ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

POTRAVINÁŘSKÉ VĚDY

FOOD SCIENCES

3

ROČNÍK 14
PRAHA 1996
CS ISSN 0862-8653

ČESKÁ AKADEMIE ZEMĚDĚLSKÝCH VĚD

POTRAVINÁŘSKÉ VĚDY
FOOD SCIENCES

An international journal published by the Czech Academy of Agricultural Sciences and with the promotion of the Ministry of Agriculture of The Czech Republic

Abstracts from the journal is comprised in Agrindex of FAO (AGRIS database), Food Science and Technology Abstracts, Dairy Science Abstracts, Chemical Abstracts, PASCAL – CD-ROM (INIST), WLAS, TOXILINE PLUS and Czech Agricultural Bibliography.

Aim and scope: The journal publishes original scientific papers, short communications, and selectively reviews, that means papers based on the study of technical literature and reviewing knowledge in the given field. Published papers are in Czech, Slovak or English.

Subscription information: Subscription orders can be entered only by calendar year and should be sent to the contact address.
Subscription price for 1996 is 264 Kc, 66 USD (Europe) and 70 USD (overseas)

Periodicity: The journal is published six times a year.

Contact address: Slezská 7, 120 56 Prague 2, Czech Republic,
tel.: 0402/25 10 98; fax: 0402/25 70 90; E-mail: braun@uzpi.agrec.cz

Potrav. Vědy, *14*, 1996 (3) : 165–174

THE pH TOLERANCE, BILE RESISTANCE AND PRODUCTION OF ANTIMICROBIAL COMPOUNDS BY LACTOBACILLI*

Milada PLOCKOVÁ, Jana CHUMCHALOVÁ, Blanka PLUHAŘOVÁ

Institute of Chemical Technology – Department of Milk and Fat Technology, Prague, Czech Republic

Abstract: Variations in low pH resistance and bile tolerance were observed among seven cultures of *Lactobacillus* sp. of various origin (human, dairy starters, dairy products) that all grow well in milk and MRS broth and produce only minimal levels of H_2O_2. The degree of bile tolerance and the low pH resistance were the highest for *Lactobacillus plantarum*, LHI10 of human origin. The strains varied in bacteriocin production and bacteriocin sensitivity. *Lactobacillus acidophilus*, CH5 produced bacteriocin acidocin CH5, to which the most sensitive strain was *Lactobacillus delbrüeckii* subsp. *lactis*, LTI30, which was chosen for acidocin CH5 activity evaluation. *Lactobacillus* sp. cultures possessing good low pH resistance, bile tolerance and bacteriocin production have an advantage over those that do not possess the activity in selecting cultures for protective and pro-biotic functions.

Lactobacillus; hydrogen peroxide; bile resistance; low pH tolerance; acidocin CH5; bacteriocin

It has been known for many years that lactobacilli may positively influence the gastrointestinal tract of humans and other mammals (Sandine, 1979). The beneficial effects include the inhibition of undesirable microorganisms, reduction in cholesterol and reduction of the risk of colon cancer (Gilliland et al., 1985; Gilliland, Speck, 1977; Goldin, Gorbach, 1984). Selection of lactobacilli to be used for the possible therapeutic value involves: pH tolerance (Hood, Zottola, 1988), bile resistance (Gilliland, Walker, 1990), the ability of the organism to adhere to and colonize the intestinal tract by means of a carbohydrate-rich layer on the bacterial cell (Brooker, Fuller, 1975) and antibacterial activity (Gilliland, Speck, 1977; Gilliland, Walker, 1990).

To reach the intestine, the organism must first pass through the stomach where the hydrochloric acid and enzymes ingesting food are present. It is

* Supported by Grant Agency of the Czech Republic Grant No. 510/95/0990.

likely that ingested organisms would come into contact with pH values in the range 2–8 (Franklin, Skoryne, 1971). The ability of bacteria to survive at pH 2, 3 and 4 is important for prediction of passing the bacteria through the hostile stomach environment (Hood, Zottola, 1988) as well as the bile resistance (Gilliland, Walker, 1990). Inhibition of undesirable bacteria including the pathogens and the competition and well growing in the presence of similar bacteria depends on the production of antibacterial compounds including organic acids, hydrogen peroxide and bacteriocins (Gilliland, Walker, 1990).

The aim of the work was to compare several strains of lactobacilli isolated from various sources (human intestinal tract, dairy cultures, milk products) in view of pH tolerance, bile resistance and production of antibacterial compounds and to find a bacteriocin producing strain of the genus *Lactobacillus*.

MATERIAL AND METHODS

Microorganisms

From the collection of 20 strains of lactobacilli isolated from various sources and confirmed at the Department of Milk and Fat Technology (Kandler, Weiss, 1986) 7 strains were chosen on the basis of their ability to grow in milk and were further studied:

- *Lactobacillus acidophilus*, A92
  isolated from dairy starter, Milcom Vokovice, Czech Republic,
- *Lactobacillus acidophilus*, CH5
  isolated from dairy starter, CHr. Hansens, Denmark,
- *Lactobacillus acidophilus*, NO1
  isolated from the fermented product Bioghurt, Germany,
- *Lactobacillus acidophilus*, D10
  isolated from the fermented product Bioghurt, Germany,
- *Lactobacillus acidophilus*, B12
  isolated from the fermented product Biogarde, Germany,
- *Lactobacillus delbrüeckii* subsp. *lactis*, LTI30
  isolated from the intestinal tract of a child,
- *Lactobacillus plantarum*, LHI10
  isolated from the intestinal tract of a child.

All strains were cultivated in MRS broth (Oxoid) or in skim milk with 0.5% of yeast extract at 37 °C for 16–18 hrs. All strains are now maintained in freeze-dried form in the Collection of Milk Microorganisms of Milcom, Prague 6, CR.

Methods

The content of hydrogen peroxide was measured according to Frew and Jones (1983). The casein from milk based media was precipitated by 5% TCA and discurded by filtration, the supernatant was mixed with potassium iodide solution and the amount of iodine proportional to hydrogen peroxide concentration was read at 352 nm by Specol Carl Zeiss Jena, Germany.

For low pH tolerance estimation (Hood, Zottola, 1988) washed cells (10^8–10^9 CFU/ml) after cultivation in MRS broth (Oxoid) were added into: a) MRS broth (Oxoid) adjusted to pH 2, 3, 4, b) KCl buffer pH 2, 3, c) potassium phthalate buffer pH 3, 4. The tubes were incubated for 2 hrs at 37 °C and the decrease of viable cells of lactobacilli was tested by plate method using MRS agar (Oxoid). The results are the average values obtained from three trials.

Comparison for bile tolerance (Gilliland, Walker, 1990) was done by a test for rapidity of growth in MRS broth (Oxoid) with and without added 0.3% w/v sodium salt of tauroglycocholic acid (Merck). 1% of freshly prepared cultures was inoculated into both broth media and incubated at 37 °C for 8 hours. Every hour the growth was monitored by measuring the optical density at 620 nm. Comparison of the cultures was based on the time required for each to increase the optical density by 0.3 units.

The ability to produce bacteriocin was screened for all tested strains. The MRS broth cultures (16 hrs at 37 °C) were centrifuged 10 min at 5 000 g and less than 5 °C to remove the bacterial cells. The supernatants were collected, adjusted to pH 6.5 with 1 mol/l NaOH to neutralize the organic acids. Then the catalase (6 mg/ml) was added to avoid the effect of hydrogen peroxide and the samples were filter sterilized and used for a cross-inhibition test. To confirm that inhibition is caused by the action of bacteriocin the broth samples were treated with trypsin (Sigma) (Ferreira, Gilliland, 1988). 7 ml of molten soft MRS agar (0.7%) tempered to 45 °C was inoculated with 0.2 ml of fresh overnight culture of each strain, mixed well and then dispensed into 82-mm sterile Petri dish. After solidification, 10 l volumes of spent broth samples were spotted onto the surface of the seeded agar. The plates were incubated for 16 hrs at 37 °C in an anaerobic jar (Oxoid). The clear zones in the place of stabbing as were taken an indication of inhibition of test culture by the spent broth samples.

RESULTS AND DISCUSION

The *Lactobacillus* sp. strains producing acids in reconstituted skim milk and MRS broth at a concentration typical of this type of starter cultures were tested from the point of view of their survival at low pH and in the presence of sodium salt of tauroglycocholic acid, for hydrogen peroxide production and production of bacteriocins.

I. Ranking of lactobacilli cultures according to decreasing hydrogen peroxide content in culture media

Rank	H_2O_2 production [mg/l]		
	culture	MRS broth	skim milk
1.	LA CH5	0.75	0.95
2.	LA B12	0.60	0.75
3.	LD LTI30	0.55	0.70
4.	LP LHI10	0.40	0.55
5.	LA NO1	0.30	0.40
6.	LA A92	<0.10	<0.10
7.	LA D10	<0.10	<0.10

In the selection of strains for protective and probiotic effects tolerance of low pH might be one of many criteria to be considered. The low pH tolerance measured in this study in several different environments is presented in Table II. The differences in the survival of each strain in different media with the identical pH value and the differences between the particular strains in the identical environment were proved.

In the presence of nutrient (MRS broth) *Lactobacillus* sp. strains survived better compared with KCl or phthalate buffers at the corresponding pH values (after 2hrs at pH 2 the number of viable cells decreased in MRS broth by

II. Low pH tolerance measured in MRS broth, KCl and phtalate buffer expressed as log CFU_0 – – log CFU_{120}[1]

Rank[2]	Culture	MRS			KCl		Phthalate	
		pH						
		2	3	4	2	3	3	4
1.	LP LHI10	1.4	0.8	0.4	2.0	1.5	2.0	1.5
2.	LA D10	1.9	1.7	0.7	2.0	1.6	2.0	1.8
3.	LA NO1	1.4	0.8	0.8	3.3	2.0	2.5	2.0
4.	LA A92	2.2	1.9	1.0	3.7	2.9	2.4	2.1
5.	LA B12	1.6	1.1	0.7	4.5	3.9	4.9	3.3
6.	LA CH5	2.0	1.7	0.7	4.3	3.7	4.0	3.7
7.	LD LTI30	1.8	1.4	0.7	4.4	4.2	4.5	3.8

[1]log CFU_0 is log CFU in time 0, log CFU_{120} is log CFU after 120 min at 37 °C at each environment

[2]Ranked from the most tolerant strain to the minimally tolerant one according to the average difference in CFU in seven tested environments

1.5–2 log cycles, in KCl buffer by 2–4.5 log cycles). In the phthalate buffer (pH 3) in spite of the fact it is not known to be toxic to bacterial cells the decrease of viable cells was higher compared with the KCl buffer (pH 3). The presented data show the survival of microorganisms in buffer systems at pH values that might be encountered in the stomach. The best surviving strain was *Lactobacillus plantarum*, LHI10 of the human origin followed by *Lactobacillus acidophilus* strains commercially used (D10, NO1, A92). The results are not in agreement with those reported by Hood and Zottola (1988), where no viable cells of *Lactobacillus acidophilus* were recovered after 45 min at pH 2.

The variations among the cultures with regard to the rapidity of growth in MRS broth (MRS) and MRS with 0.3% w/w of tauroglycocholic acid (MRST) can be read in Table III, where the strains are arranged in the order of decreasing rapidity of growth in each medium. In agreement with Gilliland and Walker (1990) it was observed that the rapidity of growth in MRS broth was not indicative of the rapidity of growth observed in MRST broth. The results indicate the wide range in the ability of the strains to grow in the presence of bile. The minimal differences in growth rates in MRS and

III. Comparison of the growth of *Lactobacillus* sp. cultures in MRS broth and MRS broth with 0.3% w/w tauroglycocholic acid

Rank	MRS		MRST			
	culture[1]	(h_0)[2]	culture	(h_T)	Culture	(Δh)
1.	LA B12	3.75	LA NO1	5.60	LP LHI10	0.65
2.	LA NO1	3.80	LA B12	5.85	LA NO1	1.80
3.	LA D10	3.90	LP LHI10	5.90	LA CH5	1.85
4.	LD LTI30	4.40	LA D10	6.55	LA B12	2.10
5.	LA A92	4.75	LA CH5	6.85	LA D10	2.65
6.	LA CH5	4.90	LA A92	>8.00	LA A92	>3.25
7.	LP LHI10	5.25	LD LTI30	>8.00	LD LTI30	>3.60

[1]Ranked from faster to the slowest growth in each medium
[2]ours required for optical density at 620 nm to increase 0.3 units at MRS (h_0) and MRST (h_T)
[3]Ranked according to decreasing value $\Delta h = h_T - h_0$

MRST indicating good tolerance to bile were proved by *Lactobacillus plantarum*, LHI10. The strains *Lactobacillus acidophilus,* A92 and *Lactobacillus delbrüeckii* subsp. *lactis,* LTI30 grew well in MRS and were totally inhibited in MRST indicating no tolerance to bile. The data are in agreement with the report of Huis in't Veld et al. (1994) where the strains of *Lactobacillus* appeared more resistant to gastric acid and bile than other bacteria. Although there exist large differences between individual strains.

When selecting the strains for use as a protective culture a number of factors including the production of antimicrobials such as hydrogen peroxide and bacteriocins should be considered (Holzapfel et al., 1995).

In this study the production of hydrogen peroxide (Table I) was realized under static cultivation conditions and reached the levels less than 1 mg/l at 16 hr of incubation at 37 °C in skim milk as well as in MRS broth for all tested strains. The increase of hydrogen peroxide production and accumulation in environment could be expected in continuously shaken incubation.

The concentration needed to prevent the growth of vegetative bacteria is about 25 mg/l H_2O_2 or less (Block, 1991), to prevent the growth of the most sensitive *Pseudomonas* sp. it is 2–8 mg/l H_2O_2 (Price, Lee, 1970). For the activation of lactoperoxidase system (LPS – nonspecific antimicrobial system present in bovine milk including three components: the lactoperoxidase enzyme, thiocyanate SCN^- and hydrogen peroxide) it is necessary to have the concentration of hydrogen peroxide higher than 8 mg/l (Reiter, Harnulv, 1984). The concentration levels observed in tested media after cultivation of starter cultures were not sufficient to inhibit either vegetative bacteria or activate LPS system in bovine milk. To eliminate the contribution of H_2O_2 to the antibacterial effect of bacteriocins catalase (6 mg/ml) was used (Barefoot, Klaenhammer, 1983).

The ability of strains to produce bacteriocin provides substantial health benefits to man by means of stabilizing the gastrointestinal tract. This ability may be an important additional advantage for the selection of strains used in the food industry for their probiotic and protective functions (Holzapfel et al., 1995).

The production of bacteriocins is presented in Table IV and it can be read from the results that some strains produce bacteriocins and some strains are sensitive to bacteriocins. The strong production of bacteriocin was observed in *Lactobacillus acidophilus*, CH5. The active substance was named acidocin CH5 and partly characterized (Chumchalová et al., 1995). From the strains sensitive to bacteriocins and the strain *Lactobacillus delbrüeckii* subsp. *lactis*, LTI30 was selected used as a target microorganism for the acidocin CH5 activity evaluation.

IV. Test of selected *Lactobacillus* sp. cultures for production of bacteriocins

Indicator culture	Spent broth from cultures of *Lactobacillus* sp.						
	LP LHI10	LD LTI30	LA CH5	LA A92	LA NO1	LA D10	LA B12
LP LHI10	–	–	–	–	–	–	–
LD LTI30	–	–	+++	–	+	+	–
LA CH5	+	+	–	+	+	+	+
LA A92	–	–	–	–	–	–	–
LA NO1	–	–	+	–	–	–	–
LA D10	–	–	+	–	–	–	–
LA B12	–	–	+	–	–	+	–

– no inhibition; + slight inhibition; +++ strong inhibition

In this work several factors to be considered in selection of *Lactobacillus* sp. strains were verified and found as useful. Applying the above mentioned methods, the folloving strains were recommended for future studies:

- *Lactobacillus acidophilus*, CH5 producing bacteriocin acidocin CH5,
- *Lactobacillus delbrüeckii* subsp. *lactis*, LTI30, a strain sensitive to acidocin CH5 suitable as a target microorganism for acidocin CH5 activity evaluation,
- *Lactobacillus plantarum*, LHI10 possessing the best low pH tolerance and bile resistance.

According to data from the present study and the previous ones (Gilliland, Walker, 1990) variations among strains with regard to bile tolerance, ability to survive at low pH and production of bacteriocins exist. The strains that exhibit desirable levels of these properties can exist in nature and so it is recommended to isolate and verify the new strains of *Lactobacillus* sp. of human origin for human nutrition and research purposes.

References

BAREFOOT, S. F. – KLAENHAMMER, T. R.: Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol., *45*, 1983: 1808–1815.

BLOCK, S. S.: Peroxygen compounds. In: BLOCK, S. S. (Ed.): Disinfection, Sterilization, Preservation. 4 ed. Philadelphia, Lea & Feabiger 1991: 169 p.

BROOKER, B. E. – FULLER, R.: Adhesion of *Lactobacillus* to the chicken crop epithelium. J. Ultrastruc. Res., *52*, 1975: 21.

CHUMCHALOVÁ, J. – JOSEPHSEN, J. – PLOCKOVÁ, M: Characterization of acidocin CH5, a saccharolytic sensitive bacteriocin of *Lactobacillus acidophilus* CH5. Chem. Mikrobiol. Technol. Lebensm., *17*, 1995: 145–150.

FERREIRA, C. L. – GILLILAND, S. E.: Bacteriocin involved in premature death of *Lactobacillus acidophilus* NCFM during growth at pH 6. J. Dairy Sci., *71*, 1988: 306–315.

FRANKLIN, M. A. – SKORYNA, S. C.: Studies on natural gastrics. Survival of bacteria in fasting human subjects. Can. Med. Assoc. J., *105*, 1971: 380–392.

FREW, J. E. – JONES, P. – SCHOLES, G.: Spectrophotometric determination of hydrogen peroxide and organic hydroperoxides at low concentrations in aqueos solution. Anal. Chim. Acta, *155*, 1983: 139–150.

GOLDIN, B. R. – GORBACH, S. L.: The effect of oral administration of *Lactobacillus* and antibiotics on the intestinal bacterial activity on chemical induction of large bowel tumors. Devel. Indust. Microbiol., *25*, 1984: 134–142.

GILLILAND, S. E. – NELSON, C. R. – MAXWELL, C.: Assimilation of cholesterol by *Lactobacillus acidophilus*. Appl. Environ. Microbiol., *49*, 1985: 371–376.

GILLILAND, S. E. – SPECK, M. L: Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associated cultures. J. Food Prot., *40*, 1977: 820–825.

GILLILAND, S. E. – WALKER, D. K.: Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. J. Dairy Sci., *73*, 1990: 905–911.

HOLZAPFEL, W. H. – GEISEN, R. – SCHILLINGER, U.: Biological preservation of foods with reference to protective cultures, bacteriocins and food grade enzymes. Int. J. Fd. Microbiol., *24*, 1995: 343–362.

HOOD, S. K. – ZOTTOLA, E. A.: Effect of low pH on the ability of *Lactobacillus acidophilus* to survive and adhere to human intestinal cells. J. Food Sci., *53*, 1988: 1514–1516.

HUIS IN'T VELD, J. H. J. – HAVENAAR, R. – MARTEAU, P.: Establishing a scientific basis for probiotic, R & D. Trends in Biotechnol., *12*, 1994: 6–8.

KANDLER, O. – WEISS, N.: Regular, nonsporing gram-positive rods, In: Bergey's Manual of Systematic Bacteriology, 9 ed. Baltimore, WI, Williams and Wilkins 1986: 1208–1234.

