The biosensor based on a Clark-type oxygen sensor with co-immobilized galactose oxidase and catalase was prepared and used for the determination of total content of α-galactooligosaccharides in peas. The optimal conditions of sample preparation and measurement as well as the properties of biosensor were determined. The galactose biosensor is known to be less specific than e.g. glucose oxidase sensors, therefore the results were compared with those obtained by HPLC (using $SiNH_2$ or strongly acidic catex columns with RI detection). For results of 16 pea cultivars the good correlation between total content of flatulence causing oligosaccharides determined by biosensor and HPLC was found.

biosensor; galactose oxidase; flatulence; legume; raffinose; stachyose; verbascose

Raffinose family oligosaccharides (raffinose, stachyose, verbascose) are not digested in human intestinal tract due to the absence of α-galactosidase. They are metabolized by colon microflora producing carbon dioxide and hydrogen (Rackis et al., 1975). The flatulence causing oligosaccharides are a limiting factor in consumption of nutritionally valuable legumes. A possible way to minimize flatulence is to select the legume varieties with the lowest content of oligosaccharides.

The content of flatulence oligosaccharides in legume seeds can be determined by various analytical methods. Chromatographic procedures, e.g. paper chromatography (Lineback, Ke, 1975; Tanaka et al., 1975),

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HPTLC (Revilleza et al., 1990), GC of silylated (Kosson, 1988) or trifluoracetylated sugars (Karoutis, Tyler, 1992), HPLC using $SiNH_2$, reverse phase or strong acid catex separation with RI or pulsed amperometric detection (Knudsen, 1986; Kvasnička et al., 1994) were described. These methods are usually relatively too intricate and time consuming to make the screening analyses of a large number of samples feasible. For this purpose, the rapid evaluation of flatulence oligosaccharides in legume seeds, the galactose oxidase biosensor could be used. The galactose oxidase biosensor – the Clark-type oxygen sensor with immobilized galactose oxidase was already prepared for the determination of D-galactose (Vrbová et al., 1992). Since the galactose oxidase (D-galactose: O_2-6-oxidoreductase) oxidises D-galactose on the 6th carbon atom, it can oxidise flatulence causing oligosaccharides (raffinose, stachyose, verbascose, ajugose) directly on the C-6 of atom having the free OH group of end-bound galactose. The response of the biosensor can then be correlated to the sum of flatulence oligosaccharides present in analysed sample.

The aim of the present paper was to evaluate the possibility of use of galactose biosensor for rapid evaluation of flatulence oligosaccharides content in pea.

MATERIAL AND METHODS

Samples of dried pea seeds of different varieties (Tyrkys, LU115, Olivín, HM1743, Bohatýr, Janus, Smaragd, Romeo, LU138, MS27150, MS28641, HM2648, rrRbRb, RRRbRb, RRrbrb, rrrbrb) were obtained from ÚKZÚZ Brno and from John Innes Centre, Norwich, UK.

Preparation of Biosensor

A nylon net was partially hydrolyzed with 100 µl of 25% HCl at 20 °C for 1 s, washed thoroughly with distilled water and dried. On the hydrolyzed area (0.2 cm^2) 20 µl of a solution of galactose oxidase (D-galactose oxidase, E.C. 1.1.3.9, 12.5 U/mg, lyophilized preparation from *Dactylium dendroides*, Sigma, USA) prepared by dissolution of 1.6 mg of powdered sample in 100 µl 0.1M potassium buffer pH 7.0 and 5 µl of a suspension of catalase (H_2O_2 : H_2O_2-oxidoreductase, E.C. 1.11.1.6, 2000 U/mg, 1 g = = 24.8 ml, Reanal, Hungary) were applied. Volumes of 5 µl of 2.5% solution of glutaraldehyde (Koch Light Laboratories, UK), 1 µl of cyclohexyl iso-

cyanide (Fluka, Switzerland) were added. After mixing, the enzyme mixture was incubated in a moist chamber at 4 °C for 72 hours. The net with immobilized enzymes was then washed with potassium buffer solution, fastened in a holder and fixed on the surface of Clark-type oxygen electrode (Theta 90, Elektrochemická čidla, Czech Republic).

Determination of Oligosaccharides

A portion of 0.5 g of milled peas was extracted in 10 ml of 0.1 M potassium buffer solution pH 7.0 for 30 min in ultrasonic bath. A volume of 0.1 to 1 ml of filtered extract was added into a thermostated (30 °C) reaction vessel with immersed biosensor containing 2–2.9 ml of the same buffer saturated by air. The enzyme electrode response was measured with a nanoampermeter having a stabilized source of direct polarized voltage (–650 ± 10 mV) and a signal deriving device. Both the single signal and its derivatives were monitored by means of TZ 4200 line recorder (Laboratorní přístroje, Praha). Quantification was made using the initial slope. Calibration curve was prepared by analysis of different quantities of D-galactose. Results were expressed as % (w/w) and mol/kg of D-galactose in analysed samples of pea.