PRICE, R. J. – LEE, J. S.: Inhibition of *Pseudomonas* species by hydrogen peroxide producing lactobacilli. J. Milk Food Technol., *33*, 1970: 13–19.

REITER, B. – HARNULV, G.: Lactoperoxidase antibacterial system: natural occurrence, biological functions and practical application. J. Food Prot., *47*, 1984: 724–729.

SANDINE, W.: Roles of *Lactobacillus* in the intestinal tract. J. Prot., *42*, 1979: 259–263.

Received March 4, 1996

Odolnost laktobacilových kmenů vůči pH a žlučovým solím a stanovení tvorby antimikrobiálních látek

Bylo testováno sedm kmenů rodu *Lactobacillus* izolovaných z různých přírodních zdrojů z hlediska jejich odolnosti vůči pH a žlučovým solím a na produkci látek antimikrobiálního charakteru (H_2O_2, bakteriociny).

Pro stanovení odolnosti buněk vůči pH bylo využito nutričně bohaté médium MRS o hodnotě pH 2, 3 a 4, KCl pufr o pH 2 a 3 a ftalátový pufr o pH 3 a 4. Ze zjištění počtu přežívajících buněk po 2 hodinách při 37 °C (resp. 30 °C) se jako nejodolnější kmen projevil *Lactobacillus plantarum* LHI10 a dále kmeny *Lactobacillus acidophilus* D10, N01 a A92.

Stanovení odolnosti kmenů vůči žlučovým solím (přídavek 0,3 % hm. tauroglykocholátu sodného) spektrofotometricky (zjištění změny A_{620} o 0,3) poukázalo, že nejrychlejší nárust indikující největší odolnost byl opět u kmene *Lactobacillus plantarum* LHI10. Naproti tomu u kmene *Lactobacillus acidophilus* A92 a *Lactobacillus delbrüeckii* subsp. *lactis* LTI30 byla zaznamenána totální inhibice růstu.

U všech kmenů testovaných na tvorbu antimikrobiálních látek byla zaznamenána produkce H_2O_2 při statické kultivaci.

Tvorba bakteriocinů sledovaná za použití zfermentovaného média vpichovou metodou poukázala na antimikrobiální vlastnosti všech sledovaných kmenů. Nejúčinnější byl kmen *Lactobacillus acidophilus* CH5, jehož růst byl však také nejčastěji inhibován.

Ze studie vyplývá, že pro aplikační možnosti lze využít sledované mikroorganismy pro tyto charakteristické vlastnosti:

– *Lactobacillus acidophilus* CH5 pro tvorbu antimikrobiální látky zvané acidocin CH5,

– *Lactobacillus delbrüeckii* subsp. *lactis* LTI30 pro testování aktivity acidocinu CH5,

– *Lactobacillus plantarum* LHI10 pro jeho zvýšenou odolnost vůči extrémnímu prostředí (nízké pH, žlučové soli).

Lactobacillus; peroxid vodíku; žlučové soli; tolerance vůči nízkému pH; bakteriocin; acidocin CH5

Contact address:

Ing. Milada Plocková, Vysoká škola chemicko-technologická,
Technická 5, 166 28 Praha 6, Česká republika,
tel.: 02/2435 3275, fax: 02/311 99 90, e-mail:milada.plockova@vscht.cz

Potrav. Vědy, *14*, 1996 (3) : 175–184

EFFECT OF PHOSPHOLIPIDS ON THE OXIDATION OF RAPESEED OIL*

Jiří LEDAHUDEC, Lenka KOUŘIMSKÁ, Hidetoshi SAKURAI[1]*, Zuzana RÉBLOVÁ, Jan POKORNÝ*

Institute of Chemical Technology – Department of Food Chemistry and Analysis, Prague, Czech Republic; [1]*Permanent address: Nihon University, Faculty of Agriculture and Veterinary Medicine, Tokyo, Japan*

Abstract: The antioxidant activity of soybean phospholipids was tested in rapeseed oil, which is particularly difficult to stabilize against oxidative deterioration. Changes of rapeseed oil were compared with those in 0.5 % and 2.0 % solution of lecithin in oil. The oxidation proceeded at 60 °C in the dark at free access of air. Lecithin inhibited the increase of lipid hydroperoxides, total oxidized fatty acids, particularly at the higher concentration, and inhibited the deterioration of flavour acceptability as well. The development of rancid off-flavour was delayed, and other off-flavours characteristic for oxidized oil were efficiently suppressed. The intensity of oily flavour increased during the induction period, but decreased again afterwards. The brown discolouration was produced during the induction period, and was decomposed at higher concentrations of hydroperoxides. The effect of different phospholipid preparations was very similar.

phospholipids; lecithin; rapeseed oil; sensory value; oxidation; stabilization

The antioxidative effect of phospholipids in fats and oils was reported by several authors (Nasner, 1985; Pokorný et al., 1976a,b; Kouřimská et al., 1995; Chen, Nawar, 1991). Phospholipids are active as inhibitors even in highly unsaturated oils, such as fish oils (Takagi et al., 1979; Hara et al., 1992). They can protect fats and oils even under frying conditions (Kouřimská et al., 1994) and in the presence of non-lipidic components (Réblová et al., 1995).

Their antioxidant activity is attributed to their synergistic effect with tocopherols, which act as primary antioxidants (Yuki et al., 1978; Hudson, Ghavami, 1984; Kajimoto et al., 1987).

---

* Supported by the Grant Agency of the Czech Republic, No. 509/93/0419.

We have found that their activity is due, at least partially, to their ability to decompose lipid hydroperoxides in a non-radical way (Pokorný et al., 1981). The peroxide decomposing activity was reported from other laboratories as well (Lee et al., 1981; Miyazawa et al., 1981), and it was explained by the reaction of choline and ethanolamine bound in phospholipids (phosphatidylcholine and phosphatidylethanol-amine, respectively) with hydroperoxides (Miyazawa et al., 1981). We have observed similar activities in the case of ammonium salts of phosphatidic acids as well (Pokorný et al., 1990, 1992).

The autoxidation of polyunsaturated oils containing phospholipids is accompanied by intensive browning (Pokorný, 1981). The browning products of phospholipids inhibited the autoxidation of methyl linoleate (Rafat Husain et al., 1986).

The antioxidant activity is usually determined on the basis of the inhibition of oxygen absorption (such as measured by weight increase) or the accumulation of hydroperoxides in oil. The reduction of the hydroperoxide content need not be, however, the most reliable indicator of the antioxidant activity. The most important factor is the stabilization of the sensory value (or at least, the inhibition of its degradation). Therefore, we have studied the effect of phospholipids on changes of the sensory value of rapeseed oil, which is particularly sensitive to off-flavours.

MATERIAL AND METHODS

Material

Three samples of soybean lecithin were produced by Lucas Meyer, Hamburg, Germany. Their composition is given in Table I. Rapeseed oil was produced from zero-erucic winter rapeseed (*Brassica napus* L.) on the plant scale by combined expeller pressing and extraction, alkali refined and deodorized (peroxide value: 0.44 mval/kg, acid value: 0.03 mg KOH/g; fatty acid composition: saturated fatty acids: 8.8%, monoenoic: 62.6%, dienoic: 20.6%, trienoic: 7.7% the content of total tocopherols was 355 mg/kg, the content of iron 0.03 mg/kg).

Methods

The analyses of lecithin and of rapeseed oil were carried out after IUPAC (1987). The peroxide value was determined iodometrically (IUPAC, 1987)

I. Composition of soybean lecithins used in the experiments

Component of lecithin	Sample		
	No. 1	No. 2	No. 3
Toluene insolubles [%]	0.26	0.18	0.41
Water [%]	0.18	0.22	0.37
Acetone insolubles [%]	56.42	61.20	63.21
Phospholipids [%]	46.41	41.22	40.06
Phosphatidylcholine [%]	9.6	13.8	11.5
Phosphatidylethanolamine [%]	10.0	9.5	11.3
Phosphatidic acids [%]	14.0	5.7	5.6
Phosphatidylinositols [%]	6.6	8.1	7.5
Free fatty acids[%] as oleic acid	3.8	3.8	4.4
Hydroperoxides [mval/kg]	2.0	1.6	1.5

and is expressed in mval/kg. The sample was saponified, fatty acids were isolated by extraction with hexane after the acidification, and the content of oxidized fatty acids was determined by reversed phase HPLC (Valentová et al., 1986; Pokorný et al., 1983). The colour of lecithin solutions in oil was measured between 380–780 nm (IUPAC, 1987).

The sensory analysis was carried out under standard conditions (ISO, 1985b) in a standardized test room provided with test booths (ISO, 1988) using a group of selected assessors (ISO, 1989), trained in a special 4-month course, with a subsequent experience of at least six months. The sensory profile method (ISO, 1985a) and the use of scales (ISO, 1978) were conform with the international standard requirements. Graphical scales were used for the sensory profiling of flavour (straight lines 150 mm long: 0 mm = imperceptible, 150 mm = very strong) and of colour (0 mm = imperceptible, 150 mm = very dark; the colour hue contribution: 0 mm = negligible, 150 mm = the only important). Results were expressed in p. c. of the graphical scale. The hedonic evaluation was carried out using 100 mm long graphical scales (0 mm = not acceptable, 100 mm = extremely pleasant). The following descriptors were used in the flavour profile: oily, rancid, buttery, green, grassy, leaves, aldehydic, metallic, nutty, sharp, pungent, total off-flavours.

RESULTS AND DISCUSSION

Rapeseed oil was stored in loosely covered Petri dishes in a 10 mm layer at 60 °C in the dark and under free access of air oxygen. A 0.5% solution of lecithin No. 1 in rapeseed oil and a 2.0% solution of lecithin No. 1 in rapeseed oil were stored at the same time. After defined time intervals, samples were taken, analyzed for the discolouration (only values at 430 nm are given in the Tables), for the content of peroxides and oxidized products. The sensory analysis was carried out at the same time. The results are given in Tables II–IV for rapeseed oil, the 0.5% solution and the 2.0% solution, respectively. To save space, only values of selected flavour descriptors are given in the Tables.

The results show that lecithin imparted moderate discolouration to the samples, which became darker on storage, but in the second stage of oxidation, when the peroxide value increased, the colour remained constant or

II. Changes during the storage of rapeseed oil at 60 °C in the dark and under free access of air

Storage time [d]	Trans-mittancy at 430 nm [%]	Peroxide value [mval/kg]	Oxidized products [%]	Flavour acceptability [%]	Oily flavour (%)	Rancid flavour [%]
0	64	4.0	1.11	121	15	0
1	46	4.6	1.16	83	62	18
2	37	8.9	1.48	63	113	23
3	40	11.5	1.84	73	69	44
4	28	24.3	2.05	57	90	25
5	30	37.6	2.84	62	51	95
7	26	45.0	2.88	57	76	68
8	25	84.2	3.56	50	84	81
18	58	135.6	6.02	46	30	95
31	54	189.4	7.24	42	27	118
38	50	206.5	9.53	45	22	109
39	49	215.8	10.12	52	58	115
42	43	336.4	18.84	41	70	136
46	49	465.2	25.65	36	38	147

III. Changes during the storage of a 0.5% solution of lecithin No. 1 in rapeseed oil at 60 °C in the dark and at free access of air

Storage time [d]	Trans-mittancy at 430 nm [%]	Peroxide value [mval/kg]	Oxidized products [%]	Flavour acceptability [%]	Oily flavour [%]	Rancid flavour [%]
0	43	4.0	1.05	89	47	6
1	47	3.7	1.10	80	71	3
2	50	3.4	1.07	101	38	2
3	32	4.2	1.12	87	94	6
4	32	3.8	1.14	96	62	3
5	33	6.4	1.65	92	85	11
7	30	6.2	1.44	84	95	8
8	24	6.3	1.48	101	89	5
18	19	19.6	2.27	73	66	51
31	22	28.5	2.68	62	69	65
38	21	29.2	3.11	67	54	41
39	21	64.0	4.30	68	69	84
42	20	68.6	4.43	61	72	86
46	25	162.4	10.21	44	85	128

even a slight bleaching was observed. The bleaching of lecithin pigments with peroxides was reported in our previous paper (Pokorný et al., 1976a,b). It is a common practice in the industry to bleach lecithin by addition of hydrogen peroxide.

The flavour acceptability of the sample without lecithin was very good, but rapidly deteriorated on storage, already during the induction period. The intensity of oily flavour increased during the induction period as it is due to small amounts of oxidation products. It decreased again on further storage, being replaced by rancid off-flavour, which is caused by higher concentrations of oxidation products. The induction period of pure oil was 4–5 days. Such an induction period was found in Czech rapeseed oils from that period (own unpublished results).

The sample containing 0.5% lecithin changed much more slowly, and the induction period approached 18 days, when the rancid flavour began to be

IV. Changes during the storage of 2.0 % solution of lecithin No. 1 in rapeseed oil at 60 °C in the dark under free access of air

Storage time [d]	Transmittancy at 430 nm [%]	Peroxide value [mval/kg]	Oxidized products [%]	Flavour acceptability [%]	Oily flavour [%]	Rancid flavour [%]
0	28	3.6	1.12	98	73	2
1	29	3.9	–	81	93	1
2	30	3.2	–	74	75	7
3	30	4.0	–	83	70	2
4	35	6.3	1.24	83	86	8
5	33	5.4	–	93	77	5
7	37	5.0	1.42	90	75	4
8	38	4.8	–	84	86	4
18	37	4.9	1.65	97	79	2
31	48	6.3	1.89	73	55	14
38	52	6.8	2.02	84	79	7
39	47	7.2	2.34	84	73	3
42	57	12.4	2.54	73	107	14
46	68	22.6	2.71	75	79	66

well perceptible. The third sample, containing 2.0% lecithin, became rancid only after 40 days of storage. It agrees well with our previous results obtained with sunflower oil (Pokorný et al., 1982).

The results confirm that the addition of lecithin not only suppresses the accumulation of hydroperoxides and oxidized fatty acids, but also it decreases the formation of rancid off-flavour and improves the flavour acceptability of sample on storage. The addition of lecithin may impart some slight off-flavour to fresh oils, but during the storage, the sample containing lecithin became soon better than the control sample.

The three samples of lecithin were dissolved in rapeseed oil, and the 0.5% solutions were stored in the same way as above. As the course of oxidation

V. Comparison of 0.5% solutions of three lecithin samples in rapeseed oil after the storage of 18 days at 60 °C

Characteristics	Sample			Mean value
	No. 1	No. 2	No. 3	
Transmittancy at 430 nm [%]	19	27	26	24
Peroxide value [mval/kg]	19.6	14.7	21.5	18.6
Oxidized products [%]	2.26	2.16	2.40	2.26
Flavour acceptability [%]	73	68	66	69
Oily flavour [%]	66	70	75	70
Rancid flavour [%]	51	41	46	46
Buttery flavour [%]	5	15	10	10
Green off-flavour [%]	19	14	13	15
Grassy off-flavour [%]	7	6	12	8
Leaves off-flavour [%]	8	4	10	7
Aldehydic off-flavour [%]	19	10	18	16
Metallic off-flavour [%]	11	10	14	12
Nutty flavour [%]	15	13	10	13
Total off-flavours [%]	33	30	43	35
Sharp pungent odour [%]	29	15	41	28
Colour acceptability [%]	78	69	65	71
Colour intensity [%]	117	104	110	110
Contribution of yellow hue [%]	147	143	139	143
Contribution of red hue [%]	47	40	35	41
Contribution of brown hue [%]	23	17	22	21

was very similar, only the values obtained after 18 days of storage are compared in Table V.

The data given in Table V show that even rather pronounced differences in the composition of lecithins have no great effect on their antioxidant activity. Small differences in the proportion of different phospholipid classes is obviously not crucial for their antioxidant activity.

References

CHEN, Z. Y. – NAWAR, W. W.: Prooxidative and antioxidative effects of phospholipids on milk fat. J. am. Oil Chem. Soc., *68*, 1991: 938–940.

HARA, S. – OKADA, N. – HIBINO, H. – TOTANI, Y.: Antioxidative behaviour of phospholipids for polyunsaturated fatty acids of fish oil. Yukagaku, *41*, 1992: 130–135.

HUDSON, B. J. F. – GHAVAMI, M.: Phospholipids as antioxidant synergists for tocopherols in the autoxidation of edible oils. Lebensm.-Wiss. Technol., *17*, 1984: 191–194.

KAJIMOTO, G. – ONISHI, H. – YOSHIDA, H. – SHIBAHARA, A.: Effects of phospholipids on the decomposition of tocopherol in heated vegetable oils. Nippon Nogeikagaku Kaishi, *61*, 1987: 191–198.

KOUŘIMSKÁ, L. – POKORNÝ, J. – RÉBLOVÁ, Z.: Phospholipids as inhibitors of oxidation during food storage and frying. Prehr.-tehnol. biotehnol. Rev., *32*, 1994: 91–94.

KOUŘIMSKÁ, L. – RÉBLOVÁ, Z. – POKORNÝ, J.: Stabilization of dietetic oils containing phospholipids against oxidative rancidity. In: CEVC, G. – PALTAUF, F. (Eds.): Phospholipids. Champaign, AOCS Press 1995: 372–377.

LEE, J.-H. – FUJIMOTO, K. – KANEDA, T.: Antioxygenic and peroxide decomposition properties of Antarctic krill lipids. Bull. Jap. Soc. Sci. Fisheries, *47*, 1981: 881–888.

MIYAZAWA, T. – YAMAGUCHI, M. – LEE, J.-H. – FUJIMOTO, K. – KANEDA, T.: Decomposition of lipid hydroperoxide by choline and ethanolamine. Agr. Biol. Chem., *48*, 1981: 881–888.

NASNER, A.: Die antioxydativen Eigenschaften von Lecithin. Fette, Seifen, Anstrichmittel, *87*, 1985: 477–481.

POKORNÝ, J.: Browning from lipid-protein interactions. Proc. Food Nutr. Sci., *5*, 1981: 421–428.

POKORNÝ, J. – ALEXOVÁ, H. – DAVÍDEK, J.: Stanovení stupně oxidace tuků použitých k průmyslovému smažení. Prům. Potr., *34*, 1983: 6–8 (Příloha).

POKORNÝ, J. – DAVÍDEK, J. – VIERECKLOVÁ, M. – RANNÝ, M.: Effect of phosphorylated acylglycerols on the relative stability of edible oils. Nahrung, *34*, 1990: 719–725.

POKORNÝ, J. – MARCÍN, A. – DAVÍDEK, J.: Stability of phospholipid-enriched sunflower oil against oxidative rancidity. Sbor. VŠCHT v Praze, E *53*, 1982: 7–23.

POKORNÝ, J. – NGUYEN, T. L. – SVOBODOVÁ, H. – JANÍČEK, G.: Antioxidative effect of soybean phospholipids. Nahrung, *20*, 1976a: K 3–4.

POKORNÝ, J. – POSKOČILOVÁ, H. – DAVÍDEK, J.: Effect of phospholipids on the decomposition of lipid hydroperoxides. Nahrung, *25*, 1981: K 29–31.

POKORNÝ, J. – TAI, P.-T. – HEGEDŰŠOVÁ, B. – JANÍČEK, G.: Bleaching of oxidized phosphatidylethanolamine with peroxides. In: Sbor. VŠCHT v Praze, E *46*, 1976b: 17–34.

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: 461–465.

RAFAT HUSAIN, S. – TERAO, J. – MATSUSHITA, S.: Effect of browning reaction products of phospholipids on autoxidation of methyl linoleate. J. am. Oil Chem. Soc., *63*, 1986: 1457–1460.