Determination of Oligosaccharides by HPLC

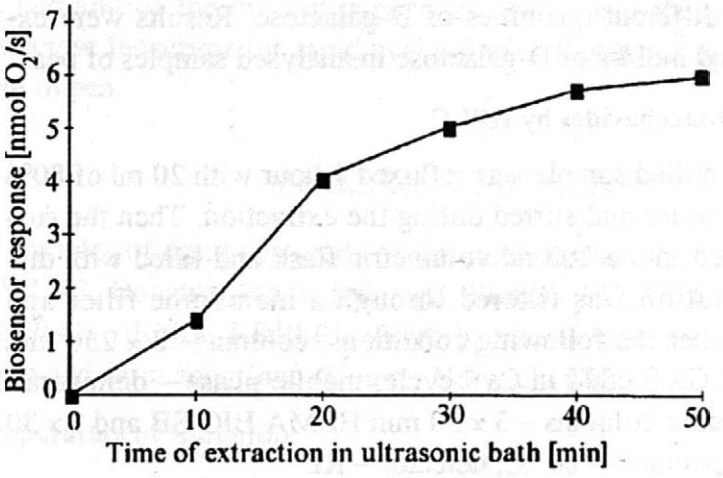
A portion of 2 g of milled sample was refluxed 1 hour with 20 ml of 80% ethanol with distilled water and stirred during the extraction. Then the suspension was transferred into a 200 ml volumetric flask and filled with distilled water. The solution was filtered through a membrane filter and analysed by HPLC under the following conditions: column – 8 x 250 mm filled with OSTION LGKS 0804 in Ca^{2+} cycle; mobile phase – demineralized distilled water; guard columns – 3 x 30 mm HEMA BIO SB and 3 x 30 mm HEMA BIO; temperature – 80 °C; detector – RI.

RESULTS AND DISCUSSION

The biosensor was prepared using the already described procedure (Vrbová et al., 1992), with co-immobilized galactose oxidase and catalase. The latter enzyme is added to remove H_2O_2 produced during the reaction and to improved the stability of biosensor. Both enzymes were co-immobilized on a nylon net activated by partial hydrolysis with hydrochloric acid. The covalent bond itself was realized by means of Ugi four-component reaction

leading to a stronger bonding of substituted amides, in contrast to the direct covalent bond formed by the reaction of amino groups with glutaraldehyde alone (Vrbová, Marek, 1990).

The optimal extraction (Fig. 1) and measurement conditions were found. The continuing increase of response during the extraction is probably caused by enzymatic hydrolysis of glycoproteins setting free other galactose oxidase substrate. The reproducibility of biosensor calculated from five repeated measurements in the same solution expressed as a relative standard deviation was less than 2.5%. In the case of the whole procedure (including the extraction) this value was not higher than 8.0%. The obtained results were correlating numbers relating to the sum of galactooligosaccharides. For practical purposes we expressed them in percentage of galactose, which was used for the calibration of biosensor. During the use of biosensor we estimated its stability, within 100 measurements of real samples we noticed only a negligible decrease of biosensor response.

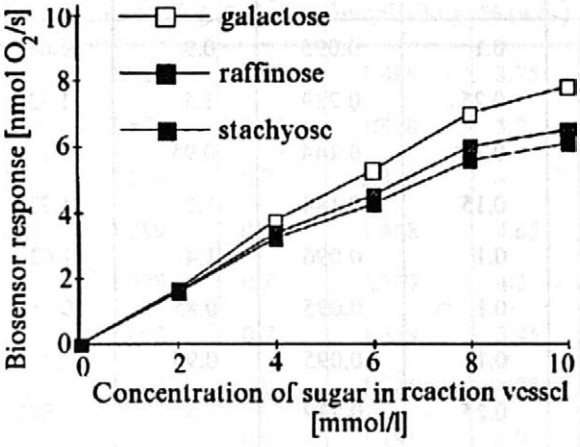


1. The effect of extraction duration on the determined quantity of galactose oxidase substrates in pea

The use of galactose biosensor to determine the flatulence causing oligosaccharides of legumes has several limitations:

1. Analysed samples should not contain free galactose, which is not flatulent but gives a misleading response in measured samples.
2. The results obtained correlate with the sum of RFO, the response, the slope of calibration curves of different oligosaccharides decreases with the increase of molecular weight of sugars (Fig. 2), the differences are more important when concentration is expressed in % (w/w) than in moles.

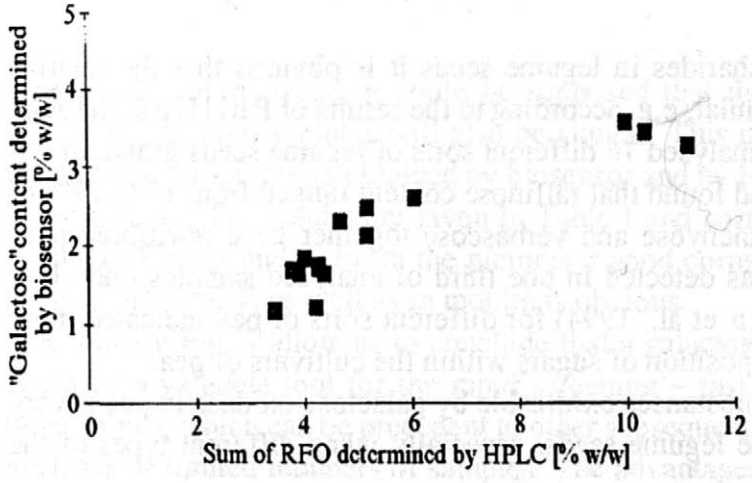
3. Galactose oxidase is less specific, it is active in oxidation of other sugars such as D-talose, L-altrose, 2-deoxy-L-lyxo-hexose (2-deoxy-D-galactose). This broad substrate specificity is dependent in a great deal on the orientation of C-4 hydroxyl group (Vrbová et al., 1992).



2. Calibration of curves of D-galactose, raffinose and stachyose

Free D-galactose is usually a minor constituent of legumes, which is not usually detected (Philips, Abbey, 1989). Our precedent chromatographic analyses of a large number of pea samples also gave only indetectable or very low content of free galactose ranging from 0 to 6% of total oligosaccharides content (Kvasnička et al., 1994).

The different response of various oligosaccharides could be a limiting factor for the use of biosensor. However, when comparing the composition of



3. Correlation of biosensor determination of RFO in 16 pea samples with the sum of RFO determined by HPLC (concentrations are expressed in % w/w)

I. Content of RFO in analysed pea samples

Sample	HPLC					
	Galactose		Ajugose		Verbascose	
	% (w/w)	mmol/100 g	% (w/w)	mmol/100 g	% (w/w)	mmol/100 g
Tyrkys	0	0	0.1	0.095	0.9	1.042
LU115	0.05	0.278	0.25	0.239	1.5	1.736
Olivín	0	0	0.15	0.144	0.95	1.1
HM1743	0	0	0.15	0.144	1.5	1.736
Janus	0	0	0.1	0.096	1.4	1.62
Smaragd	0	0	0.1	0.095	0.85	0.984
Romeo	0	0	0.1	0.095	0.95	1.1
LU138	0	0	0.25	0.239	1.6	1.852
MS27150	0	0	0.2	0.192	1.2	1.389
MS28641	0	0	0.2	0.192	1.6	1.852
HM2641	0	0	0.3	0.287	2.1	2.431
rrRbRb	0.19	1.056	0.07	0.067	5.47	6.331
RRRbRB	0.14	0.778	0.06	0.057	3.12	3.611
RRrbrb	0.22	1.222	0.09	0.096	4.71	5.451
rrrbrb	0.24	1.333	0.17	0.163	3.99	4.618

flatulence oligosaccharides in legume seeds it is obvious that the relative compositions are similar, e.g. according to the results of Phillips and Abbey (1989). They analysed 16 different sorts of legume seeds grown in Africa and America and found that raffinose content ranged from 17 to 29% of oligosaccharides. Stachyose and verbascose together gave remaining part, when verbascose was detected in one third of analysed samples only. Our results (Kvasničkа et al., 1994) for different sorts of pea indicated more similar relative composition of sugars within the cultivars of pea.

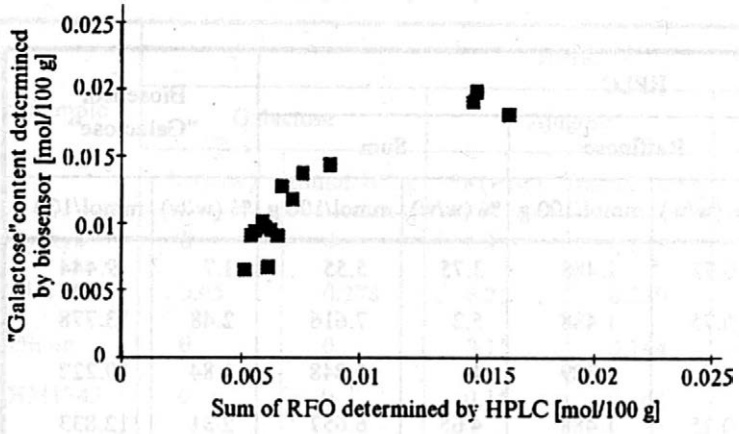
The presence of substances oxidizable by galactose oxidase in pea is very probable, but for the legume seeds, especially when different types of the

Table I. continue

HPLC						Biosensor "Galactose"	
Stachyose		Raffinose		Sum			
% (w/w)	mmol/100 g	% (w/w)	mmol/100 g	% (w/w)	mmol/100 g	% (w/w)	mmol/100 g
2	2.924	0.75	1.488	3.75	5.55	1.7	9.444
2.65	3.874	0.75	1.488	5.2	7.616	2.48	13.778
2.2	3.216	0.7	1.389	4	5.848	1.84	10.222
2.25	3.289	0.75	1.488	4.65	6.657	2.31	12.833
1.9	2.778	0.8	1.587	4.2	6.081	1.23	6.833
1.8	2.632	0.7	1.389	3.45	5.1	1.18	6.556
2.4	3.509	0.9	1.786	4.35	6.49	1.64	9.111
1.45	2.12	0.6	1.19	3.9	5.402	1.64	9.111
2.2	3.216	0.7	1.389	4.3	6.186	1.72	9.556
1.75	2.558	0.7	1.389	4.25	5.991	1.76	9.778
2	2.924	0.75	1.488	5.15	7.13	2.14	11.889
3.48	5.088	1.15	2.282	10.36	14.823	3.44	19.111
2.03	2.968	0.69	1.369	6.04	8.783	2.61	14.5
4.86	7.105	1.26	2.5	11.14	16.365	3.28	18.222
4.32	6.316	1.28	2.54	10	14.97	3.58	19.889

same seeds are analysed, it could be supposed that the content of disturbances in analysed samples will also be similar. This is obvious also from the comparison of results obtained by biosensor and by HPLC on strong acid catex column. The results are given in Table I and correlation of results is shown in Figs. 3 and 4. From the pictures a good correlation of results expressed in % (w/w) as well as in mol/kg is obvious.

Results in Fig. 3 allow us to conclude that a galactose oxidase biosensor could be a valuable tool for the rapid screening – fast sorting of different types of pea, which can be precedent to other subsequent, more sophisticated analyses of limited numbers of samples. The advantages of biosensor assay



4. Correlation of biosensor determination of RFO in 16 pea samples with the sum of RFO determined by HPLC (concentrations are expressed in mol/kg)

comparing with chromatographic procedure are: no needs of complicated equipment, rapid and simple sample preparation and speed of analysis. Disadvantages are: low specificity, obtaining only a relative "number" corresponding with the sum of sugars and sensitivity of sensor to the mode of its use.

References

KAROUTIS, A. I. – TYLER, R. T.: Analysis of legume oligosaccharides by high resolution gas chromatography. J. Chromatogr., *623*, 1992, 186–190.

KOSSON, R.: Flatulence-causing galactooligosaccharides of *Phaseolus coccineus* L. and *Phaseolus vulgaris* L. Acta Soc. Bot. Poloniae., *57*, 1988, 493–497.

KNUDSEN, I. M.: High-performance liquid chromatographic determination of oligosaccharides in leguminous seeds. J. Sci. Food Agric., *37*, 1986: 560–566.