RÉBLOVÁ, Z. – POKORNÝ, J.: Effect of lecithin on the stabilization of foods. In: CVEC, G. – PALTAUF, F. (Eds.): Phospholipids. Champaign, AOCS Press 1995: 378–383.

VALENTOVÁ, H. – DAVÍDEK, J. – POKORNÝ, J.: Stanovení polárních oxidovaných mastných kyselin tukového podílu potravin kapalinovou chromatografií s vysokou účinností. Potrav. Vědy, *4*, 1986: 1–8.

TAKAGI, T. – ISHIZAWA, T. – IIDA, T.: Antioxidants for highly unsaturated oils. Yukagaku, *28*, 1979: 548–551.

YUKI, E. – MORIMOTO, K. – ISHIKAWA, Y. – NOGUCHI, H.: Inhibition effect of lecithin on the thermal oxidation of tocopherols. Yukagaku, *27*, 1978: 425–430.

ISO 4121: Sensory analysis – Grading of food products by methods using scale categories. Geneva, International Organisation for Standardization 1978.

ISO 6564: Sensory analysis – Sensory profile. Geneva, International Organization for Standardization 1985a.

ISO 6658: Sensory analysis – Methodology – General guidance. Geneva, International Organization for Standardization 1985b.

ISO 8586: Sensory analysis – General guidance for the selection, training and monitoring of assessors. Geneva, International Organization for Standardization 1989.

ISO 8589: Sensory analysis – General guidance for the design of test rooms. Geneva, International Organization for Standardization 1988.

IUPAC: PAQUOT, C. – HAUTFENNE, A. (Eds.): Commission on Oils, Fats and Derivatives: Standard Methods for the Analysis of Oils, Fats and Derivatives. 7th ed. Oxford, Blackwell 1987.

Received January 22, 1996

Vliv fosfolipidů na oxidaci řepkového oleje

Antioxidační aktivita sójových fosfolipidů byla zkoumána v řepkovém oleji, který se obecně velmi obtížně stabilizuje proti oxidačnímu žluknutí. Změny při skladování v 10 mm tlusté vrstvě oleje byly sledovány při 60 °C ve tmě a za volného přístupu vzdušného kyslíku. Řepkový olej bez přídavku lecithinu byl srovnáván s oleji obsahujícími 0,5 % a 2,0 % lecithinu, což odpovídá řádově obsahu v surovém oleji a přichází v úvahu u některých dietních olejů. Lecithin inhiboval růst hydroperoxidů a oxidovaných mastných kyselin zvláště při vyšší koncentraci a účinně také brzdil zhoršování přijatelnosti chuti oleje. Olejovitá chuť narůstala během indukční periody, potom zvolna mizela a narůstala intenzita škodlivých pachutí, které byly silněji zastoupeny až při peroxidových číslech vyšších než 50 mval/kg. Byla provázena dalšími pachutěmi, jako aldehydickou, trávovou nebo fermežovitou. Tmavnutí narůstalo během indukční periody, ale vyšší obsahy hydroperoxidů měly mírně bělicí účinek. Různé fosfolipidové přípravky se chovaly podobně.

fosfolipidy; lecithin; řepkový olej; senzorická jakost; oxidace; stabilizace

---

Contact address:

Ing. Zuzana Réblová, CSc., and Prof. Dr. Jan Pokorný, DSc.,
Vysoká škola chemicko-technologická, Ústav chemie a analýzy potravin,
Technická 5, 166 28 Praha 6, Česká republika,
tel.: 02/2435 3264, fax: 02/31199 90, e-mail: jan.pokorny@vscht.cz

Potrav. Vědy, *14*, 1996 (3) : 185–195

EFFECT OF SODIUM SOAPS AND OTHER POLAR SUBSTANCES ON FOAMING OF FRYING OILS*

Zuzana RÉBLOVÁ, Jan BEDNÁREK, Jan POKORNÝ

Institute of Chemical Technology – Department of Food Chemistry and Analysis, Prague, Czech Republic

Abstract: The effect of sodium soaps on the foaming of frying oils (sunflower, rapeseed and olive oils) was studied by immerging pieces of moistened cellulose or potato cubes into preheated oil, and measuring the development of foam in the interval of up to 45 s or 100 s, respectively. The repeatability is not very high as the intensity of foaming depends very much on the water content, therefore, 6–8 parallel determinations are necessary to obtain reliable results. The addition of soaps was based on the content of soaps after frying french fries or luncheon meat in a household fryer. The effect of sodium soaps was not pronounced in fresh frying oils, and did not differ significantly from that of free fatty acids. Soaps of saturated fatty acids are moderately more effective than soaps of unsaturated fatty acids. Partial esters of fatty acids with glycerol have comparable effects on foaming. An increasing amount of sodium soaps slightly increases foaming, but higher concentrations are less effective and can even decrease foaming. Oxidized triacylglycerols increase the foaming of frying oils. During the foaming, the peroxide value of frying oil moderately increases.

deep fat frying; alkaline soaps; foaming; frying oil; free fatty acids; monoacylglycerols; diacylglycerols; oxidation products; potato frying

Frying oils deteriorate by multiple processes during deep-fat frying, due to the effect of fried food, the effect of air oxygen, and the effect of high temperature (Varela et al., 1988; Blumenthal, 1987). Hydrolytical reactions are caused by steam originating from water present in the fried material. Free fatty acids and partial esters of glycerol are produced by hydrolysis of triacylglycerols.

Oxygen dissolved in oil before frying is consumed in a few minutes for heating (Fedeli, 1974) so that oxidation reactions are due only to oxygen passing from air through the interface between air and frying oils. The inter-

* Supported by the Grant Agency of the Czech Republic, No. 509/93/0419.

face is increased by foaming, stimulated during the dehydration of fried material. Foaming is influenced by the interphase surface tension, therefore, the surfactant theory (Blumenthal, 1991; Blumenthal, Stier, 1991) is of great importance. Limited oxidation is favourable for the sensory quality of fried products as fried flavour substances are produced by oxidation of polyunsaturated oils (Pokorný, 1989). Higher levels of oxidized products are, however, objectionable as fried-flavour substances are objectionable at higher concentrations.

The interface between air and frying oil is increased very much by foaming, which is stimulated by steam produced during the dehydration of fried material. Therefore, excessive foaming is an indicator to replace used (or abused) frying oil with fresh oil.

Various substances are released from the fried substrate into frying oil, including mineral components. Sodium and potassium ions combine with free fatty acids produced in frying oil by hydrolytical reactions, forming alkaline soaps (Blumenthal, Stockler, 1986). Traces of alkaline soaps may be present even in fresh oil as residues after the alkali refining, if the washing after neutralization is not sufficient (Blumenthal et al., 1985). The highly polar material, non-eluted from the chromatographic column during the analysis of frying oil after the DGF-IUPAC-AOAC Standard method (IUPAC, 1987) consisted of sodium oleate as the major constituent (Blumenthal, Stockler, 1986). As sodium soaps are well known emulsifiers in aqueous medium, which might stimulate the foaming, a quick spectrophotometric test was proposed for the determination of alkaline soaps in frying oils (Blumenthal et al., 1985).

Alkaline soaps enhance foaming in aqueous solutions as they can be ionized in those media or form polar micelles. In this paper, we present our results on the foaming effect of sodium salts in relatively non-polar medium, such as fresh frying oil. The effect of alkaline soaps is compared with that of other polar products, such as free fatty acids, monoacyl- and diacylglycerols, and oxidized products under conditions of simulated frying.

MATERIAL AND METHODS

Material

Frying oils were produced by industrial alkali refining and deodorization (high-linoleic sunflower and zero-erucic winter raperseed oils in SETUZA a. s., Ústí nad Labem, Czech Republic; olive oil, Linea Oro, imported by Olitalia, Italy). Emulsifier C (containing monoacylglycerols as major components) was produced in SETUZA a. s., Ústí nad Labem, by glycerolysis of hydrogenated rapeseed oil; diacylglycerol concentrate was produced after the plant-scale removal of monoacylglycerols from the emulsifier C by molecular distillation. Fatty acids were commercial chemically pure products. Sodium salts were prepared by neutralization of fatty acids in methanol solution, and by evaporation of the solvent *in vacuo*.

Solutions of sodium salts in oil were prepared by addition of their methanol solutions to frying oil, heating to 50 °C for 3 min, and by evaporation of the solvent in a rotating vacuum evaporator; control oils were treated in the analogous way.

Methods

Concentration of alkali in oil was determined by a two-phase titration of acetone solution (containing 2% water) at 50 °C under stirring, using bromophenol blue as an indicator.

The peroxide value (expressed in mval/kg) was determined iodometrically (IUPAC, 1987). Polar products were determined by reversed phase high-performance liquid chromatography (Pánek et al., 1989): Hewlett-Packard No. 1050, provided with the autosampler and the integrator HP 3396 Series II, and the differential refractometer HP 1047 with automatic washing of the reference cell after each measurement. The stainless-steel column 250 mm x 4 mm, packed with Separon SGX C-18 (5 μm) was manufactured by Tessek, Prague; mobile phase: acetone–acetonitrile–methanol (4 : 2 : 1, v/v/v); flow rate: 1.00 ml/min; column temperature: 40 °C; sample: 30 μl of oil was dried with anhydrous sodium sulphate, dissolved in 1.5 ml of the mobile phase, and 50 μl were injected. Polar substances were eluted with the capacity factor of 0.50–1.45, and quantified using inner standardization.

Procedure

Vegetable oils (25 ml) were preheated (sunflower and rapeseed oils to 180 °C, olive oil to 160 °C) for 8–10 min in an aluminium heating block (kept at 205 °C and 185 °C, respectively) in ground glass test tubes (inner diameter: 20 mm). The foaming was achieved by throwing a piece of folded filter paper (130 mg) moistened with 60 mg water (31.57% with the precision of 0.41%), if not otherwise stated (in experiments with moistened cellulose) or a piece of potato (cubes 7 mm x 7 mm x 6 mm, weight 625 mg with the precision of 4%, dried on the surface with a piece of cotton). The height of foam (*H*) was recorded (in mm) for 45 s (moistened cellulose) or for 100 s (potato cubes). The foaming activity was calculated by integrating the height-time plot (with respect to the change of volume during heating), where $t = 45$ s (moistened cellulose) or $t = 100$ s (potato cubes).

For the Schaal oven test, batches of 25.0 g of oil were weighed into 150ml glass beakers, which were stored at 60 °C in the dark under free access of oxygen (Pokorný et al., 1985), and weight changes were determined by weighing and by determination of polar products.

In frying experiments, batches of 2.6 kg of oil were used in the household fryer Moulinex at 180 °C (recorded value). Batches of 350 g of potato strings (cut from fresh potatoes and rapidly dried with a piece of cellulose cotton) were fried for 4 min, and the frying was repeated 10 times without adding fresh oil. In case of frying luncheon meat, 220 g batches were fried for 3 min, and the frying was repeated 25 times without adding fresh oil.

RESULTS AND DISCUSSION

The repeatability of foaming experiments is evident from the results given in Table I, which shows rather high variation coefficients. The variations are partially due to quite an important effect of water present in the sample (Table II). Therefore, each determination should be based on several runs (six to eight parallel repetitions).

I. Repeatability of the determination of foaming activity

Determination No.	Maximum foaming [mm]	Foam height after 10 s	Foam height after 20 s	Foaming activity [mm.s]
1	25.0	18.1	14.3	186.2
2	30.0	19.2	14.1	203.3
3	28.0	19.9	14.0	214.0
4	30.0	17.0	13.2	186.2
5	30.0	16.0	13.4	159.1
6	26.0	10.2	13.8	231.5

II. Effect of water in moisted cellulose on the foaming of rapeseed oil

Amount of water [%]	Maximum foaming [mm]	Foam height after 10 s	Foaming activity [mm.s]
14.3	16.0	12.8	36.6
23.3	23.8	15.8	114.1
31.5	27.0	19.5	185.7
38.0	31.0	22.5	265.9
43.4	37.7	25.0	341.4

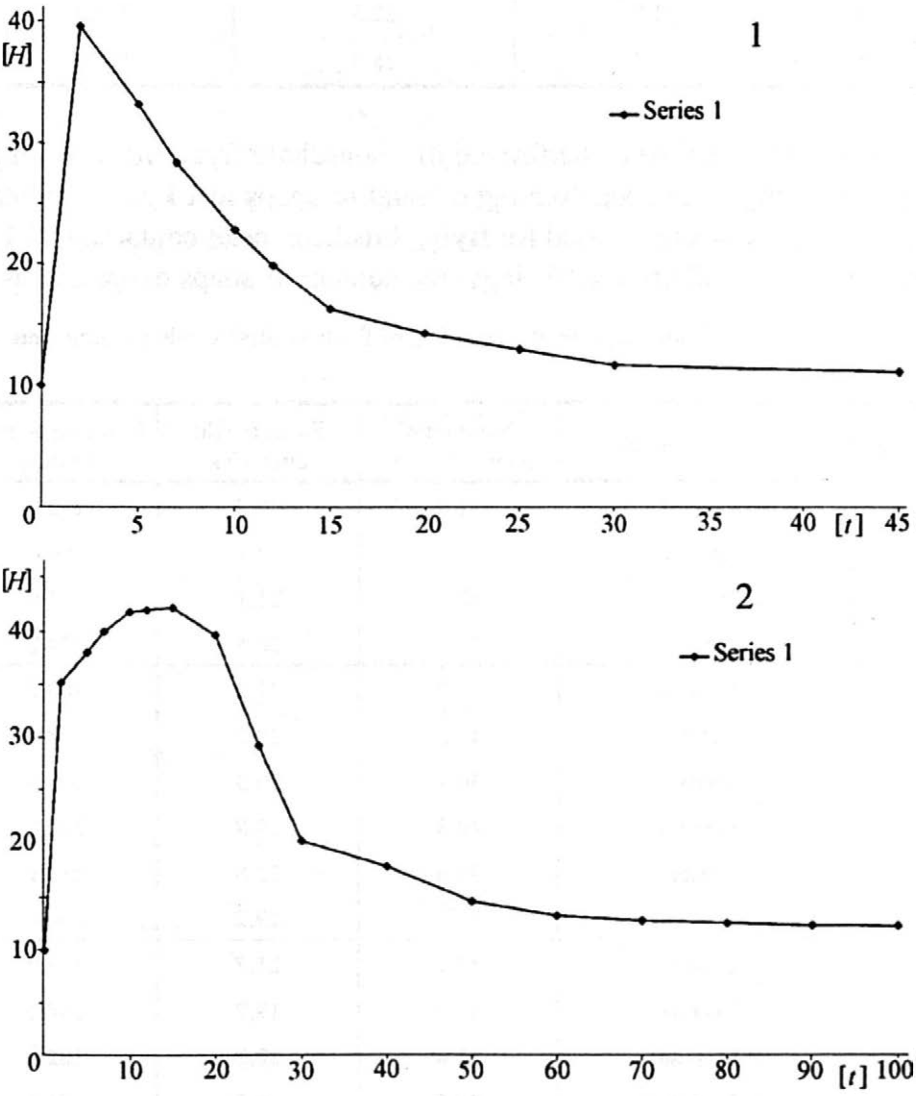
In the frying experiments performed in a household fryer, oil used for frying potato strings contained 40.5 mg of alkaline soaps in 1 kg of oil after 10 repeated fryings, while oil used for frying luncheon meat contained 80.1 mg soaps per 1 kg of oil after 25 fryings (the content of soaps expressed as so-

III. Effect of 0.01% sodium soaps on the foaming of fresh vegetable oils (experiments with moistened cellulose)

Vegetable oil	Sodium soap	Maximum foaming [mm]	Foam height after 10 s	Foaming activity [mm.s]
Sunflower	Control	31.0	20.5	292.4
	Laurate	43.1	24.1	390.0
	Oleate	40.9	21.1	323.0
	Linoleate	32.5	20.5	294.2
Rapeseed	Control	41.0	21.2	260.7
	Laurate	44.2	20.0	258.4
	Myristate	50.7	20.3	259.8
	Palmitate	49.8	19.8	260.0
	Oleate	39.6	22.8	231.1
	Linoleate	35.3	19.2	190.6
Olive	Control	39.2	21.7	258.0
	Laurate	36.0	19.7	248.1
	Myristate	33.8	22.0	262.8
	Palmitate	36.5	20.2	264.7
	Oleate	34.3	19.5	229.0
	Linoleate	34.2	21.2	246.2

dium oleate). Therefore, concentrations between 5–200 mg of soaps in 1 kg of frying oil were tested, which correspond to real concentrations in frying oils.

Maximum foaming was attained after 2 s in simulated frying experiments with moistened cellulose, and after 10–20 s in frying potato cubes. A typical course of foaming is shown in Fig. 1.



H = height of foam [mm]; *t* = time of foaming [s]; 1 = moistened cellulose; 2 = potato cube

1. Course of foaming by immersion of moistened cellulose or a potato cube in preheated rapeseed oil

IV. Effect of sodium soaps on the foaming of rapeseed oil, experiments using potato cubes

Sodium soap	Maximum foaming [mm]	Foam height after 10 s	Foam height after 20 s	Foaming activity [mm.s]
Control	46.8	46.2	43.5	927.4
Laurate	42.7	41.8	42.0	998.2
Palmitate	45.8	45.8	39.5	880.9
Oleate	42.2	41.8	39.6	851.8
Linoleate	46.3	43.2	40.2	845.7

V. Effect of the concentration of sodium soaps on the foaming of edible oils (experiments with moistened cellulose)

System used	Concentration [mg/kg]	Maximum foaming [mm]	Foam height after 10 s	Foaming activity [mm.s]
Sodium oleate in sunflower oil	0	31.0	20.5	227.2
	26	37.7	20.6	242.7
	50	40.8	21.0	265.6
	60	39.5	20.7	268.5
	75	40.3	21.9	273.5
	100	40.9	21.1	281.2
	200	43.8	21.5	295.0
Sodium oleate in rapeseed oil	0	36.5	20.0	234.5
	25	36.8	20.3	263.2
	50	41.4	21.6	277.0
	75	39.7	21.4	285.2
	100	44.5	22.1	299.6
	200	59.1	21.1	321.4
Sodium palmitate in rapeseed oil	0	36.5	20.0	234.5
	25	43.0	21.9	251.3
	50	47.8	21.8	283.8
	75	46.3	22.6	288.3
	100	59.6	20.0	283.0
	150	60.3	19.1	266.2
	200	47.1	18.1	217.9

VI. Effect of fatty acids on the foaming of fresh refined rapeseed oil (concentration of fatty acids: 0.1%)

Fried substrate	Fatty acid	Maximum foaming [mm]	Foam height after 10 s	Foaming activity [mm.s]
Moistened cellulose	Control	28.7	18.7	171.3
	Lauric	27.2	19.8	190.6
	Myristic	28.2	20.0	191.4
	Palmitic	29.0	19.2	175.5
	Oleic	27.7	20.0	190.7
	Linoleic	29.5	18.8	188.4
Potato cubes	Control	34.3	29.7	479.4
	Lauric	46.7	35.2	783.3
	Myristic	46.6	33.6	767.9
	Palmitic	41.2	34.8	694.6
	Oleic	43.3	23.8	771.3
	Linoleic	39.5	38.0	648.4

The effect of 100 mg of soaps on the foaming of oil was tested in sunflower and rapeseed oils (Table III) in the experiment with moistened cellulose, and in the experiments with potato cubes (Table IV). Sodium laurate and sodium oleate increased moderately the foaming of sunflower oil while no effect was observed in the case of potato frying. The effect of soap concentration is evident from Table V. The foaming increased by addition of small amounts of soaps, but higher amounts had only a very slight effect or even decreased the foaming, probably by forming lipophilic micelles.