KVASNIČKA F. – VAVROUSOVÁ R. – ŽÁČKOVÁ J.: Determination of α-galactosides in Pea Seeds. NEWSLINE (Newsletter of the LINE Network), Issue 4, winter 1994

LINEBACK, D. R. – KE, C. H.: Starches and low molecular-weight carbohydrates from chick pea and horse bean flours. Cer. Chem., *52*, 1975, 108–114.

PHILIPS, R. D. – ABBEY, B.N.: Composition and flatulence-producing potential of commonly eaten Nigerian and American legumes. Food Chem., *33*, 1989, 271–280.

RACKIS, J. J. – JEANS, A. – HODGE, J. (Eds): Physiological Effect of Food Carbohydrates. (ACS Symposium series, No. 15), Washington, DC, American Chemical Society 1975.

REVILLEZA, J. R. – MENDOZA, E. M. T. – RAYMUNDO, L. C.: Oligosaccharides in several Philippine indigenous food legumes: Determination, localisation and removal. Plant Foods Human Nutr., *40*, 1990, 83–90.

TANAKA, M. – THANANUNKUL, D. – LEE, T. C. – CHICHESTER, C. O.: A simplified method for the quantitative determination of sucrose, raffinose and stachyose in legume seeds. J. Food Sci., *40*, 1975, 1087–1088.
VRBOVÁ, E. – MAREK, M.: Application of the Ugi reaction for the preparation of enzyme electrode. Anal. Chim. Acta, *239*, 1990: 263–268.
VRBOVÁ, E. – PECKOVÁ, J. – MAREK, M.: Preparation and utilization of a biosensor based on galactose oxidase. Collect. Czech Chem. Commun., *57*, 1992, 2287–2294.

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Biosenzor pro rychlé stanovení flatulentních oligosacharidů hrachu

Širšímu uplatnění luštěnin v lidské výživě brání jejich negativní nutriční vlastnosti, zejména flatulentní účinky, které jsou mimo jiné způsobeny také přítomností α-galaktooligosacharidů (rafinosy, stachyosy, verbaskosy a ajugosy). Lidský trávicí systém nemá α-galaktosidasu, proto jsou nestrávené cukry utilizovány bakteriemi tlustého střeva za vývoje plynů. Zvýšení podílu luštěnin v lidské výživě je možné dosáhnout výběrem a šlechtěním odrůd s nízkým obsahem uvedených oligosacharidů. Pro podobné studie je nezbytné analyzovat velké soubory vzorků. Metody stanovení flatulentních oligosacharidů zahrnují širokou škálu metod, zejména chromatografických (papírovou chromatografii, TLC, HPTLC, HPLC). Stanovení je však poměrně pracné a časově náročné. Jednou z možností může být stanovení flatulentních oligosacharidů pomocí biosenzoru pro stanovení D-galaktosy na bázi koimobilizované galaktosaoxidasy a katalasy ve spojení s kyslíkovým Clarkovým článkem. Galaktosaoxidasa (D-galactosa: O_2-6-oxidoreductasa) oxiduje D-galaktosu na 6. uhlíku, proto biosenzor poskytuje odezvu také na galaktooligosacharidy. Použití má několik omezení: přítomnost volné D-galaktosy, různá odezva na jednotlivé cukry, nízká substrátová specificita galaktosaoxidasy. Z údajů v literatuře i z našich výsledků vyplynulo, že volná D-galaktosa je přítomna pouze v nízkých koncentracích, relativní složení cukrů je podobné v řadě testovaných luštěnin, zejména mezi různými odrůdami hrachu. Rovněž přítomnost rušících látek je srovnatelná.

Biosenzor připravený koimobilizací galaktosaoxidasy s katalasou na nylonovou síťku Ugiho reakcí byl použit k analýze 16 vzorků různých odrůd hrachu, nalezené obsahy – sumární hodnoty vyjádřené jako D-galaktosa – byly porovnány s výsledky stanovení oligosacharidů kapalinovou chromatografií na silně kyselém katexu v Ca^{2+} cyklu s RI detekcí. Byla zjištěna těsná korelace mezi oběma metodami. Biosenzor na

stanovení D-galaktosy by mohl posloužit k rychlým analýzám, k roztřídění vzorků a snížení počtu analýz prováděných náročnějšími instrumentálními metodami.

biosenzor; galactosaoxidasa; flatulence; luštěniny; rafinosa; stachyosa; verbascosa

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COLOUR OF SAUSAGES INFLUENCED BY NON-MEAT PROTEIN ADDITION

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Abstract: The colour of meat products is influenced by the addition of non-meat proteins. The brightness is increased and the a* value (the coefficient for red colour) is reduced. Up to certain concentration of additives, the changes are not important for consumer.

colour; meat products; protein; soy isolates; pH; brightness

The colour is one of the most important organoleptic properties of meat products. The pink sausage colour is caused by the relative stable dinitrosylhaem produced by reaction between haem pigments of meat (myoglobin and haemoglobin) with the nitrite added to the meat in form of curing mixture.

The colour may be characterised as a dinitrosylhaem ratio or by three values L*, a* and b* of the C.I.E. system. The dinitrosylhaem ratio is defined as a ratio of dinitrosylhaem concentration to the total haem pigment one. The brightness L* is expressed in percentage where 0% is equal to the black colour and 100% to the white one. The a* value characterises the relation between red and green, and the b* value characterises the relation between yellow and blue colours.

Changing the recipes of sausages the colour can be significantly influenced. Lately great attention has been given to application of non-meat additives, such as hydrocoloids and non-meat proteins. The addition of such proteins into the meat products is of the biggest importance for economic aspects. It is caused by the substitution of expensive lean meat (beef) by means of relatively cheaper raw materials. The frequently mentioned nutritional effects are often problematical in view of the used amount. Among different protein additives (soybean, milk, wheat, pie, etc.) the most perspective are the soy protein isolates.

The character and quality of meat products should be maintained or improved after of non-meat proteins applications. According to the type and

quality of protein additive different changes of organoleptic and technological properties can occur. The consistency may be positively or negatively influenced, the flavour is reduced or changed and the changes of colour can take place, too.

The addition of non-meat proteins causes the reduction of haem pigment concentration. Thus the brightness (L*, Y resp.) of such meat products may be higher. As the protein additives increase the water-holding capacity (WHC), i.e. they reduce the free water content and consequently the reflection on the sausage surface may be influenced so that this increase of brightness is partially compensated (Pipek, 1986).

In case when 12% meat is replaced by textured soy proteins, the brightness of Bologna-type sausages increased (Ambordiasis, 1993). The Serbian fermented sausages produced using 2% of soybean floor or soy textured proteins had lighter colour than controls without such additives. Although the colour during ripening became lighter, the brightness of controls was lower than at sausages with soy proteins (Grujič et al., 1990).

Besides brightness the changes in hue may occur. The products with too extensive water and protein addition contain too little haem pigments for stable colour forming. Thus such products become brownish. The brownish colour appears also in the case when too much brine is injected that contains less refined proteins (e.g. soy concentrates) (Hoogenkamp, 1992).

Such changes of hue value may be caused by alteration of condition for the reaction of haem pigments with nitrites. The level of sulphyhydrylic bonds that are important for the conversion of haem iron to ferrous form, is different at various proteins. The other condition is pH value of sausage mixture that can be altered by the protein addition. Although some authors had not observed any changes (the pH value of sausages ranged between 6.2 and 6.4) (Katsaras,Peetz, 1994), in other cases such changes occurred because the pH value of pure soy protein isolate ranged between 6.4 and 7.0 (Gorbatov, 1984).

If the reaction between nitrites and haem pigments is influenced, the changes in a* and b* values and dinitrosylhaem ratio may occur.

The addition of soy proteins into meat products caused the decrease of a* value and the increase of b* one; in the same time the colour became red-orange (Katsaras,Peetz, 1994). The a* value is proposed for characterisation of Bologna-type sausages colour. The b* value is considered to be less suitable because of irregular changes (Klettner,Ott, 1993).

During the sensory evaluation of meat products colour the pink colour became worse with increasing addition of isolated soy proteins together with the decrease of a* value and the increase of pH (Reichert, 1991).

MATERIAL AND METHODS

In our experiments we studied different types of protein additives. The results of experiment with milk and wheat proteins were published earlier. This time the influence of the soy protein isolates upon the colour of different smoked minced meat products was estimated..

The isolated soybean protein SUPRO 500E (produced by Protein Technologies International) containing at least 90% of protein was tested. The protein concentrates (i.e. the additives with only 70% protein) were not investigated.

The samples were prepared under the industrial conditions using the usual recipes for two types of frankfurters ("Libové párky", "Jemné párky") and the fine comminuted Bologna-type sausage "Junior". The soy protein isolates were added (following the producer recommendation) in hydrated form. The ratio protein and water was 1 to 4, if no other ratio is said. The soy proteins were added to the sausage mixture replacing the corresponding amount of beef in the recipe.

Methods

The colour of meat products was estimated by the reflectance spectrometry and by nitrosohaem/total haem pigments ratio.

The reflectance of the meat product slices was measured using the spectrophotometer SPECORD in the whole visual area from 380 to 770 nm. The desk of the barium sulphate was used as the standard of the white colour. The own software CIELAB was used to calculate the values of the C.I.E. systems at the light source A (evening light): the brightness L*, coefficient for red a* and coefficient for yellow b*.

The chemical analysis of the nitrosohaem ratio was performed using the modified Hornsey method. For the nitrosylhaem content estimation the samples of meat products were extracted for 20 minutes by neutral acetone in dark and after filtration the optical density of extract was measured at the 540 nm using the spectrophotometer SPEKOL. Another part of the sample was extracted by acidified acetone (1.125 ml of HCl for 100 ml neutral ace-

tone) for 60 minutes in the light to estimate the total haem pigment content. After the filtration the optical density of the extract was measured at 640 nm. The dinitrosylhaem ratio, e.g. the ratio of dinitrosylhaem concentration to total haem pigment concentration, was then calculated.

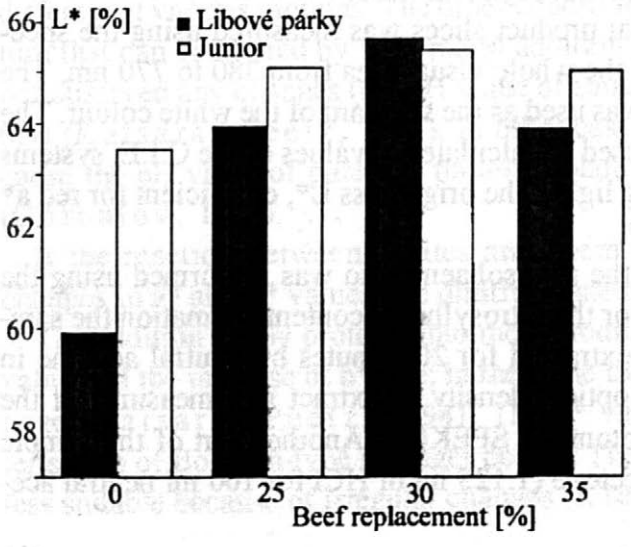
The pH value of the meat product was measured by pH-meter Testoterm.