VII. Effect of partial glycerol esters on the foaming of fresh refined rapeseed oil

Fried substrate	Glycerol ester added	Maximum foaming [mm]	Foam height after 10 s	Foaming activity [mm.s]
Moistened cellulose	MG	27.7	19.8	192.3
	DG	28.2	20.0	197.7
Potato cubes	MG	43.5	35.7	786.6
	DG	29.7	29.7	472.2

Concentration of the emulsifier: 0.1% MG = emulsifier C containing mostly monoacylglycerols; DG = the residue after distillation of monoacylglycerols containing mostly diacylglycerols

VIII. Effect of the content of oxidation products on the foaming of refined rapeseed oil, oxidized under conditions of the Schaal oven test

Time of heating [d]	Oxidized products [%]	Maximum foaming [mm]	Foam height after 10 s	Foaming activity [mm.s]
0	0.63	26.0	18.5	185.2
3	1.08	30.5	17.0	145.5
6	1.19	29.0	18.5	167.2
8	2.13	30.5	19.0	195.1
10	2.39	32.5	20.5	251.8
13	5.10	29.5	17.8	194.5
15	6.01	29.0	19.0	218.0
17	8.02	32.5	21.5	237.0
20	11.58	40.5	24.0	266.5
22	14.01	52.0	27.1	380.2

Free fatty acids had only an unsubstantial effect on foaming in fresh oils in experiments with moistened cellulose, but increased the foaming in frying potato cubes (Table VI). The lack of foaming activity is very important as in the presence of oxygen, free fatty acids are active pro-oxidants (Mistry, Min, 1987).

An addition of emulsifier C (containing mainly monoacylglycerols) increased foaming in frying experiments (Table VII, values of controls: see Table VI), diacylglycerols increased foaming only in the experiment with frying of moistened cellulose. The increase of foaming may thus be due not only to alkaline soaps, but to other hydrolytic products of triacylglycerols as well. Mono- and diacylglycerols moderately increase the rate of oxidation of edible oils (Mistry, Min, 1988), but their effect is rather insignificant.

Oxidized products are also mainly polar compounds (but less polar than hydrolytic products). Rapeseed oil was oxidized under conditions of the Schaal oven test (Table VIII), and the foaming intensity (in experiments with moistened cellulose) increased with the increasing content of oxidized products. We assume that the effect of alkaline soaps is greater in frying oils containing higher amounts of oxidized products. Therefore, we intend to study the effect of sodium soaps on the foaming of frying oils containing higher amounts of polar and polymeric products than fresh frying oils.

IX. Effect of sodium soaps on the oxidation of frying oil during the foaming (experiments with moistened cellulose)

Sodium soap added	Peroxide value before foaming [mval/kg]	Peroxide value after foaming [mval/kg]	Increase of peroxide value [mval/kg]
Control	0.44	1.38	0.94
Laurate	0.38	1.21	0.82
Myristate	0.45	0.54	0.08
Palmitate	0.35	0.87	0.52
Oleate	0.41	1.91	1.50
Linoleate	0.49	1.12	0.63

The effect of sodium soaps on the oxidation of oil was studied in Schaal oven test, when no foaming occurs. At the concentration of 0.01% (which is rather low), sodium soaps had no significant effect on the oxidation of rapeseed oil under the above conditions. During the foaming (following the addition of the fried substrate, in our experiments, moistened cellulose), the peroxide value of frying oil moderately increased (Table IX).

References

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

BLUMENTHAL, M. M.: A new look at the chemistry and physics of deep-fat frying. Food Technol., *45*, 1991: 68–71, 94.

BLUMENTHAL, M. M. – STIER, R. F.: Optimization of deep-fat frying operations. Trends Food Sci. Technol., 1991: 144–148.

BLUMENTHAL, M. M. – STOCKLER, J. R.: Isolation and detection of alkaline contaminant materials in used frying oils. J. am. Oil Chem. Soc., *63*, 1986: 687– 688.

BLUMENTHAL, M. M. – STOCKLER, J. R. – SUMMERS, P. J.: Alkaline contaminant materials and frying oils: a new quick test. J. am. Oil Chem. Soc., *62*, 1985: 1373– 1374.

FEDELI, E.: Transformation of the oxygen dissolved in fats. In: Proc. 3e Symp. int. oxyd. Lipides catalysées Métaux. Paris 1974: 39– 43.

MISTRY, B. S. – MIN, D. B.: Effects of fatty acids on the oxidative stability of soybean oil. J. Food Sci., *52*, 1987: 831–832.

MISTRY, B. S. – MIN, D. B.: Prooxidant effects of monoglycerides and diglycerides in soybean oil. J. Food Sci., *53*, 1988: 1896–1897.

PÁNEK, J. – POKORNÝ, J. – DAVÍDEK, J.: Stanovení oxidovaného podílu jedlých rostlinných olejů kapalinovou chromatografií s vysokou účinností. Potrav. Vědy, 7, 1989: 253–261.

POKORNÝ, J.: Flavor chemistry of deep fat frying in oil. In: MIN, D. B. – SMOUSE, T. H. (Eds.): Flavor Chemistry of Lipid Foods. Champaign, AOCS 1989: 113–155.

POKORNÝ, J. – DOBIÁŠOVÁ, S. – DAVÍDEK, J.: Repeatability of the determination of oxidative stability of vegetable oils using the Schaal test. In: Sbor. VŠCHT Praze, E *58*, 1985: 163–173.

VARELA, G. – BENDER, A. E. – MORTON, I. D.: Frying of Food. Principles, Changes, New Approaches. Chichester, Ellis Horwood 1988.

IUPAC, Commission of fats, oils and derivatives: Standard methods for the analysis of fats, oils and derivatives. 7th ed. (PAQUOT, C. – HAUTFENNE, A., Eds.). Oxford, Blackwell 1987.

Received February 5, 1996

Vliv sodných mýdel a jiných polárních látek na pěnění smažicích olejů

Vliv sodných mýdel na pěnění olejů při smažení byl sledován u několika smažicích olejů (slunečnicového, řepkového a olivového) v modelových pokusech s ovlhčenou celulosou nebo bramborovými kostkami. Výsledky jsou zatíženy značnou chybou, způsobenou do určité míry proměnlivým obsahem vody ve smaženém materiálu, proto je nutné opakovat pokus 6–8krát, aby se dosáhlo dostatečně přesného výsledku. Přídavek mýdel byl volen na základě výsledků pokusů se smažením bramborových hranolků a luncheon meatu. Sodná mýdla neměla velký vliv na pěnění čerstvého smažicího oleje a také vliv koncentrace nebyl příliš výrazný. Nižší přísady sodných mýdel zvyšovaly pěnění, ale vyšší obsahy již neměly skoro žádný vliv, nebo naopak pěnění potlačovaly. Mýdla nasycených mastných kyselin měla poněkud větší vliv než mýdla nenasycených mastných kyselin. Volné mastné kyseliny měly přibližně stejný účinek jako sodná mýdla. Ani vliv parciálních esterů glycerolu s mastnými kyselinami nebyl zvláště výrazný. Oxidační produkty zvyšovaly pěnění a během pěnění se poněkud zvýšilo peroxidové číslo oleje.

smažení v tuku; alkalická mýdla; pěnění; smažicí olej; volné mastné kyseliny; monoacylglyceroly; diacylglyceroly; oxidační produkty; bramborové hranolky

---

Contact address:

Ing. Zuzana Réblová and prof. Dr. Jan Pokorný, DSc., Ústav chemie a analýzy potravin, Vysoká škola chemicko-technologická, Technická 5, 166 28 Praha 6, Česká republika, tel.: 02/2435 3264, fax: 02/311 99 90,
e-mail: jan.pokorny@vscht.cz

Potrav. Vědy, *14*, 1996 (3) : 197–210

THE SIGNIFICANCE OF GLUCOSINOLATES AND THEIR BREAKDOWN PRODUCTS IN THE FORMATION OF THE COOKED CABBAGE FLAVOUR*

Bahru W. KASSAHUN, Jan VELÍŠEK, Jiří DAVÍDEK

Institute of Chemical Technology – Department of Food Chemistry and Analysis, Prague, Czech Republic

Abstract: Following the cooking of fresh and fermented cabbage, the volatile constituents were analysed by gas-liquid chromatography. Out of more than 30 identified compounds about 20 of them were the breakdown products of glucosinolates. Aldehydes, alcohols and sulfides were amongst the rest. Nitriles were the major products of glucosinolate hydrolysis during cooking. Beside 1-cyano-3-methylsulfinylpropane, the fermented and cooked cabbage contained comparable amounts of the corresponding isothiocyanate. The contribution of glucosinolates and their breakdown products to the formation of the flavour profile of cabbage meals depends on the processing conditions. When the cabbage is cooked for more than 30 min, the content of intact glucosinolates can be significantly reduced and their participation in the flavour formation will be minimised or negligible.

cabbage; glucosinolate; volatiles; flavour-active compounds

Glucosinolates are a class of ionic compounds distributed throughout the plant family *Brassicaceae*. On the disruption of the plant tissue or the damage of seeds in the presence of water, glucosinolates can be hydrolysed by a co-existing enzyme called myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) to a wide range of physiologically active compounds such as isothiocyanates, nitriles, oxazolidinethiones and other sulfur-containing products (McGregor et al., 1983). Glucosinolates are relatively labile compounds when *Brassica* vegetables are treated by heat.

Glucosinolates and their degradation products contribute to the flavour profile of meals from *Brassica* plants (Fenwick et al., 1989). The flavour profile of these meals depends on the method of preparation. For example, the flavour of cooked and fermented cabbage is different. Similarly, the iden-

* Supported by Grant Agency of the Czech Republic Grant No. 525/96/0163.

tity of the glucosinolate breakdown products also depends on the processing conditions. When cabbage is cooked in water both nitriles and isothiocyanates are the major products and when cooked in vinegar nitriles predominate.

Investigations were, therefore, made to examine the effect of different processing conditions similar to consumer common practice and the results were partly reported (Kassahun et al., 1995b). This report deals with the volatile breakdown products of glucosinolates and other volatile compounds formed in cooked (under different conditions) and fermented cabbage. The significance of these compounds to the formation of the processed cabbage flavour is also discussed.

MATERIAL AND METHODS

Material

Fresh samples of head cabbage (Aneto F1 – summer early variety, Dobrovodské polopozdní – summer semilate variety) and packed (commercial) fermented cabbages were collected from local shops and supermarkets in and around Prague.

Chemicals and Reagents

Chemicals and reagents used for the determination of pertrimethylsilyl derivatives of desulfoglucosinolates were already described (Kassahun et al., 1995a). Diethyl ether (Labscan Analytical Sciences, Dublin, Ireland) and acetonitrile (Merck, Darmstadt, Germany) were of HPLC grade. Methanol and methylene chloride were obtained from Penta Chemie, Chrudim, Czech Republic. Allyl isothiocyanate was purchased from Aldrich Chemical Company, USA and allyl cyanide from Fluka Chemika-BioChemika, Switzerland. All the other chemicals and reagents were of analytical grade and products of Lachema a. s. Brno, Czech Republic. Allyl thiocyanate (1-thiocyanato-2-propene) was synthesized in our laboratory according to the procedure of Slater (1992).

Analytical Methods

Extraction, purification, GLC and HPLC analysis of glucosinolates were carried out according to Spinks et al. (1984). The analysis of the volatile products was performed according to the previously described method (Velíšek et al., 1995).

Non-volatile degradation products of indole glucosinolate (indoleacetonitriles) from the distillate and the cooking water were extracted with methylene chloride (Slominski, Campbell, 1989) or with ethyl acetate and analysed. Identification of these breakdown products was performed by gas-chromatography/mass spectrometry.

For the confirmation of the identity of breakdown products of glucosinolates formed during cooking, glucosinolates were extracted from cabbage seeds (200 mg) according to Minchinton et al. (1982) and purified on a DEAE Sephadex A-25 anion exchanger column. The intact glucosinolates eluted with water were then cooked in 100 ml distilled water for 60 min and analysed by gas-liquid chromatography/mass spectrometry (GC/MS).

Instrumentation

A Hewlett-Packard (HP) Model 5890A gas chromatograph (HP Palo Alto, CA, USA) equipped with a fused silica capillary column with a stationary phase of SE-54 (25 m x 0.25 mm i.d., film thickness of 0.25 µm) and a flame ionization detector (FID) were employed for both intact glucosinolate and their breakdown products analysis. The column temperature for the intact glucosinolate analysis was programmed from 200 to 285 °C at an increase of 4 °C/min. Injection and detector temperatures were set to 285 °C and 300 °C, respectively. Two columns were used for the determination of the glucosinolate breakdown products: the temperature of the fused silica SE-54 column was held at 40 °C for 10 min and further increased to 250 °C at a rate of 5 °C/min, the fused silica capillary column with the stationary phase SPB-1 (30 m x 0.2 mm i. d., film thickness 0.80 µm) was employed with initial temperature set to 40 °C, and the temperature was increased to 250 °C at a rate of 10 °C. Indole acetonitriles were analysed using the above mentioned gas chromatograph equipped with FID and SE-54 capillary column according to Slominski and Campbell (1989).

GC/MS analysis of the glucosinolate breakdown products was performed on the above gas chromatograph, HP 5890 series II, equipped with a HP quadruple mass spectrometer Model 5972 and a fused silica capillary column HP-5 of identical dimension as the SE-54 column. The column temperature was held for 5 min at 45 °C and increased to 250 °C at a rate of 5 °C. Mass spectra were obtained by electron impact ionisation over the range of 40–400 mass units. The ion source temperature was held at 180 °C and the electron impact energy was 70 eV.

For the HPLC analysis, Thermo Separation Constametric® 3200 gradient pump system (Riviera Beach, Florida, USA) with a Constametric® control panel, reversed phase C_{18} column (4 µm, 4.6 x 250 mm, Waters Nova-Pack®, Milford, MA, USA) and Thermo Separation Auto Injection System (Spectra Series AS100) were used. Detection of desulfoglucosinolates was made by a SpectroMonitor 3200 spectrophotometric detector (Thermo Separation Products) at 230 nm. Chromatographic data were recorded using Chromatographic Station for Windows (CSW) computer program.

RESULTS AND DISCUSSION

The identity of the quantified glucosinolates was in agreement with the findings of Etten et al. (1976) and Spinks et al. (1984). Glucosinolate content of the cabbage samples was determined before and after cooking.

The major glucosinolates were found to be allyl-(sinigrin), 3-methylsulfinylpropyl- and 3-indolylmethyl glucosinolate amounting to 44, 25 and 13% of the total content of the fresh material glucosinolate, respectively.

In the boiled cabbage (60 min) the contents of the above mentioned and all other glucosinolates were significantly decreased either by leaching into the cooking water or by hydrolysis (Table I). Hydrolysis of glucosinolates can arise from the combined effect of endogenous myrosinase and heat applied to cook the sample (Kassahun et al., 1995a). The amount of sinigrin, glucoiberin and glucobrassicin leached into the cooking water represents 53, 25 and 25% of their initial level in the fresh material. Thus, the major part of glucoiberin and glucobrassicin has been transformed to other products. On the other hand, about one half of the sinigrin content was found in its intact form. This indicates that sinigrin is less thermo-labile than glucoiberin and glucobrassicin. Although progoitrin is one of the minor glucosinolates in the analysed samples, it was relatively thermo-stable, and this finding is consistent with that reported by Betz and Fox (1994) and Kassahun et al. (1995a). About 30% of its initial content (when it was cooked for 60 min) was found in the cooking water and about 8% in the cooked material. In contrast to this, the other glucosinolates occurring in the raw material, excluding the three main glucosinolates mentioned above, were almost completely lost

I. Average concentration of glucosinolate in cooked cabbage [mg.kg^{-1} dry weight]

Time [min]	Glucosinolates (numbers 1 to 11 refer to names below)											
	1	2	3	4	5	6	7	8	9	10	11	remark
0	3 911	90	29	186	767	49	1 995	370	1 118	309	47	in fresh cabbage
15	1 213	40	1	131	113	26	883	83	482	138	33	in boiled cabbage
	1 115	7	16	15	8	–	697	17	45	21	10	in liquor
30	1 438	36	24	162	50	tr	745	88	608	39	21	in boiled cabbage
	1 201	15	tr	20	27	tr	921	–	40	17	10	in liquor
60	93	–	–	14	–	–	tr	–	–	–	–	in boiled cabbage
	2 108	4	–	55	–	–	489	–	280	1	–	in liquor

1 – sinigrin, 2 – gluconapin, 3 – glucobrassicanapin, 4 – progoitrin, 5 – glucoibervirin, 6 – glucoerucin, 7 – glucoiberin, 8 – gluconasturtiin, 9 – glucobrassicin, 10 – 4-methoxyglucobrassicin, 11 – neoglucobrassicin

Gluconapoleiferin, glucoraphanin and 4-hydroxyglucobrassicin were present in trace amounts and are not included

on cooking. During cooking, it should be taken into account that the release of glucosinolates from the cabbage tissue can be influenced by the maturity stage, seasonal variety, the presence of surface wax and some other factors.

In the volatile portion of the cooked cabbage, about 20 compounds were found to be the degradation products of glucosinolates. Out of these, allyl cyanide, allyl isothiocyanate, 3-methylthiopropyl cyanide, 3-methylthiopropyl isothiocyanate and 3-indolylacetonitrile were the major compounds (Table II). Fig. 1 is an example of gas chromatographic separation of cabbage volatiles arised during 60 min cooking in water.

The results also show that the production of glucosinolate breakdown products depends on the length of time within which the sample is cooked (Figs. 2 and 3). The amount of the breakdown products is almost twice to three times higher in the 30 min cooked sample than in the 15 min cooked one. MacLeod and MacLeod (1970) reported a certain increase in allyl cyanide content and other volatile products when the cabbage was cooked for 30 min and a decrease when it was cooked longer. In agreement with this

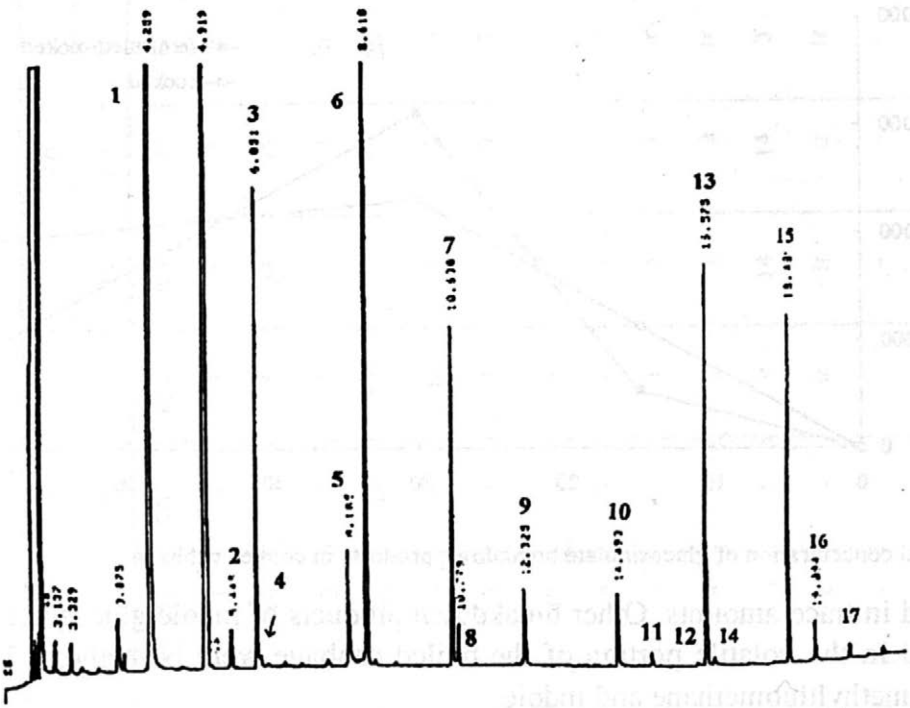
II. Content [mg.kg^{-1} dry weight] of glucosinolate breakdown products in cabbage cooked in water

Breakdown product		Cooking time [min]		
		15	30	60
1.	Allyl cyanide	263	415	245
2.	Allyl thiocyanate	54	22	22
3.	Allyl isothiocyanate	269	568	628
4.	3-Butenylisothiocyanate	8	8	–
5.	3-methylthiopropyl cyanide	292	593	233
6.	Benzeneacetonitrile	7	23	22
7.	Benzenepropionitrile	6	20	tr
8.	4-methylthiobutyl cyanide	tr	16	tr
9.	3-methylthioproppyl isothiocyanate	97	233	155
10.	Benzyl isothiocyanate	tr	tr	tr
11.	4-Methylthiobutyl isothiocyanate	28	5	70
12.	2-Phenethyl isothiocyanate	1	12	–
13.	5-Vinyloxazolidine-2-thione (goitrin)	–	12	18
14.	3-Methylsulfinylpropyl cyanide	5	18	15
15.	3-Methylsulfinylpropyl isothiocyanate	4	–	58
16.	3-indolylacetonitrile	97	367	450

finding, such results can be seen in the majority of the breakdown products content when comparing the 30 min and 60 min cooked samples (Table II). This may probably be caused by the fact that the breakdown products of glucosinolates can undergo a secondary transformation reaction. Characterisation of this process however needs further investigation.