RESULTS AND DISCUSSION

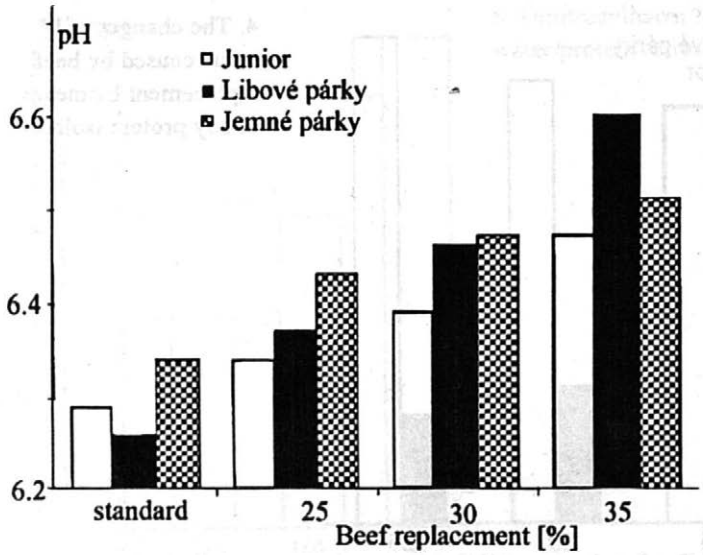
The addition of the hydrated soy protein resulted in all cases in colour changes of the meat products. These changes concerned both the lightness and the hue value.

The brightness L* was higher every time the soy isolates were added (Fig. 1). At the larger addition of protein the lightness was not so high. The change of water-holding capacity and thus the change of light distraction on the sausage surface could be the reason for this effect as mentioned earlier (Pipek et al., 1986). Such an increase of lightness is not visually important. Without the control sample the untrained person can hardly observe any changes. In opposite the trained panel was able to distinguish, by means of triangle test, between samples with soy protein addition and control. The differences were statistically significant.

The replacement of beef by soy protein isolates caused the increase of pH value (Fig. 2). This change influenced the water-holding capacity and the light distraction. The pH value was also away of optimum for haem pigment



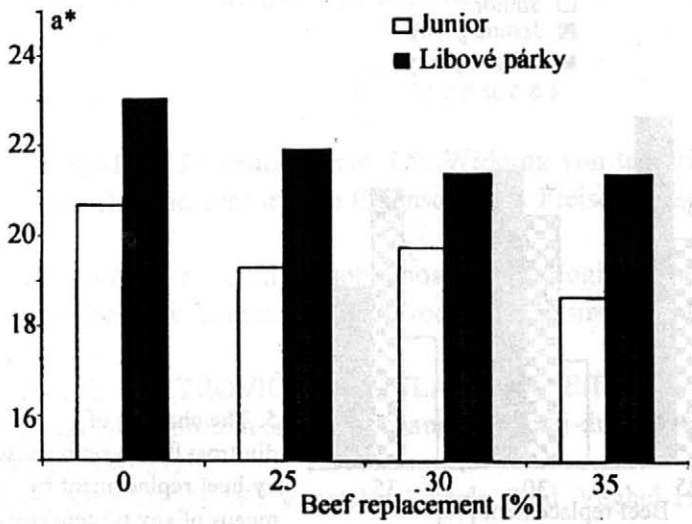
1. The changes of meat products brightness caused by beef replacement by means of soy protein isolates



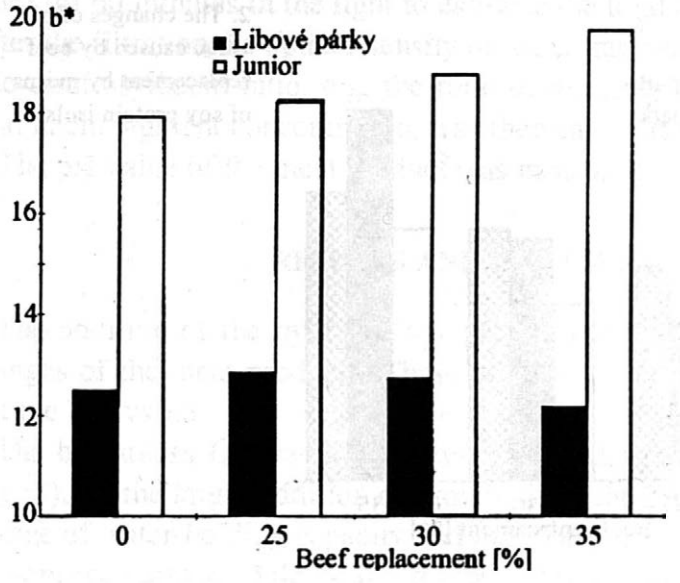
2. The changes of pH value caused by beef replacement by means of soy protein isolates

conversion, that is reaction of haem pigments with nitrites that is influenced by presence of H^+ ions. The reaction was slower and the extent of conversion smaller – see below.

The values a* and b* were influenced by soy protein addition, too. The a* value characterising the red colour decreased with rising meat replacement (Fig. 3). In the same time the b* value changes (yellow colour) were not univocal, this value seemed to increase a little (Fig. 4). These changes were also small and for untrained observer not important.



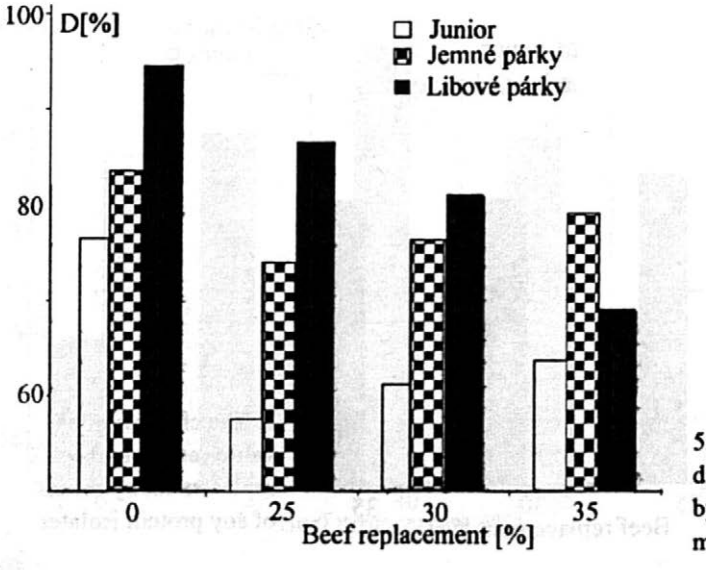
3. The changes of a* value caused by beef replacement by means of soy protein isolates



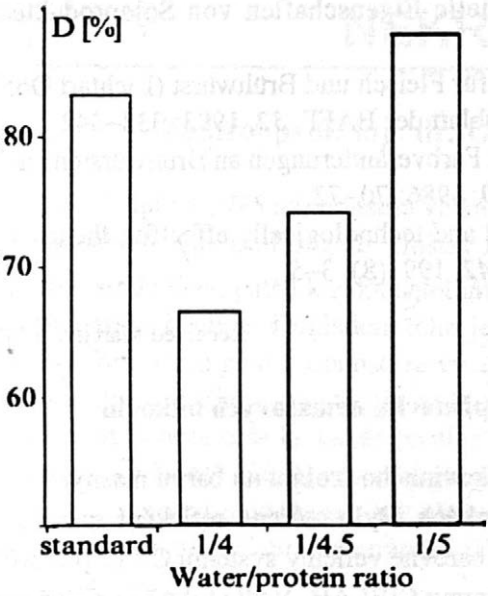
4. The changes of b* value caused by beef replacement by means of soy protein isolates

There may exist two reasons for such changes here. First the decrease of beef ratio in sausage recipe causes the decrease of haem pigment content in sausage mixture, that is one of the two reaction partners. Together the pH value increases and the reduction properties of in sausage mixture are changed and the conversion of haem pigments into nitrosohaem is reduced.

This fact could be demonstrated on the dinitrosylhaem ratio that decreases after soy protein addition as shown in Fig. 5. In opposite at the higher addition of protein isolates the decrease of nitrosohaem ratio was smaller. It can



5. The changes of dinitrosylhaem ratio caused by beef replacement by means of soy protein isolates



6. Dinitrosylhaem ratio at various water/protein ratios (15% of beef was replaced)

be related to the higher viscosity of sausage mixture, which affect againts the evaporation of nitrogen oxide that can be better used in the reaction with haem pigments (Hoogenkamp, 1992).

The changes of dinitrosylhaem ratio were other. If the water and protein ratio was greater than 4 to 1 as recommended by producer (Fig. 6). When 4.5 parts of water were added together with one part of protein, the decrease of dinitrosylhaem ratio was smaller, at 5 to 1 it was even higher than in control. Although it seems to be positive, other properties (WHC, cooking yield) were negatively influenced in the same time.

References

AMBROSIADIS, I.: Bruchwürste. Die Wirkung von texturiertem Sojaeiweiss auf technologische und sensorische Eigenschaften. Fleischwirtschaft, *73*, 1993: 1128–1160.

GORBATOV, V. M. et al.: Osobennosti technologii primeneniya izolirovanogo soyevogo belka v kolbasnom proizvodstve. Mjasnaja industrija SSSR, *62*, 1984: 14–17.

GRUJIČ, R. – PETROVIČ, L. – MULALIČ, N.: Effects of addition of soy protein or brewers' yeast protein on colour changes of semi-dry sausages. Tehnologija Mesa, *31*, 1990: 208–212.

HOOGENKAMP, H. W.: Vegetable Protein. 2.ed. Veghel 1992.

KATSARAS, K. – PEETZ, P.: Funktionelle Eigenschaften von Sojaprodukten. Fleischwirtschaft, *74*, 1994: 89–91.
KLETTNER, P. G. – OTT, G.: Farbwerte für Fleisch und Brühwürst (Lichtart D65) im Qualitätssicherungssystem. Mitteilungsblatt der BAFF, *32*, 1993: 338–342.
PIPEK, P. – SIELAFF, H. – THIEMIG, F.: Farbveränderungen an Brühwürsten nach Zugabe von Eiweisspräparaten. Fleisch, *40*, 1986: 70–72.
REICHERT, J. E.: Nutritionally important and technologically effectful: the use of soy protein in meat products. Fleischerei, *42*, 1991(8): 3–5.

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Barva masných výrobků po přídavku nemasových bílkovin

Byl sledován vliv přídavku sójového bílkovinného izolátu na barvu masných výrobků připravených v provozních podmínkách. Bylo měřeno reflekční spektrum (spektrofotometr SPECORD) a vypočteny barevné veličiny systému C.I.E. pro světelný zdroj A s využitím výpočetního programu CIELAB. Vedle toho byl zjišťován stupeň vybarvení, tj. poměr nitroxyhemochromu k obsahu všech hemových barviv, s použitím modifikované Hornseyovy metody spektrofotometrického stanovení extraktů hemových barviv v okyseleném a neutrálním acetonu. V důsledku přídavku sójových bílkovin došlo ke změně barvy. Světlost se zvýšila; vliv mělo jak snížení koncentrace hemových barviv, tak i ovlivnění odrazu světla na povrchu v důsledku změny vaznosti. Byly ovlivněny i hodnoty barevných koeficientů a* a b*. Koeficient a* pro červenou barvu klesal, zatímco koeficient b* pro žlutou jen nepatrně rostl. Uvedené změny souvisí i se zhoršením stupně vybarvení, tj. vzniká méně nitroxyhemochromu. Vedle snížení obsahu hemových barviv se zde projevuje i změna pH, které se vzdalovalo od optima reakce hemových barviv a dusitanů. Barva masných výrobků je ovlivněna přídavkem sójových bílkovin. Při dodržení doporučených dávek a způsobu aplikace však tyto změny nejsou příliš významné.

barva; masné produkty; proteiny; sójový izolát; pH; světlost

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NEKROLOG

Zemřel prof. ing. dr. Gustav Janíček, DrSc.