The presence of benzeneacetonitrile and traces of benzyl isothiocyanate shows that the occurrence of the parent glucosinolate glucotropaeolin in some cabbage varieties cannot be excluded, as it was proved by Etten et al. (1976).

The contents of 3-methylthiopropyl cyanide, 3-methylthiopropyl isothiocyanate, 3-methylsulfinylpropyl cyanide and 3-methylsulfinylpropyl isothiocyanate are in contrast to the contents of their parent glucosinolates, glucoibervirin and glucoiberin. The content of the first two products is rather

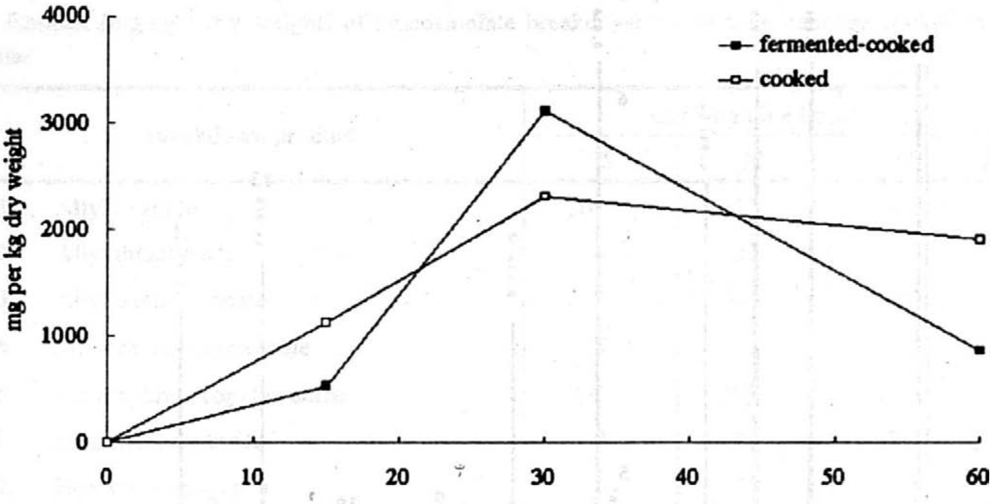


1. 1-cyano-2-propene, 2. 2-hexenal, 3. 3-hexenol, 4. dimethyl disulfide, 5. 1-thiocyanato-2-propene, 6. allyl isothiocyanate, 7. dimethyl trisulfide, 8. 3-butenyl isothiocyanate, 9. 1-cyano-3-methylthiopropane, 10. benzenepropionitrile, 11. 1-cyano-4-methylthiobutane, 12. 5-vinyloxazolidine-2-thione, 13. 3-methylthiopropyl isothiocyanate, 14. 1-cyano-3--methylsulfinylpropane, 15. 4-methylthiobutyl isothiocyanate, 16. 2-phenylethyl isothiocyanate, 17. 3-methylsulfinylpropyl isothiocyanate

1. Separation of volatile flavour-active products of cabbage cooked in water for 30 min

high and the content of the latter two compounds is unexpectedly low (Tables I and II). Certain amounts of 3-methylsulfinylpropyl cyanide and 3-methylsulfinylpropyl isothiocyanate were found in the cooking water. The breakdown products (mainly nitriles) of other glucosinolates in the water were also present in traces as it was described previously (Velíšek et al., 1995). This is because of the partial solubility of these compounds in the cooking water that condenses in the condenser of the extraction apparatus used.

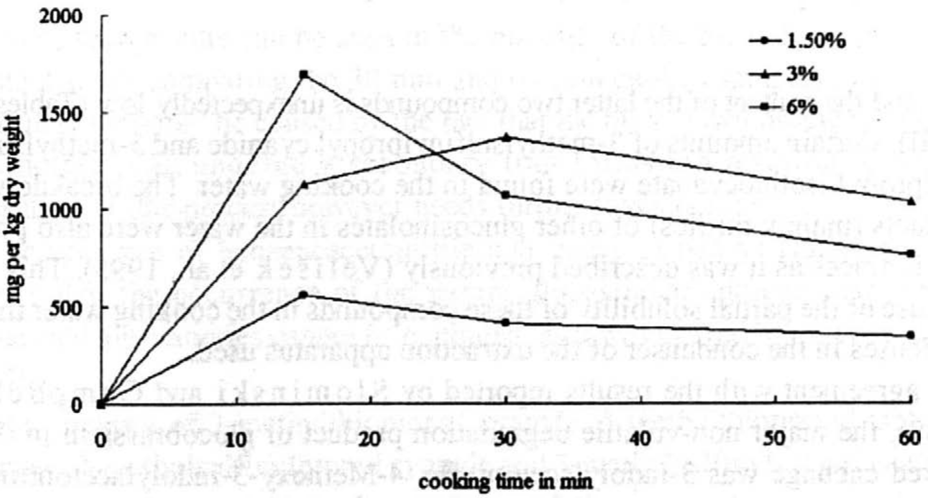
In agreement with the results reported by Slominski and Campbell (1989), the major non-volatile degradation product of glucobrassicin in the cooked cabbage was 3-indolylacetonitrile. 4-Methoxy-3-indolylacetonitrile and N-methoxy-3-indolylacetonitrile were also present, with the latter being



2. Total concentration of glucosinolate breakdown products in cooked cabbage

found in trace amounts. Other breakdown products of indole glucosinolates found in the volatile portion of the boiled cabbage were N-methoxy-3-indolylmethylthiomethane and indole.

Indoleacetonitriles can be well separated by gas-liquid chromatography. However, the corresponding chromatograms of 4-methoxy-3-indolylacetonitrile and N-methoxy-3-indolylacetonitrile were accompanied by contaminant peaks. So, improvements should be made using authentic standards of these compounds.



3. Total concentration of glucosinolate breakdown products in cabbage cooked in vinegar

III. Content [mg·kg^{-1} dry weight] of glucosinolate breakdown products in cabbage cooked in acetic acid

Acetic acid (vinegar) concentration [%]	1.5			3.0			6.0		
Time [min]	15	30	60	15	30	60	15	30	60
1. Allyl cyanide	453	276	209	1 551	966	636	964	1 209	983
2. Allyl thiocyanate	23	tr	tr	1	4	32	3	6	3
3. Allyl isothiocyanate	28	21	23	19	32	25	39	45	8
4. 3-Butenylisothiocyanate	2	5	3	2	1	2	3	1	tr
5. 3-Methylthiopropyl cyanide	14	63	78	86	34	38	59	50	26
6. Benzenacetonitrile	2	3	tr	5	8	tr	1	2	–
7. Benzeneproinitrile	4	25	–	3	3	4	7	9	7
8. 4-Methylthiobutyl cyanide	4	6	7	10	1	2	7	2	3
9. 3-Methylthiopropyl isothiocyanate	11	8	8	8	8	8	12	12	4
10. Benzyl isothiocyanate	11	–	tr	–	–	1	2	1	tr
11. 4-Methylthiobutyl isothiocyanate	1	tr	–	4	1	2	1	2	tr
12. 2-Phenethyl isothiocyanate	1	–	–	–	1	9	14	14	3
13. 5-Vinyloxazolidine-2-thione	2	–	–	tr	tr	tr	tr	tr	tr
14. 3-Methylsulfinylpropyl cyanide	2	4	4	–	–	–	–	–	–
15. 3-Methylsulfinylpropyl isothiocyanate	2	–	10	3	9	tr	14	18	2

The content of nitriles and isothiocyanates determined in the cabbage cooked in vinegar are given in Table III. Details of this analysis were described elsewhere (Velíšek et al., 1995), but without quantitation of the individual breakdown products. The major products formed from glucosinolates were nitriles and their content decreased when the sample had been cooked for more than 30 min.

With only one exception, the degradation products found in the fermented cabbage (Table IV) were similar to those found in the sample cooked in vinegar: the fermentation resulted in the formation of a higher amount of 3-methylsulfinylpropyl cyanide and 3-methylsulfinylpropyl isothiocyanate. Generally, the identification of the isothiocyanates was dependent on the abundance of the parent glucosinolate in the native sample. For example, allyl isothio-cyanate was present (in the fermented cabbage) while 4-methylthiobutyl isothiocyanate was not detected and 3-indolemethanol was found in low amounts. The level of 3-indolylacetonitrile and other indoleacetonitriles was lower than that found in the cabbage cooked in water. The formation of breakdown products of glucosinolates under different processing conditions is shown in Figs. 2 and 3.

IV. Content [mg.kg^{-1} dry weight] of glucosinolate breakdown products in fermented-cabbage cooked in water

Time [min]	Breakdown products no.													
	1	2	3	4	5	6	7	8	9	13	14	15	16	17
15	278	76	106	–	13	tr	tr	7	16	tr	35	tr	tr	tr
30	37	62	241	18	57	13	12	17	39	16	1 333	1 126	14	19
60	345	33	302	tr	79	tr	tr	2	8	13	7	6	55	21

Numbers in row "breakdown products" refer to names of compounds in Table II, tr = traces

Although the mechanism is still not well understood, it is well known that the hydrolysis products of glucosinolates with aromatic side-chain, i.e. glucotropaeolin (Walker, Gray, 1970) and sinalbin (Kassahun, Velíšek, 1993 – unpublished), can yield methylthio-substituted compounds. The same seems to happen with the indole compounds, but the formation of N-methoxy-3-indolylmethylthiomethane and other compounds would not attract attention because of their low levels in the boiled cabbage.

Isothiocyanates can possibly be transformed to sulfides, dimethyl disulfide, dimethyl trisulfide and dimethyl tetrasulfide (Fisher, 1992). The major precursor of these compounds in sliced, chopped or cooked cabbage, cauliflower and other Brassicas, is S(+)-methylcysteine sulfoxide (Marks et al., 1992). The presence of these alkyl sulfides, either in the volatile portion or in the cooking liquor of the boiled cabbage, may have substantial influence on the overall flavour of the cooked meal. The contribution of dimethyl tetrasulfide to this flavour would be insignificant because of its very low content.

Nitriles and isothiocyanates (Table II) were found when purified glucosinolates were cooked in water, the latter products being present in trace amounts. Aldehydes such as (*Z*)-3-hexenal, 5-hydroxymethyl-2-furancarboxaldehyde etc. were also detected. In addition, allyl methyl sulfide, allyl thiol (allyl mercaptan), dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide were found. Dimethyl tetrasulfide and dimethyl sulfoxide were present in traces.

The individual glucosinolates in the cooked cabbage sample were not purified prior to cooking (see methods of analysis above). No quantitative analysis was made. Other unidentified heat degradation products of glucosinolates were present in the analysed model sample but the characterisation of products which were not detected in the volatile portion of the cooked cabbage was beyond the scope of this experiment.

It is very important to emphasise that extreme care should be taken during the extraction and concentration of the extract in order to minimise loss of the volatile components. The extraction of the volatile components using diethyl ether in the vapour phase should also be complemented with extraction employing other suitable solvents (e.g. methylene chloride) if there is a need to quantify all breakdown products of glucosinolates in the boiled cabbage.

Using ethyl acetate to extract the breakdown compounds of indole glucosinolates from the distillate of the cooking liquor is necessary to quantitatively extract all of the breakdown products of the indole glucosinolates. Methylene chloride does not quantitatively extract these compounds.

From the sensorial point of view, nitriles are compounds with unpleasant to irritating odour, for example, allyl cyanide. This was not, however, perceptible even in the concentrated volatile portion of the analyte, which may be influenced by the complex nature of the extract. One has to keep also in mind that most of the nitriles formed are highly or relatively (e.g. allyl, 3-butenyl, 3-metylthiopropyl cyanides) volatile constituents which can be lost during cooking, under common consumer practice conditions, unless the cabbage is cooked in a sealed dish or container; their contribution to the overall flavour or taste would be minimised.

The sensorial character of some nitriles and isothiocyanates was reported by Lange et al. (1992) and Fenwick et al. (1983). In agreement with this report, the results (including the subjective judgements) showed that the degradation products of glucosinolates contribute to the bitterness, sulfidic, biting (in raw cabbage only) and to some extent also to sour taste of the cabbage meals. This taste can be, however, influenced by the cooking conditions or by the way of meal preparation. For example, if the cabbage is cooked in vinegar, the dominant taste can clearly be described as being acidic and sour, and in fermented cabbage slightly bitter or bitter, and non-intensive but pleasant acidic sour taste can be recognised. Furthermore, cooking can also significantly reduce the amount of glucosinolates and their breakdown products in the cooked material. From the results of this experiment in boiled cabbage (especially the cabbage cooked for 60 min and more) or fermented cabbage, the contribution of glucosinolates to bitterness or other taste is unlikely. The participation of allyl glucosinolate, as proved by Fenwick et al. (1983) in Brussels sprouts, in the creation of the bitter taste, can be taken into account in the 15 to 30 min boiled cabbage, whereas in the cabbage meals cooked for 60 min this participation is unaccountable because of very low level of this compound.

References

BETZ, J. M. – FOX, W. D.: In: HUANG, M. T. – OSWA, T. – HO, C.-T. – ROSEN, R.-T. (Eds.): Food Phytochemical for Cancer Prevention I. 1994: 181.

ETTEN, C. H. van – DAXENBICHLER, M. E. – WILLIAMS, P. H. – KWOLEK, F.: Glucosinolates and derived products in cruciferous vegetables: Analysis of the twenty-two varieties of cabbage. J. Agric. Food Chem., *24*, 1976: 452–455.

FENWICK, G. R. – HEANEY, R. K. – MAWSON, R.: In: CHEEKE, P. R. (Ed.): Toxicants of Plant Origin. Vol. II, Glycosides; Glucosinolates. Florida, Boca Raton, CRC Press INC.; 1989: 1–41.

FENWICK, G. R. – GRIFFITH, N. M. – HEANEY, R. K.: Bitterness in Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*): The role of glucosinolates and their breakdown products. J. Sci. Food Agric., *34*, 1983: 73–80.

FISHER, J.: Sulfur- and nitrogen-containing volatile components of kohlrabi (*Brassica oleracea* var. *gongylodes* L.). Z. Lebensm. Unters. Forsch., *194*, 1992: 259–262.

KASSAHUN, B. W. – VELÍŠEK, J. – DAVÍDEK, J.: Glucosinolates in cooked cabbage. Potrav. Vědy, *13*, 1995a: 93–102.

KASSAHUN, B. W. – VELÍŠEK, J. – DAVÍDEK, J.: Glucosinolates and their degradation products in cooked cabbage. In: Proc. Euro Food Chem. VIII., Vol. II, 18–20 September, Austria, Vienna 1995b: 402–405.

LANGE, R. – DIEDRICH, B. – HENSCHEL, K-P. – KUJAWA, M.: Glucosinolate in der Ernhrung - Pro Und Contra Einer Naturstoffklasse; Teil II: Abbau und Stoffwechsel. Lebensm.-Wiss. Technol., *39*, 1992, 292–296.

MacLEOD, A. J. – MacLEOD, G.: Effects of variations in cooking methods on flavor volatiles of cabbage. J. Food Sci., *35*, 1970: 744–750.

MARKS, H. S. – HILSON, J. A. – LECHTWEIS, H. C. – STOEWSAND, G. S.: S-methylcysteine sulfoxide in *Brassica* vegetables and formation of methyl methanethiosulfinate from Brussels sprouts. J. Agric. Food Chem., *40*, 1992: 2098–2101.

McGREGOR, D. I. – MULLIN, W. I. – FENWICK, G. R.: Analytical methodology for determining glucosinolate composition and content. J. Assoc. Off. Anal. Chem., *66*, 1983: 825–849.

MINCHINTON, I. – SANG, J. – BURKE, D. – TRUSCOTT, R. J. W.: Separation of desulfoglucosinolates by reverse-phase high-performance liquid chromatography. J. Chromatogr., *247*, 1982, 141–148.

SLATER, G. P.: Allyl thiocyanate – natural product or artifact in crucifer extracts? Chromatographia, *34*, 1992: 461–467.

SLOMINSKI, B. A. – CAMPBELL, L. D.: Formation of indole glucosinolate breakdown products in autolysed, steamed and cooked *Brassica* vegetables. J. Agric. Food Chem., *37*, 1989: 1297–1302.

SPINKS, E. A. – SONES, K. – FENWICK, G. R.: The quantitative analysis of glucosinolates in cruciferous vegetables, oilseeds and forages using high-performance liquid chromatography. Fette Seifen Anstrichm., *86*, 1984: 228–231.

VELÍŠEK, J. – KASSAHUN, B. W. – DAVÍDEK, J.: The effect of cooking on cabbage glucosinolates. In: Abstr. Agri-Food Conf. 95, 25 - 29 June, UK, Norwich 1995.

WALKER, N. J. – GRAY, I. K.: The glucosinolates of land cress (*Coronopus didymus* L.) and its enzymic degradation products as precursors of off-flavour in milk – a review, J. Agric. Food Chem., *18*, 1970: 346–352.

Received April 22, 1996

Potrav. Vědy, *14*, 1996 (3) : 197–210

Vliv glukosinolátů a jejich degradačních produktů na aroma vařeného zelí

Těkavé produkty vznikající během vaření čerstvého a kysaného zelí byly izolovány extrakcí nepolárními rozpouštědly a analyzovány metodou kapilární plynové chromatografie. Identifikováno bylo více než 30 sloučenin. Asi dvacet z těchto sloučenin vzniklo degradací glukosinolátů. Další látky byly aldehydy, alkoholy a sulfidy vzniklé z jiných prekurzorů. Bylo zjištěno, že účast glukosinolátů, resp. jejich degradačních produktů na tvorbě aroma vařeného zelí závisí na způsobu tepelného zpracování (přípravy) pokrmů.

Při vaření zelí dochází ke snížení obsahu glukosinolátů několika způsoby. V syrovém zelí a také během vaření do doby, než dojde k inaktivaci enzymů, jsou ztráty glukosinolátů způsobeny hlavně hydrolýzou endogenní myrosinasou. V enzymově neaktivních materiálech (při vaření po dobu delší než asi 30 minut) je hlavní příčinou ztrát chemická degradace glukosinolátů. K značným ztrátám dochází také výluhem.