Dne 24. dubna 1995 nás opustila velká osobnost naší potravinářské vědy prof. ing. dr. Gustav Janíček, DrSc.

Profesor Janíček patřil k zakladatelům naší moderní potravinářské chemie. Dokladem toho je celý jeho život. Na formování jeho osobnosti se významným podílem uplatnilo rodinné prostředí, ve kterém vyrůstal. Především to byla úcta ke každé poctivé práci. Sám se k tomu, co v životě získal, dostával právě tímto způsobem. Již samotná skutečnost, jak absolvoval fakultu chemickotechnologického inženýrství ČVUT v Praze, vzbuzuje obdiv.

I jeho další životní dráha je spojena s láskou k práci, s osobní zodpovědností a angažovaností. Důkazem toho jsou výsledky jeho práce dosažené během jeho aktivní činnosti jak v průmyslu, tak i ve výzkumu a na vysoké škole.

Vysoké škole, jmenovitě fakultě potravinářské a biochemické technologie, věnoval nejproduktivnější část svého života. Zde také dosáhl největších životních úspěchů. Ve své vědecko-výzkumné, pedagogické i společenské práci ideálně sladil zkušenosti získané působením v hospodářské sféře. Odtud pramení i jeho schopnost správně určit hlavní problémy, vybrat si vhodný kolektiv a řídit ho tak, aby získané výsledky mohly být co nejrychleji využity jak pro další výzkum, tak i co nejrychleji uplatněny v průmyslové i kontrolní praxi.

Profesor Janíček nikdy nezapomínal na harmonický vývoj svých spolupracovníků a žáků. Byl si vždy vědom toho, že životní pohoda inženýra je možná jen tehdy, je-li technika v rovnováze s kulturou. Pokládal tuto stránku výchovy za velmi důležitou. Jak sám říkal, pouze jedinec, který dovede sladit tyto dvě stránky vzdělání, může nejen sám úspěšně pracovat, ale také může účinněji řídit svěřený kolektiv, neboť lépe chápe i jeho těžkosti a životní starosti. Proto i v dobách svého nejvyššího pracovního zatížení si vždy našel alespoň chvilku času na své komorní „muzicírování".

Představovat profesora Janíčka chemické a speciálně pak potravinářské veřejnosti s pohledu dosažených výsledků ve vědecko-výzkumné činnosti pokládám za zbytečné. Všichni známe jeho aktivitu v oblasti výzkumu potravinářských kontaminantů, lipidů, Maillardových reakcí a senzorického hodnocení potravin. Všichni víme, že na základě své práce obdržel nejen řadu ocenění, ale je všeobecně pokládán za zakladatele moderní české školy potravinářských chemiků.

Výsledky jeho práce i jeho osobní vlastnosti ho předurčovaly k nejrůznějším funkcím jak v oblasti vědecké a pedagogické, tak i společenské. Rovněž zde dosáhl mimořádných úspěchů. Bylo to především proto, že se mu vždy při řešení svěřeného úseku jednalo o podstatu věci, a nikdy o to, jaké výhody může navrhované řešení přinést jeho pracovišti, nebo dokonce jemu osobně. Jistě i tyto jeho charakterové vlastnosti nemalou měrou přispěly k tomu, že za svoji práci obdržel řadu vyznamenání.

Je pravdou, že skutečnou ztrátu člověka si plně uvědomujeme, až když nás navždy opustil. Profesor Janíček patřil mezi mimořádné osobnosti nejen jako vědec a pedagog, ale především jako člověk a přítel s neobvykle širokým kulturním a politickým rozhledem.

Měl jsem tu výsadu, že jsem se s ním spolu s mojí ženou Evou a mým přítelem Pavlem Rauchem pravidelně u něho v bytě po dlouhá léta setkával při našem měsíčním posezení. Vždy jsme se na tyto chvíle s naším učitelem těšili. Byly to chvíle plodných debat, úvah a názorů na vývoj vědy, společnosti i života. Profesor Janíček byl až do posledních chvil svého života duševně svěží a bylo přímo potěšením sledovat jeho úvahy a názory na nejrůznější lidské činnosti. Měl jsem v životě to štěstí, že jsem měl tak výborného učitele a později přítele. Nesmírně jsem si vážil toho, že jsem, tak jak on říkal, jeho druhým synem. S bolestí, ale rád jsem s ním trávil jeho poslední hodiny života. Pro mne a jistě pro celou řadu svých žáků a přátel zůstane profesor Janíček nezapomenutelnou osobností. Hluboce se skláním před jeho životem a bude mi opravdu chybět. Chybět bude i řadě svých žáků a přátel. V našich myslích zůstane spojen s představou pracovitého, čestného a upřímného vědce, učitele a přítele.

Prof. ing. Jiří Davidek, DrSc.

Prof. Ing. Dr. Gustav Janíček, Emeritus Professor died on April 24 1995. He was 83. Gustav Janíček retired from Faculty of Food and Biochemical technology in 1982 as Professor of food chemistry and analysis. He served as head of the Department of food chemistry and analysis between 1952–1971 and as rector of Institute of Chemical Technology between 1962–1966. He was also active as Corresponding member of Czechoslovak Academy of Science and as Member of Czechoslovak Academy of Agriculture.

Prof. Janíček lifelong research emphasis was the food chemistry and food analysis, especially lipid's oxidation, Maillard reaction and contaminants.

He was active as Chairman of Working Party on Food and Agricultural Chemistry, Czechoslovak Chemical Society and Czechoslovak representative in Working Party on Food Chemistry, Federation of European Chemical Societies.

He was author and co-authors of several scientific papers on lipid oxidation, Maillard reaction and contaminants.

Instructions for authors

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

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