Degradačními produkty glukosinolátů vznikajícími při vaření zelí ve vodě jsou zejména isothiokyanáty a nitrily. Jako hlavní produkty byly identifikovány allylisothiokyanát a příslušný nitril 1-kyano-2-propen vznikající enzymovou degradací sinigrinu (allylglukosinolátu) a dále 3-methylthiopropylisothiokyanát a odpovídající nitril 1-kyano-methylthiolpropan vnikající z 3-methylthiopropylglukosinolátu. Jako další významný produkt degradace sinigrinu byl prokázán také allylthiokyanát. Při vaření zelí ve vodě okyselené octem byly dominantními produkty hydrolýzy glukosinolátů nitrily, především byl přítomen 1-kyano-3-methylsulfinylpropan spolu s malým množstvím 3-methylsulfinylisothiokyanátu.

Téměř ve všech vzorcích vařeného zelí byly jako složky těkavých aromatických látek identifikovány dimethylsulfid, dimethyldisulfid, dimethyltrisulfid a dimethyltetrasulfid. Prekurzorem těchto látek je v brukvovitých zeleninách především sirná aminokyselina S(+)-methylcysteinsulfoxid.

zelí; glukosinoláty; těkavé produkty; aroma zelí

Contact address:

Prof. Ing. Jan Velíšek, DrSc., Vysoká škola chemicko-technologická, Ústav chemie a analýzy potravin, 166 28 Praha, Česká republika, tel.: 02/311 52 17, fax: 02/311 99 90, e-mail: jan.velisek@vscht.cz

Potrav. Vědy, *14*, 1996 (3) : 211–218

KINETICS OF CONTAMINATION OF WATER WITH BENZO(A)PYRENE FROM RECYCLED LOW DENSITY POLYETHYLENE PACKAGING

Peter ŠIMKO, Viera KHUNOVÁ[1]

Food Research Institute, Bratislava; [1]Slovak Technical University – Faculty of Chemical Technology, Bratislava, Slovak Republic

Abstract: A possibility of contamination of water with benzo(a)pyrene (BaP) by re-diffusion from polyethylene packaging made of recycled polyethylene (PE) was tested. PE packaging material was contaminated primarily with BaP by diffusion from water medium which was spiked with BaP at the level 41.6 μg per kg. When the polycyclic aromatic hydrocarbons concentration in the water dropped to 0.6 μg/kg, the PE packaging material was subjected to a recycling process. As observed, the recycling process did not affect the BaP concentration in PE at all. From the recycled PE, a packaging material was made again, and a possibility of re-diffusion of BaP from the packaging into water was followed for 600 hrs. As found, the packaging material prepared in this way is a potential source of food contamination since the final concentration of BaP in the water reached 1.04 μg/kg at the end of experiment when the BaP concentration in water was linearly dependent on the square root of the time of interaction.

HPLC; benzo(a)pyrene; diffusion; polyethylene packaging

Polycyclic aromatic hydrocarbons (PAHs) comprise the largest class of known environmental carcinogens; some, while not carcinogenic, may act as synergists. As a result of industrial activity, PAHs are present in water, air, soil and subsequently also in foods (Bartle, 1991). PAHs, however, can arise also in food products directly during processing, especially in thermal operations of flavouring, frying, and drying (Fretheim, 1983; Stahl, Eisenbrandt, 1988). A non-traditional source of contamination was described by Grob et al. (1991), who found PAHs in the batching oil used as a softener for jute bags.

In many studies the attention is focused on the determination of benzo(a)pyrene, which is widely considered to be a general indicator of the presence of PAHs in a sample (Kruijf et al., 1987).

In the last time, a number of papers have dealt with migration of chemical compounds from packaging into food products (Mercer et al., 1990; Begley, Hollifield, 1990; Kondyli et al., 1992). On the other hand, some papers have also reported the sorption of flavour compounds (carbonyls, alkylesters, sulfur compounds, and alkylpyrazines) from foods by polyethylene package (Nielsen et al., 1991; Linssen et al., 1992). Hence, interactions between packaging materials and foods are intensively studied with the aim to define the physico-chemical processes taking place in the boundary layer as well as inside the food-packaging systems.

Because of the permeable nature of plastic materials and their possible commercial and consumer use as containers for various chemicals, the recycled polymers could contain potentially toxic contaminants, which could migrate from the package into the food (Begley, Hollifield, 1993).

High ability of PAHs to penetrate into PE from liquid media has been already proven (Šimko, Bruncková, 1993), and the kinetic equation of the PAHs decrease in liquid media has been derived (Šimko et al., 1994).

As the contamination of non polar foods with PAHs from recycled polyethylene packaging was shown to be highly possible (Šimko et al., 1995), the aim of this work was to investigate the degree of contamination of a polar medium, i.e. water from PE packaging material, primarily contaminated with BaP, then recycled and used again for packaging of the water medium.

MATERIAL AND METHODS

A packaging material used in the experiment was made of low density polyethylene, type Bralen RA2-19, with a density of 922 kg/m^3 (Slovnaft, Bratislava, Slovak Republic).

Benzo(a)pyrene – BaP

BaP was of analytical grade, purchased from Supelco (Gland, Switzerland).

Solvents

All organic solvents (acetonitrile was HPLC grade purchased form Fluka, Buchs, Switzerland; methanol and hexane were of analytical grade purchased from Lachema Brno, Czech Republic) were purified before use by rectification.

Experiment

First, all organic solvents, water and PE were analyzed for the presence of BaP to eliminate an accidental contamination of materials used. Water was spiked with methanolic solution of BaP at the level 41.6 µg/kg. Then, PE foil (thickness 0.5 mm) was prepared from PE granulate using a hydraulic press where the pressing temperature was 140 °C, time of preheating 5 min, pressing time 3 min, working pressure 25 MPa, cooling time 10 min. The foil was sheared and put into beakers to obtain cylindrical shape of packages with the inside diameter of 5 cm. The cylinders were filled with 80 g of the contaminated water and the beakers were divided into two groups and placed in dark at 24 °C. The water of the first group was analyzed successively to determine the drop in BaP concentration in liquid media, i.e., the degree of BaP sorption on PE at times 17, 24, 41, 66, 111, and 208 hrs. When the total BaP concentration in the liquid media decreased to 0.6 µg/kg, PE from the second group was recycled as follows: PE was cut into small pieces, stirred up (at this stage analyzed for BaP concentration), then heated to 150 °C for 10 min. PE recycled in such a way was used again for the foil preparation, an PE packages of cylindrical shape were prepared for the next part of the experiment as mentioned above. Beakers were then filled with 80 g of distilled water. At the beginning of this part of experiment, BaP concentrations were determined in recycled PE, as well as in the water; then, a re-diffusion of BaP from PE, as well as into water was followed for 600 hrs at the temperature 24 °C.

To prevent the penetration of liquids between PE cylinders and beaker walls, minimal amounts of silicone paste were used for sealing in both stages of the experiment to guarantee only one dimensional diffusion, or re-diffusion, respectively.

Sample Preparation

Polyethylene: PE was cut into small pieces (ca. 1 cm x 1 cm) and put into Erlenmeyer flask with 100 ml of hexane, shaken for 1 h, and sonicated for 5 min. After filtering, the hexane was evaporated to near dryness and the residue dissolved in 1 ml of methanol.

Water: Water was extracted three times with 50 ml of hexane. Combined hexane layers were dried with anhydrous Na_2SO_4, evaporated to near dryness, and dissolved in methanol.

HPLC Conditions

HPLC was performed isocratically on a Separon SGX C_{18} reverse phase column (particle size 5 μm, length 30 cm, i.d. 3 mm purchased from Tessek, Praha, Czech Republic) at ambient temperature. The mobile phase was a mixture of acetonitrile and water (3 : 1, v/v) with a flow rate of 1.15 ml/min.

Instrumentation consisted of a high pressure pump and the syringe loop injector. The eluent from the column was directed to a Perkin-Elmer filter fluorescence detector (Beaconsfield, England), the model LS-2B, which operated at 310 nm excitation wavelength, and 410 nm emission wavelength.

All determinations were performed in triplicate.

RESULTS AND DISCUSSION

Since the sample preparation involved several steps, the efficiency of these procedures was determined by recovery studies. This was carried out by adding BaP solution into "blank" medium to give the concentration of 10 μg/kg. The recovery of BaP from water varied between 95.6 and 96.7% ($n = 3$) with a standard deviation 0.3% at most.

As showed additional recovery studies of BaP from PE, the one-step extraction was sufficient to reach more than 97% extractive efficiency.

The measured values of BaP concentrations in water decreased followingly during 208 hrs by two orders – from 41.6 to 0.9 μg/kg, which was in agreement with our previous observations (Šimko, Brunckov á, 1993; Šimko et al., 1994):

Time of interaction [hrs]	0	17	24	41	66	11	208
BaP	41.6	37.4	32.7	24.2	17.1	4.9	0.9

The recycling process itself did not affect the BaP concentration in PE at all because the boiling point of BaP is much higher ($b.p._{BaP} = 495$ °C) in comparison with the temperature of recycling process and the recycling time itself was too short for losses of BaP by photooxidation. The difference between BaP concentration in PE before recyclation (797.7 μg/kg) and after recyclation (801.2 μg/kg) is statistically insignificant within the error of measurement.

As follows from Table I (rows A), the PAHs concentration started to increase immediately after filling the recycled PE cylinders with the water, and this increase was observed- during the whole period.

I. The increase of BaP concentration [μg/kg] in the water

	Time of interaction [h]						σ
	0	28	67	254	480	600	
A	0.00	0.10	0.22	0.44	0.89	1.04	0.1
B	0.00	0.18	0.30	0.58	0.78	0,89	

A – measured values
B – values calculated by equation [2]

For this case – difussion of compounds from cylindrical shape packaging into liquid medium – kinetic equation [1] has already been derived (Šimko et al., 1995) in the form

$$c_s = s_0 + c_\infty \frac{r+h}{r.h}(4D/\Pi)^{1/2} . t^{1/2} \quad [1]$$

which at initial concentration $c_0 = 0$ takes the form

$$c_s = c_\infty \frac{r+h}{r.h}(4D/\Pi)^{1/2} . t^{1/2} \quad [2]$$

where: c_s – concentration of given compound in liquid media in the time t
r – diameter of cylinder
h – thickness of the package material
c_∞ – concentration of given compound in liquid phase in infinite time t_∞

The value of equilibrium concentration (c_∞ = 4 μg/kg) was taken from Björseth (1983) and the diffusion coefficient of BaP in PE (D_{BaP} = = $4.4.10^{-11}$ cm^2/s) from Šimko et al. (1995). For comparison, by equation [2] BaP concentrations were calculated in the same time intervals (Table I, rows B) as measured values. As follows from the value of standard deviation σ, measured and calculated values are in close agreement, which makes it possible to use equation [2] for the calculation of any BaP concentration at whatever time of the experiment. As follows from equation [2], the rate of BaP diffusion into water depends on thickness of PE film, diameter of cylinder as well as diameter of cylinder and the diffusion coefficient, and it is directly proportional to the square root of the time of interaction. Although the diffusion rate constant ($k_{BaP/H_2O} = 0.101$) of non polar compound (BaP) from non polar phase (LDPE) into polar liquid (water) is 12.7 times lower than the rate constant into non polar liquid (vegetable oil) (Šimko et al., 1995), the degree of contamination of polar medium can also reach relatively high values, mainly after a long time interaction of contaminated PE and polar liquid media.

Equation [2] may be suitable for calculation of concentration of various compounds, not only contaminants, but also plasticizers, softeners, colourants and other compounds that are able to diffuse from plastic packaging of cylindrical shapes, i.e. bottles into liquid contents.

References

BARTLE, K. D.: Analysis and occurrence of polycyclic aromatic hydrocarbons in food. In: CREASER, C. – PURCHASE, R. (Eds.): Food Contaminants, Sources and Surveillance. Cambridge, Royal Soc. Chem. 1991: 41–59.

BEGLEY, T. H. – HOLLIFIELD, H. C.: High performance liquid chromatographic determination of migrating polyethylene–terephthalate oligomers in corn oil. J. Agric. Food Chem., *38*, 1990: 145–148.

BEGLEY, T. H. – HOLLIFIELD, H. C.: Recycled polymers in food packaging: Migration consideration. Food Techn., *47*, 1993: 109–112.

BJÖRSETH, A. Handbook of Polycyclic Aromatic Hydrocarbons. New York, Marcel Dekker 1983: 258 pp.

FRETHEIM, K.: Polycyclic aromatic hydrocarbons in grilled meat products. Food Chem., *10*, 1983: 129–139.

GROB, K. – BIEDERMANN, M. – CARAMASCHI, A. – PACCIARELLI, B.: LLC–GC analysis of the aromatics in mineral oil fraction: Batching oil for jute bags. J. High Resol. Chromatogr., *14*, 1991: 33–39.

KONDYLI, E. – DEMERTIZIS, P. G. – KONTOMINAS, M. G.: Migration of dioetylphthalate and dioetyladipate plasticizers from food grade PVC film into ground meat products. Food Chem., *45*, 1992: 163–168.

KRUIJF, N. – SCHOUTEN, T. – STEGEN, G. H. D.: Rapid determination of benzo(a)pyrene in roasted coffee and coffee brew by HPLC with fluorescence detection. J. Agric. Food Chem., *35*, 1967: 545–549.

LINSSEN, J. P. H. – VERHEUL, A. – ROOZEN, J. P. – POSTHUMUS, M. A.: Absorption of flovour compounds by packaging material: Drink yogurt in polyethylene bottles. Int. Dairy J., *2*, 1992: 33–40.

MERCER, A. – CASTLE, L. – COMYN, J. – GILBERT, J.: Evaluation of a predictive mathematical model of di-(2-ethylhexyl)adipate plasticiser migration from PVC film into foods. Food Addit. Contam., *7*, 1990: 497–507.

NIELSEN, T. J. – JÄGERSTAD, I. M. – OSTE, R. E. – SIVIK, B. T. G.: Supercritical fluid extraction coupled with gas chromatography for the analysis of aroma compounds adsorbed by low-density polyethylene. J. Agric. Food Chem., *39*, 1991: 1234–1237.

STAHL, W. – EISENBRAND, G.: Determination of polynuclear aromatic hydrocarbons and nitrosamines. In: MACRAE, R. (Ed.): HPLC in Food Analysis. London, Academic Pres 1988: 377–412.

ŠIMKO, P. – BRUNCKOVÁ, B.: Lowering of polycyclic aromatic hydrocarbons in a liquid smoke flavour by sorption into polyethylene packaging. Food Addit. Contam., *10*, 1993: 257–263.

ŠIMKO, P. – ŠIMON, P. – KHUNOVÁ, V. – BRUNCKOVÁ, B.: Kinetics of polycyclic aromatic hydrocarbon sorption from liquid smoke flavour into low density polyethylene packaging. Food Chem., *50*, 1994: 65–68.

ŠIMKO, P. – KHUNOVÁ, V. – ŠIMON, P. – HRUBÁ, M.: Kinetics of sunflower oil contamination with polycyclic aromatic hydrocarbons from contaminated recycled low density polyethylene film. Int. J. Food Sci. Technol., 1995 (in press).

Received December 8, 1995

Kinetika kontaminácie vody benzo(a)pyrénom z recyklovaného vysokotlakového polyetylénu

Práca sa zaoberá možnosťou kontaminácie vody benzo(a)pyrénom (BaP), ktorý môže difundovať do vodného (polárneho) prostredia z recyklovaných polyetylénových obalových materiálov. Z granulovaného vysokotlakového polyetylénu (LDPE) bola najprv zhotovená fólia o hrúbke 0,5 mm za použitia hydraulického lisu a nasledujúcich podmienok: teplota lisovania 140 °C, doba prehrievania 5 min, doba lisovania 3 min, pracovný tlak 25 MPa, doba chladenia 10 min. Z fólie boli pripravené telesá cylindrického tvaru s vnútorným priemerom 5 cm, ktoré sa naplnili vodou kontaminovanou BaP. V dôsledku difúzie BaP z vodného prostredia do LDPE počas 208 hodín sa znížila koncentrácia BaP z hodnoty 41,6 na 0,6 μg/kg. Takýmto

spôsobom kontaminovaný LDPE sa podrobil recyklačnému procesu tak, že dezintegrované časti (cca 1 x 1 cm) boli premiešané a zahriate na teplotu 150 °C počas 10 min. Po ochladení bola opäť z takto recyklovaného LDPE uvedeným spôsobom vyrobená fólia. Porovnaním koncentrácií BaP v PE pred recykláciou (797,7 µg/kg) a po recyklácii (801,2 µg/kg) bolo zistené, že počas recyklačného procesu nedochádza k zmene koncentrácie BaP v PE, pretože bod varu BaP je ďaleko vyšší (495 °C) ako je teplota recyklačného procesu. Z recyklovanej PE fólie boli opäť zhotovené telesá cylindrického tvaru, ktoré sme naplnili destilovanou vodou. Počas 600 hodín experimentu sa sledovala možnosť redifúzie BaP z PE do polárneho prostredia, pričom bol zaznamený nárast koncentrácie BaP z nulovej hodnoty na počiatku na hodnotu 1,04 µg/kg na konci experimentu. Na základe nameraných hodnôt bolo možné postulovať tieto závery:

1. Rýchlosť redifúzie BaP z LDPE cylindrického tvaru do vodného prostredia je možné popísať kinetickou rovnicou.

2. Recyklovaný PE po kontaminácii počas prvotného použitia môže byť zdrojom kontaminácie pri jeho sekundárnom využití.

3. Rýchlosť difúzie je priamo úmerná druhej odmocnine doby interakcie PE a kvapalného prostredia.

4. Hoci rýchlosť redifúzie do polárneho prostredia (vody) je mnohokrát nižšia ako do nepolárneho prostredia, stupeň kontaminácie môže dosiahnuť relatívne vysokú hodnotu, najmä po uplynutí dlhšej doby interakcie kvapalnej a tuhej fázy.

5. Kinetická rovnica [2] môže byť použitá na výpočet koncentrácie rôznych zlúčenín, ako sú napr. plastifikátory, pigmenty, ale i ďalšie látky, ktoré sú schopné difundovať z obalov cylindrického tvaru do kvapalného obsahu.

HPLC; benzo(a)pyrén; difúzia; polyetylén; obalový materiál

Contact address:

Doc. Ing. Peter Šimko, CSc., Výskumný ústav potravinársky, Priemyselná 4, 820 06 Bratislava, Slovenská republika,
tel.: 07/526 46 22, fax: 07/526 14 17, e/mail: mkovac@cvt.stuba.sk

Potrav. Vědy, *14*, 1996 (2): 219–227

VYUŽITIE GÉLOVEJ CHROMATOGRAFIE PRI ŠTÚDIU KVALITY CEREÁLNYCH BIELKOVÍN

Use of Gel Chromatography by Study of Cereal Proteins Quality

Katarína JANEKOVÁ, Bernadetta KRKOŠKOVÁ, Daniela TEPEROVÁ

Food Research Institute, Bratislava, Slovak Republic

Abstract: Cereal preparates research was aimed on isolation and fractionation processes of wheat and maize proteins. Contens of different protein groups was determined in various methods of isolation from selected wheat and maize preparations. Influence of extrusion and expansion technology on protein quality was studied. Gel chromatography method was used for qualitative characterisation of cereal proteins.

cereal proteins; isolation; fractionation; gel chromatography

Abstrakt: Výskum preparátov na báze cereálií bol zameraný na postupy izolácie a frakcionácie bielkovín pšenice a kukurice. Stanovil sa obsah jednotlivých skupín bielkovín pri rôznych postupoch izolácie z vybraných pšeničných a kukuričných prípravkov. Ďalej sa študoval vplyv extrúznej a expanznej technológie na kvalitu bielkovín. Expanzné spracovanie, resp. extrúzia sa prejavila zmenou pomerného zastúpenia jednotlivých skupín bielkovín. Na kvalitatívne charakterizovanie cereálnych bielkovín sa použila metóda gélovej chromatografie. Táto metóda vo vybranej modifikácii sa ukázala ako vhodná na sledovanie skladby cereálnych bielkovín.

cereálne bielkoviny; izolácia; frakcionácia; gélová chromatografia

Rastlinné suroviny a bielkovinové preparáty z rastlinných zdrojov možno využiť ako potravinárske aditíva s výhodnými funkčnými vlastnosťami, ktoré priaznivo ovplyvňujú aj výživovú a senzorickú úroveň potravín. Navyše prednosťou rastlinných bielkovín je ich nízka cena v porovnaní so živočíšnymi bielkovinami.

Proteíny cereálnych zŕn sa z hľadiska biologických funkcií delia na biologicky aktívne enzýmy a biologicky inaktívne zásobné bielkoviny, ktoré predstavujú okolo 80 % z celkového množstva proteínov. I naďalej zostáva v platnosti klasifikácia bielkovín podľa Osborna, založená na rozpustnosti bielkovín v rôznych rozpúšťadlách, a to vo vode (albumíny), v roztoku solí (globulíny), vo vodnom roztoku alkoholu (prolamíny) a v roztoku alkálií alebo kyselín (glutelíny) (Lorenz, Kupl, 1991). Morfologicky je možno rozdeliť bielkoviny do troch skupín: bielkoviny klíčka, bielkoviny endospermu a bielkoviny aleurónovej vrstvy. Zásobné bielkoviny (prolamíny a glutelíny) sú typické bielkoviny endospermu, hoci ich možno nájsť aj v ďalších dvoch skupinách.

Prolamíny pšenice – gliadíny – sa vyznačujú kompaktnou štruktúrou molekúl. Zvyčajne sa gliadíny delia na frakcie gélovou filtráciou a následnou elektroforézou. Takto boli stanovené alfa, beta, gama a omega frakcie gliadínu, ktoré sú zložené z individuálnych 40 až 50 komponentov. Je pre ne charakteristický vysoký obsah kyseliny glutámovej, prolínu a neveľký obsah ďalších aminokyselín.

Molekulová hmotnosť (MH) alfa, beta, gama gliadínov je 31 000 až 34 700, omega gliadínov 73 000 až 78 000. Okrem toho gliadínová frakcia obsahuje 6 % vysokomolekulovej bielkoviny, ktorej MH je asi 100 000, a na Sephadexe G 100 sa delí ako prvá. Označuje sa tiež ako „vysokomolekulový gliadín" alebo „nízkomolekulový glutenín" (Lorenz, Kupl, 1991).

Glutelíny pšenice – gluteníny – sú charakteristické MH 500 000 až 3 000 000. V porovnaní s gliadínmi majú gluteníny vyšší obsah glycínu, tryptofánu, lyzínu a nižšiu koncentráciu kyseliny glutámovej, prolínu a fenylalanínu.

Proteíny gliadínu a gluteninu sa v procese technológie koncentrujú v pšeničnom lepku. Lepkom sa obohacujú rôzne cereálne výrobky a výhodne sa využíva pri výrobe cestovín zo slabých typov múky a pri výrobe mäsových výrobkov (Perlín, 1985).

Zásobným prolamínom kukuričného zrna je zeín. Hydrolyzáty zeínu sú chudobné na lyzín a tryptofán a majú vysoký obsah leucínu, prolínu a kyseliny glutámovej (Paulis, 1982). Gélovou filtráciou natívneho zeínu sa získajú dve frakcie s MH 22 000 a 45 000 (Landry, Moureaux, 1981).

Glutelíny kukuričného zrna pozostávajú zo štyroch frakcií s rôznou molekulovou hmotnosťou. Rozpustné frakcie sú označované ako G1, G2 a G3 glutelíny (Landry, Moureaux, 1981, 1980).

Štúdiu rastlinných bielkovín sa venuje intenzívna pozornosť. Bez použitia nových separačných techník a postupov spolu s modernými analytickými prístrojmi by nebolo možné zvládnuť náročnosť a zložitosť ich štúdia. V predkladanej práci informujeme o využití gélovej chromatografie pri sledovaní kvality pšeničných a kukuričných bielkovín.

MATERIÁL A METÓDY

V experimentoch sme pracovali s cereálnymi surovinami vystavenými účinku expanzného a extrúzneho procesu, a to s expandovanou pšenicou (PD Slatinské Lazy) a s experimentálne pripraveným extrúdovaným kukuričným gluténom (Slovamyl Boleráz). Ako porovnávacie vzorky sa použili: pšenica pomletá na šrot (odroda Hanka, PD Slatinské Lazy), pšeničná múka hladká T650 (z obchodnej siete), kukurica pomletá na šrot (Slovamyl Boleráz), kukuričná krupica (PD Vlára Nemšová) a kukuričný glutén (Slovamyl Boleráz). Vzorky použité na analýzu bielkovinového podielu boli odtučnené extrakciou dietyléterom na prístroji Soxtec.

Na separáciu a frakcionovanie bielkovín sa použili tieto postupy:
- postup izolácie bielkovín na základe rozpustnosti v charakteristických rozpúšťadlách (Davídek, 1981)
- postupná izolácia bielkovín podľa Paulisa (Paulis, 1982)
- modifikovaný postup frakcionácie kukuričných bielkovín, ktorý publikovali Landry a Moureaux (1980, 1981)

Pomer hmotnosti vzorky a extrakčného činidla bol 1 : 10. Disperzia bola odstreďovaná na odstredivke 5 až 7 minút pri otáčkach 4 000 za minútu.

Kvantitatívna analýza dusíkatých látok vo vstupných vzorkách, ako aj v jednotlivých frakciách bola vykonaná stanovením celkového dusíka metódou podľa Kjeldahla a obsah bielkovín bol vypočítaný po vynásobení N x 5,7 (pre pšenicu a prípravky) a N x 6,25 (pre kukuricu a prípravky).

Bielkovinové frakcie získané pri kvantitatívnej separácii sa ďalej analyzovali pomocou gélovej chromatografie.

Vlastná separácia sa uskutočnila na kolónach:
- Sephadexu G-100 umožnujúcich gélovú chromatografiu v rozsahoch MH 1.10^3 až 1.10^5
- Sephacrylu S-200 v rozsahu MH 1.10^3 až 8.10^4
- Sephacrylu S-300 v rozsahu MH 2.10^3 až 4.10^5

Vzorky boli delené v sústavách kyselina octová – voda, resp. alkohol – voda. Obsah bielkovín bol v jednotlivých frakciách stanovovaný spektrofotometricky pri 280 nm. Elúcia vzorky v jednotlivých pokusoch variovala, nepresiahla však 1 ml za min.

Jednotlivé vzorky prezentovali biologický materiál rôznej úrovne rozpustnosti. U niektorých bola pozorovaná nerozpustná frakcia bielkovín ako zákal, niektoré boli číre. Z uvedeného dôvodu boli uskutočnené pokusy v rôznych médiách, zabezpečujúcich rozpustnosť vzorky. Zakalené vzorky boli centrifugáciou rozdelené na rozpustný podiel a sediment, ktoré boli analyzované jednotlivo.

VÝSLEDKY A DISKUSIA

Výsledky kvantitatívneho stanovenia obsahu jednotlivých skupín bielkovín v pšeničných a kukuričných prípravkoch pri rôznych postupoch izolácie sú zhrnuté v tab. I až III.

I. Obsah [g] jednotlivých skupín bielkovín v pšeničných a kukuričných prípravkoch (stanovené metódou podľa Kjeldahla, po izolácii podľa Osborna) – Contents [g] of the particular groups of proteins in wheat and maize preparations (determined by Kjeldahl method, after isolation according to Osborn)

	Celkový obsah bielkovín[1]	Albuminy[2]	Globuliny[3]	Prolaminy[4]	Gluteliny[5]	NB	Straty[6]
Pšeničný šrot[7]	12,995	2,927	1,425	2,136	3,971	1,459	1,067
Expandovaná pšenica[8]	12,726	2,609	0,481	0,378	1,455	6,543	1,269
Pšeničná múka hladká[9]	11,769	0,648	0,841	2,995	4,815	1,309	1,201
Kukuričný šrot[10]	10,867	1,533	1,438	1,252	2,329	3,575	0,740
Kukuričná krupica[11]	10,554	0,843	0,921	1,536	2,583	3,978	0,693
Kukuričný glutén[12]	25,178	0,473	0,271	10,546	7,302	5,514	1,108
Extrudovaný kukuričný glutén[13]	23,433	0,219	0,084	5,080	3,201	12,452	2,397

NB = nerozpustné bielkoviny – insoluble proteins

[1]total protein content; [2]albumins; [3]globulins; [4]prolamines; [5]glutelins; [6]losses; [7]wheat groats; [8]expanded wheat; [9]fine wheat flour; [10]maize groats; [11]maize semolina; [12]maize gluten; [13]extruded maize gluten

Pre pšeničné prípravky bol použitý spôsob izolácie bielkovín podľa Osborna. Podiel jednotlivých skupín bielkovín v celkovom bielkovinovom komplexe sledovaných vzoriek je zhrnutý v tab. II.

II. Podiel [%] jednotlivých skupín bielkovín v pšeničných prípravkoch – Percentage [%] of the particular protein groups in wheat preparations

Vzorka[1]	Albumíny[2]	Globulíny[3]	Prolamíny (gliadíny)[4]	Glutelíny[5]	Nerozpustné bielkoviny a straty[6]
Pšeničná múka hladká[7]	5,306	7,146	25,109	40,912	21,327
Pšeničný šrot[8]	22,524	11,043	16,437	30,558	19,438
Expandovaná pšenica[9]	20,501	3,780	2,971	11,433	61,315
Zmena obsahu[10]	–2,023	–7,263	–13,466	–19,125	+41,877

[1]sample; [2]albumins; [3]globulins; [4]prolamines (gliadins); [5]glutelins; [6] insoluble proteins and losses wheat groats; [7]fine wheat flour; [8]wheat groats; [9]expanded wheat; [10]content change

V porovnaní s literárnymi údajmi vzorka pšenice mala vyšší obsah celkových bielkovín. Stanovené podiely jednotlivých skupín bielkovín (tab. II) sú v dobrej zhode s literárnymi údajmi (Lorenz, Kupl, 1991); v zastúpení prolamínov sa zistila odchýľka. Z výsledkov kvantitatívnej analýzy vyplýva, že vplyvom expanzie dochádza k zníženiu obsahu bielkovín skupiny globulínov, gliadínov a glutelínov. Možno predpokladať, že dochádza k ich deštrukcii, resp. denaturácii, čo sa prejavilo vo zvýšení podielu skupiny nerozpustných bielkovín.

Pre skupinu vzoriek z kukurice sme použili tri postupy frakcionácie. Ako najvhodnejší a podľa literárnych údajov najčastejšie používaný je postup frakcionácie podľa Landry-Moureauxa. Z tab. III je zrejmé, že v prípravkoch kukuričného gluténu natívneho aj extrudovaného sa nestanovila frakcia albumínov a globulínov. Príčinou môžu byť podmienky izolácie.

Podľa výsledkov kvantitatívneho stanovenia obsahu jednotlivých skupín bielkovín dochádza u kukuričného gluténu vplyvom extrúzie k zníženiu obsahu bielkovín frakcie zeínu, frakcií G2 a G3 a nárastu obsahu bielkovín frakcie G1 (tab. III). V prípade kvantitatívneho stanovenia obsahu jednotlivých skupín bielkovín natívneho a extrudovaného gluténu získaných metó-

III. Obsah [g] jednotlivých skupín bielkovín v kukuričných prípravkoch (stanovené metódou podľa Kjeldahla, po izolácii podľa Landryho a Moureauxa) – Content [g] of the particular protein groups in maize preparations (determined by Kjeldahl method, after isolation according to Landry and Moreaux)

Vzorka[1]	Celkový obsah bielkovín[2]	Albumíny, globulíny[3]	Zeín[4]	G1	G2	G3	NB	Straty[5]
Kukuričný šrot[6]	10,867	2,343	0,996	0,790	1,019	2,032	2,012	1,675
Kukuričná krupica[7]	10,554	0,693	2,330	1,610	0,741	1,739	1,333	2,108
Kukuričný glutén[8]	25,178	nestanov.[10]	10,502	3,384	2,279	6,215	2,036	0,762
Extrudovaný kukuričný glutén[9]	23,433	nestanov.	3,804	14,528	0,732	1,169	2,365	0,834

NB = nerozpustné bielkoviny – insoluble proteins

[1]sample; [2]total protein content; [3]albumins, globulins; [4]zeain; [5]losses [6]maize groats; [7]maize semolina; [8] maize gluten; [9]extruded maize gluten; [9]not evaluated

dou izolácie podľa Osborna (tab. I) sme zaznamenali pokles obsahu bielkovín, najvýraznejšie vo frakcii zeínov a glutelínov. Výrazne sa zvýšil obsah bielkovín vo frakcii nerozpustných bielkovín.

Pšeničné prípravky, ako aj jednotlivé bielkovinové frakcie získané uvedeným postupom boli podrobené analýze pomocou gélovej chromatografie. Na základe získaných výsledkov gélovej chromatografie jednotlivých vzoriek je možné vysloviť niektoré predbežné závery.

Vo väčšine vzoriek boli pozorované nízkomolekulové fragmenty s MH pod 10^3 nezávisle od spôsobov delenia. Pri gélovej chromatografii pšeničnej múky hladkej sa okrem nízkomolekulárnych frakcií bielkovín s MH 10^3 a vysokomolekulárnych frakcií bielkovín nezistili žiadne albumíny a globulíny, ktorých prítomnosť sa naopak potvrdila v pšeničnom šrote a v malej miere v expandovanej pšenici. Pri porovnaní s výsledkami stanovenia obsahu jednotlivých skupín bielkovín v pšeničnej múke (tab. I) možno konštatovať, že použité postupy chromatografického delenia nezabezpečili oddelenie uvedených bielkovín.

Porovnaním bielkovinového profilu frakcií albumínov, resp. globulínov pšeničného šrotu a expandovanej pšenice bol pozorovaný výrazný zánik bielkovinových frakcií s MH 10^4 v prospech bielkovinových zložiek s nižšou

MH (pod 10^3). Frakcia nerozpustných bielkovín sa v chromatografických záznamoch prejavovala ako bielkoviny s MH nad 10^5. V získanom chromatografickom zázname pre frakcie gliadínov a gluteninov boli pozorované dva výrazné pásy zodpovedajúce bielkovinám o MH približne 10^4 a nad 10^5.

Na overenie účinnosti delenia bola použitá gélová chromatografia na Sephadexe G-100 a Sephacryle S-200 v sústavách: CH_3COOH – voda, resp. C_2H_5OH – voda. Výraznejšie delenie zabezpečil Sephadex. V gliadínovej frakcii pšeničného šrotu boli pozorované štyri chromatografické signály, rovnako ako u pšeničnej múky hladkej.

Chromatografické záznamy sa navzájom líšili hlavne zastúpením nízkomolekulových fragmentov s MH pod 10^3. Expandovaná pšenica vykazovala v uvedenej sústave len jeden intenzívny signál pre MH pod 10^3, čo vedie k predpokladu, že termickou expanziou zrna dochádza k výraznej deštrukcii gliadínov zrna na nízkomolekulové bielkovinové frakcie.

V prípade použitia alkoholu ako extrakčného a deliaceho média chromatografický profil gliadínov v pšeničnom šrote a pšeničnej múke hladkej rovnako vykazuje štyri chromatografické signály s rozdielnym elučným objemom. U expandovanej pšenice sa na chromatografickom zázname nezistili nízkomolekulové bielkovinové látky a boli pozorované len vysokomolekulové bielkoviny o zvýšenej intenzite absorpčného signálu voči východiskovému materiálu (šrot, múka). V prípade izolátov gluteninov boli pozorované rovnaké chromatografické signály pre expandovanú pšenicu i pšeničný šrot. Nebol pozorovaný nárast, resp. úbytok uvedenej bielkovinovej frakcie vplyvom expanzného procesu. Nezistil sa ani vznik štiepnych, resp. polymerizačných produktov gluteninov.

Pre gélovochromatografickú analýzu bielkovín kukurice boli použité vzorky pripravené metódou, ktorú publikovali L a n d r y a M o u r e a u x (1980, 1981), resp. postupom, který opísal P a u l i s (1982), z kukuričného šrotu, kukuričnej krupice, kukuričného gluténu a extrudovaného kukuričného gluténu. Pre gélovú chromatografiu boli použité náplne Sephadex G-100 a Sephacryl S-200, resp. S-300.

Analýza frakcie albumínov a globulínov izolovaných z kukuričného šrotu, resp. z kukuričnej krupice vykazovala na Sephadexe G-100 rovnaký bielkovinový profil s dvomi chromatografickými signálmi:

- pre MH väčšiu ako 10^5 (elučný objem 63 ml),
- pre MH $1,8.10^4$ (elučný objem 177 ml).

Izoláty tejto bielkovinovej frakcie z kukuričného gluténu a extrudovaného kukuričného gluténu vykazovali odlišný bielkovinový profil s tromi chromatografickými signálmi. Zistila sa prítomnosť tretieho signálu pri elučnom objeme 32 ml.

Analýza frakcií prolamínov izolovaných zo všetkých kukuričných preparátov sa realizovala na Sephacryle S-200. Izoláty pripravené postupom, ktorý publikoval Paulis (1982), vykazovali v chromatografickom zázname väčší počet signálov ako izoláty pripravené postupom podľa Landryho a Moureauxa (Landry, Moureaux 1980, 1981). Zistilo sa päť chromatografických signálov, ku ktorým nemožno jednoznačne priradiť fragmenty s určitou molekulovou hmotnosťou. Vzhľadom na uvedené okolnosti nemožno urobiť závery o vplyve extrúzie na zeínovú frakciu.

Poslednou izolovanou frakciou bielkovín kukurice boli glutelíny, ktoré boli idetifikované pomocou gélovej chromatografie na géli Sephacryl S-300, resp. S-200. Na základe porovnania jednotlivých chromatografických záznamov možno konštatovať, že zistené chromatografické profily sa u jednotlivých vzoriek výrazne podobajú. Líšia sa len intenzitou signálov minoritných fragmentov, ktoré sa eluujú pred hlavnou frakciou glutelínov. Pozorovali sa dva hlavné chromatografické signály. Kvantitatívne vyhodnotenie týchto signálov neukázalo podstatné rozdiely v skladbe glutelínovej frakcie rozličných kukuričných prípravkov.

Záver

Použitý spôsob izolácie bielkovín podľa Osborna sa ukázal ako vhodný pre pšeničné prípravky. Pre prípravky na báze kukurice bol najvhodnejší postup izolácie podľa Landryho a Moureauxa. Kvantitatívna analýza ukázala vplyv expanzného spracovania, resp. extrúzie na obsah jednotlivých skupín bielkovín. Pšeničné a kukuričné prípravky, ako aj jednotlivé bielkovinové frakcie získané uvedenými postupmi izolácie boli podrobené analýze pomocou gélovej chromatografie. Gélova chromatografia je vhodnou metódou na sledovanie skladby a kvality cereálnych bielkovín. Na základe doterajších výsledkov nemožno robiť rozsiahlejšie závery o vplyve technologických operácií (expanzné a extrúzne spracovanie) na kvalitu bielkovín.

Literatúra

DAVÍDEK, J. a kol.: Laboratorní příručka analýzy potravin. Praha, SNTL-ALFA 1981.

LANDRY, J. – MOUREAUX, T.: Physicochemical properties of maize glutelins as influenced by their isolation conditions. J. Agric. Food Chem., *29*, 1981: 1205–1212.

LANDRY, J. – MOUREAUX, T.: Distribution and amino acid composition of protein groups located in different histological parts of maize grain. J. Agric. Food Chem., *28*, 1980: 1186–1191.

LORENZ, K. J. – KUPL, K.: Handbook of Cereal Science and Technology. New York-Basel-Hong Kong, Marcel Dekker,INC. 1991.

PAULIS, J. W.: Recent developments in corn protein research. J. Agric. Food Chem., *30*, 1982: 14–20.

PERLÍN, C.: Zhodnocení využívání bílkovinových zdrojů v potravinářském průmyslu. [Záverečná správa.] Praha, VÚEZV 1985: 83.

Došlo 16. 3. 1995

Kontaktní adresa:

Ing. Katarína Janeková, Výskumný ústav potravinársky, Priemyselná 4, 820 06 Bratislava, Slovenská republika,
tel.: 07/526 17 01, fax: 07/526 14 17, e-mail: mkovac@cvt.stuba.sk

Potrav. Vědy, *14*, 1996 (3) : 229–237

PŘEHLEDY

NOVŠIE ASPEKTY MIKROBIOLOGICKÉHO HODNOTENIA AKOSTI MATERINSKÉHO MLIEKA

Mária GREIFOVÁ, Bernadetta HOZOVÁ, Juraj HAMZA

Slovenská technická univerzita – Chemickotechnologická fakulta, Bratislava, Slovenská republika

Materinské mlieko, ako je známe, je ideálnou potravinou, ktorá je schopná v dostatočnom množstve a v optimálnom pomere poskytnúť detskému organizmu všetky potrebné látky pre jeho zdravý vývoj v prvých šiestich mesiacoch života (M i k o, 1991). V súčasnosti nastáva obdobie renesancie dojčenia, nakoľko nové poznatky o výžive, hodnotení zdravotného stavu a individuálneho vývoja umelo živených detí poukazovali na omyl v preferovaní umelej výživy. Aj Svetová zdravotnícka organizácią roku 1992 vyhlásila Prolaktačný program a zákaz propagácie mliečnych prípravkov pre umelú výživu (M i c h a l i č k o v á, 1993). Ľudské mlieko má jedinečné zloženie, ktoré je nielen zdrojom výživy a energie, ale má aj profylaktický a sčasti i terapeutický význam (Š v e j c a r, 1990; K l e n et al., 1987).

Na druhej strane sa s postupujúcou chemizáciou životného prostredia a šírením civilizačných chorôb zvyšuje potenciálne riziko prítomnosti ekologických jedov v materinskom mlieku (cudzorodé látky, lieky, narkotiká, ťažké kovy, polychlórované bifenyly atď.).

Tento príspevok podáva stručný literárny prehľad dostupných poznatkov z oblasti mikrobiológie materinského mlieka v zahraničí a u nás, a to nielen zo známych výživových, antimikrobiálnych a medicínskych aspektov, ale aj z hľadiska možnej mikrobiologickej a chemickej kontaminácie, negatívnych vplyvov technológie, ako aj otázok prevencie a legislatívy.

Zloženie materinského mlieka

Materinské mlieko je svojím zložením unikátne. Je donátorom energie, nutričných látok (bielkoviny, cukry, tuky, vitamíny, minerálne látky), vody, hormónov, enzýmov, imunoglobulínov atď.

Dominantnou bielkovinou je β-kazeín, zatiaľ čo α-kazeín je najmenej zastúpenou zložkou. 40 % energetickej hodnoty predstavuje laktóza, menej galaktóza. Tukový podiel zaberá 50 % celkového energetického obsahu a je najpremenlivejšou zložkou čo do množstva i zloženia (0,3–18 g/100 ml). Rozdiely sú spôsobené najmä genetickými vplyvmi, dĺžkou laktácie, výživou atď.

Pomer nasýtených a nenasýtených mastných kyselín je v kravskom mlieku 1,8 : 1, v materinskom 1 : 1. Hlavným zdrojom energie je kyselina palmitová ($C_{16:0}$) a kyselina olejová ($C_{18:1}$).

Vitamíny sú významné pre mentálny a fyzický vývoj dieťaťa a ich obsah závisí predovšetkým od výživy a zdravotného stavu matky. Oproti kravskému mlieku obsahuje približne dvoj- až šesťnásobne vyššie množstvo vitamínov (A – 1,5x; D – 6x; C - 2,5x; B vitamíny: tiamín – 3x; riboflavín – 4,5x; niacín – 2,5x; 6 – 5x; kyselina pantoténová – 1,5x).

Minerálne látky sú zastúpené v priaznivom pomere vápnika a fosforu, ktorý nezaťažuje obličky.

Materinské mlieko obsahuje v neposlednom rade hormóny a enzýmy. Doteraz bola dokázaná prítomnosť ovariálnych a testikulárnych steroidov, kalcitonínu, inzulínu, hypofyzárnych hormónov - prolaktínu, ACTH, TSH, hypotalamických hormónov LHRH a TRH, a i; z enzýmov α a β-amyláza, laktóza-syntetáza, L-laktátdehydrogenáza, lipáza, kyslá fosfatáza, proteáza, ribonukleáza atď. (Klen et al., 1987).

Antimikrobiálne vlastnosti

Ochranným látkam v materinskom mlieku je v poslednom čase venovaná mimoriadna pozornosť. Výskumy potvrdzujú, že dojčenie znižuje počet ochorení predovšetkým gastrointestinálneho traktu a respiračných orgánov.

Ochranné látky sa rozdeľujú na špecifické (imunoglobulíny) a nešpecifické (prirodzené inhibítory). Špecifické ochranné látky sú obsiahnuté v imunoglobulínovej frakcii sérových bielkovín. Imunoglobulíny (Ig) majú na mikroorganizmy, najmä gastrointestinálneho traktu aglutinačný účinok a synergicky s ostatnými ochrannými látkami neutralizujú toxíny, inaktivujú vírusy (Barret, 1988).

Ig A poskytuje široké spektrum imunity, je aktívny proti klostrídiám, salmonelám, šigelám, hemofilom, *E. coli* a enterotoxínu *Vibrio cholerae* (May, 1988; Barret, 1988) a tiež proti vírusom obrny, rubeoly, *herpes simplex*, mumpsu, chrípky, atď., ako aj parazitom (May, 1988).

Ig G sa prejavuje protivírusovými účinkami (cytomegalovírus, rubeola) a schopnosťou viazať bakteriálne toxíny (*Clostridium tetani*, *Corynebacterium diphteriae*) (Klen et al., 1987; Junker et al., 1991).

Ig M a Ig D sú účinné najmä proti G-mikroorganizmom (*E. coli, Vibrio cholerae*) (Klen et al., 1987) a vírusom (cytomegalovírus, rubeola) (May, 1988).

Vo všeobecnosti možno povedať, že hladiny všetkých imunoglobulínov sú najvyššie v mledzive a potom prudko klesajú, čo dokazujú i pokusy, pri ktorých kolostrum bolo menej kolonizované baktériami pri izbovej teplote ako zrelé materinské mlieko v priebehu sledovaných časových jednotiek (Atalay et al., 1989; Nwankwo et al., 1988).

Medzi bunkové ochranné faktory patria fagocytujúce leukocyty – z nich 80 až 90 % tvoria makrofágy syntetizujúce komplementy C_1–C_9, lyzozým, laktoferín, transferín. Tieto sa zúčastňujú na obrane proti baktériám, vírusom a plesniam, zvlášť v prítomnosti lymfokinázy vylučovanej T lymfocytmi. Okrem T lymfocytov sú v materinskom mlieku prítomné aj lymfocyty B, ktoré okrem lymfokinázy vytvárajú aj interferón (Klen et al., 1987; Barret, 1988).

Lyzozým je hlavným predstaviteľom nešpecifických ochranných látok, spôsobuje bakteriolýzu G^+ a niektorých G^- baktérií (*E. coli, Salmonella*). V zrelom mlieku sa nachádza priemerne 39 mg/100 ml – o dva poriadky viac ako v kravskom mlieku.

Ďalším nešpecifickým ochranným faktorom je laktoferín a transferín. Laktoferín je zvlášť účinný proti G^- mikroorganizmom (*E. coli*), jeho hladina je 100–200 mg na 100 ml, transferínu 5 mg/100 ml (Klen et al., 1987).

Laktoperoxidázový systém (LS) pozostávajúci z laktoperoxidázy, tiokyanátu a H_2O_2 je účinný na G^+ aj G^- baktérie (*Pseudomonas, Salmonella typhimurium, Klebsiella aerogenes*). Zvláštne postavenie v ochrannom systéme dojčiat má tzv. bifidus faktor (*Bifidobacterium bifidus*) determinujúci mikrobiálne osídlenie čreva (anti G^-), chemicky identifikovaný ako N-substituovaný glukózamín. Pri negatívnom pôsobení troch faktorov ovplyvňujúcich rovnováhu črevnej mikroflóry (výživa, lieky, stres) môže tento faktor vyvážiť nežiadúci stav organizmu (Hoover, 1993). Bifidobaktérie sa podieľajú na trávení, ochrannej imunite a inhibícii patogénov (*E. coli, Clostridium, Proteus* a iné baktérie), ktorých produktom metabolizmu sú nežiadúce látky, ako napr. amíny, indol, H_2S alebo fenoly, zapríčiňujúce intestinálne poruchy (Ishibashi, Shimura, 1993).

Druhovo špecifický interferón obsahuje 20 glykoproteínov, ktoré ovplyvňujú rezistenciu proti vírusovým ochoreniam (Záhradnický et al.,1991).

Do skupiny ochranných látok sa zaraďuje aj konglutinín, properdín, niektoré nenasýtené mastné kyseliny, pôsobiace najmä na G^+ baktérie, ale aj na vírusy (*herpes simplex,* chrípky, vírusu japonskej B encefalitídy atď.) a na prvoky *Giardia lambia, Entmoeba histolytica* a *Trichomonas vaginalis* (May, 1988).

Materinské mlieko obsahuje jeden alebo viac komponentov inaktivujúcich HIV-1 vírus. Tieto komponenty nie sú zatiaľ identifikované, ale ich fyzikálne vlastnosti nasvedčujú, že sú príbuzné lipidom (Newburg et al.,1992). Vírus HIV sa prenáša najmä kontaktom „cell -to-cell" v T_4 lymfocytoch a mliekom sa prenáša najmä pri nedostatku antiinfekčných substancií, najmä Ig M (Perre et al., 1993; Buranasin et al., 1993).

Praktický dosah antibakteriálneho, antivírusového a antiparazitárneho pôsobenia ochranných látok prítomných v materinskom mlieku spočíva v jeho vyššej skladovateľnosti pri teplote miestnosti (20 ± 2 °C).

Výsledky našich vlastných experimentálnych skúseností z oblasti mikrobiológie materinského mlieka, vykonaných počas trojmesačného obdobia roku 1994 (celkový počet, nefermentujúce baktérie, koliformné a G^+ baktérie, kvasinky a plesne pri rôznych podmienkach a dynamika rastu *E. coli, S. aureus* a *S. epidermidis* umelo kontaminovaného mlieka) budú uverejnené v samostatnom príspevku.

Prítomnosť mikroorganizmov

Mikroorganizmy prítomné v materinskom mlieku sú zastúpené psychrofilnými aj mezofilnými baktériami. Z mezofilných bol najčastejšie izolovaný *Staphylococcus epidermidis* (47,4 %), *E. coli* (21 %), *Enterobacter* sp. (7,4 %), z psychrofilných *Pseudomonas* sp. (3,4 %) a *Acinetobacter* (11,9 %) (Blahutová, 1991), *Klebsiella, Flavobacterium, Moraxella, Serratia*; okrem nich boli v mlieku zistené aj *Mycobacterium tuberculosis, M. leprae, Salmonella* sp. (Al Mafadsa et al., 1993). Ako uvádzajú Ajusi et al. (1989), neošetrené odstredené mlieko 30 laktujúcich žien obsahovalo baktérie normálnej kožnej flóry, *Staphylococcus albus* (76,3 %), *Streptococcus viridans* (40 %), fakultatívne patogény – *E. coli* (26 %), *Enterococcus faecalis* (13,6 %) a patogénny *Staphylococcus aureus* (6,7 %).

Z vírusov sa vyskytuje cytomegalovírus (May, 1988; Zhang, 1990), *herpes simplex*, rotavírusy, vírus hepatitídy, oncorna vírus typu B, nezanedbateľné sú aj nálezy vírusu HIV-1 v mlieku zatiaľ zdravých nosičiek (Klen et al.,1987).

Chorobný stav môže byť zapríčinený nielen pôsobením mikroorganizmov, ale aj ich toxínmi a enzýmami (enterotoxín *Staphylococcus aureus*, dekarboxylázy *E. coli* a *Enterococcus faecalis* atď.) (Klen et al., 1987).

Kontaminácia materinského mlieka

Materinské mlieko môžu kontaminovať baktérie, vírusy, plesne a kvasinky. Dvorce i bradavky dojčiacich žien sú kontaminované tiež mikroflórou úst a nosnej dutiny dojčených detí. Samotné mlieko môže byť kontaminované infekciou a nasledovným zápalom mliečnej žľazy v dôsledku prítomnosti stafylokokov, streptokokov, *Coxiella burnetti* atď. Podstatný vplyv na rozsah kontaminácie má spôsob a sezónnosť odberu (odstriekavanie mlieka ručne, pumpou, čistenie prsníkov). V súčasnosti existuje vážne riziko prenosu HIV-1 mliekom infikovaných matiek (Perre et al., 1993; Dunn et al., 1992), ktoré je v priamej závislosti od času dojčenia, veku, hmotnosti a pohlavia dojčaťa, klinických podmienok atď.) (Martino et al.,1992).

El Mohandes et al. (1993) zistili, že najčastejšími mikrobiologickými kontaminantami zberového a zmrazeného mlieka je *Staphylococcus epidermidis* a *Acinetobacter* (89 a 9 %) zo 108 vyšetrovaných vzoriek. Výsledky mikrobiologických štúdií v mliečnych bankách poukazujú na výskyt kvasiniek *Candida albicans* (kandidóza), prenosnej z dojčaťa na matku. Preto sa odporúča darcovské mlieko transportovať pri 4–8 °C a uskladňovať pri –20 °C (Blaschke-Hellmensen et al., 1991).

Na mikrobiologické osídlenie mlieka má vplyv aj prítomnosť cudzorodých látok – liekov (acylpyrín, antibiotiká), sociálnych jedov (alkohol, nikotín, kofeín, morfín, a pod.) a ekologických jedov (insekticídy, pesticídy, PCB a i.) (Martino et al., 1992).

Ďalšou skupinou sú ťažké kovy (Pb, Cd, Hg) ako závažné rizikové faktory v dojčeneckej výžive. Okrem vymenovaných základných typov kontaminácie cudzorodými látkami možno spomenúť umelú kontamináciu materinského mlieka, čiže riedenie mlieka vodou, príp. mliekom iných živočíšnych druhov, čo môže mať za následok mikrobiologickú alebo chemickú kontamináciu (Klen et al., 1987).

Zmeny mlieka tepelným ošetrením a skladovaním

Kontaminujúce mikroorganizmy v mlieku môžu byť odstránené pasterizáciou. Tak napr. streptokoky skupiny B, stafylokoky, *Mycobacterium tuberculosis*, *Coxiella burnetti*, vírus Q horúčky, rubeoly, *herpes simplex*, HIV-1 a cytomegalovírus môžu byť inaktivované pri 62,5 °C/30 min, vírus hepatitídy B varom a pasterizáciou sa dekontaminujú aj parazity (May, 1988). Ako uvádza Blahutová (1991), pasterizáciu pri 63 °C/30 min prežíval v 68,4 % skúmaných vzoriek mlieka *Staphylococcus epidermidis*, v 8,5 % *Escherichia coli*, v 6,7 % viridujúce streptokoky, v 5 % *Enterococcus faecalis*, v 3,9 % sporuláty a v 0,5 % *Enterobacter*.

Iným, netradičným spôsobom je použitie mikrovlnného ohrevu, ktorý je účinnejší ako dlhodobá pasterizácia (Sigman et al., 1989).

Konzervácia materinského mlieka sa vykonáva fyzikálne, a to hypotermicky (nad 0 °C tekuté) a kryotermicky pri –22 °C, kedy je zabrzdené pomnožovanie baktérií, kvasiniek, plesní činnosť lipáz.

Štúdiom termického účinku pasterizácie materinského mlieka sa zaoberali viacerí autori. Goldberg (cit. Klen et al.,1987) uvádza, že teplota 62,5 °C/20 min pôsobí letálne na termolabilné mikroorganizmy v prípade, že ich koncentrácia v mlieku nepresahuje 10^6 KTJ/ml (pre stafylokoky len 10^5 KTJ/ml).

Boli tiež robené pokusy s materinským mliekom umelo kontaminovaným *Salmonella typhimurium*, *Staphylococcus aureus*, *Streptococcus haemolyticus*, *Pseudomonas aeruginosa* a polyrezistentným kmeňom *E. coli* v denzitách nepresahujúcich 10^6 KTJ/ml. Po 30-minútovej pasterizácii a dvojmesačnom skladovaní pri –20 °C bolo mlieko dekontaminované. Skladovaním pri 4 °C sa počty uvedených mikroorganizmov signifikantne nezvýšili. Ak však bolo mlieko uskladnené pri 6-8 °C, bolo zistené postupné zvyšovanie počtu mikroorganizmov, ktoré sa prispôsobili nízkym teplotám. Naproti tomu v pasterizovanom mlieku bolo množenie mikroorganizmov rýchlejšie.

Selektívne pôsobenie chladenia sa zvyčajne prejavilo zvýšením počtu nefermentujúcich G^- mikroorganizmov, ako je *Pseudomonas, Alcaligenes* a skupiny *coli-aerogenes*, znehodnocujúcich akosť mlieka. Pri porovnaní účinku zmrazenia sa javil *Achromobacter* odolnejší ako pseudomonády. Po dvoch týždňoch skladovania pri -16 °C klesol počet *S. aureus* o 1,6 %, *E. coli* o 9,7 %, *Streptococcus agalactiae* o 12 % a počet ostatných streptokokov sa znížil o 30,8 %. Po 14 dňoch došlo k opätovnému poklesu o 5 až 20 %. Naproti tomu bol toxín *Clostridium botulinum* produkovaný nielen v chladenom, ale i v mrazenom mlieku.

Deodar a Joshi (1991) testovali 65 vzoriek materinského mlieka pre mliečnu banku. Po zahriatí na 100 °C žiadna vzorka neobsahovala mikroorganizmy, zatiaľ čo po zmrazení vzoriek (5 dní/–20 °C) bol zaznamenaný pokles počtu KTJ/ml vo všetkých vzorkách a žiadna z nich nebola pozitívna na cytomegalovírus. Vírus *herpes simplex* nebol v materinskom mlieku zistený po 10 dňoch skladovania pri –15 °C. Bolo preukázané, že pasterizácia inaktivuje cytomegalovírus a že v neošetrenom mlieku skladovanom pri 4 °C prežíval 1 týždeň (Klen et al., 1987).

Perspektívnou metódou prevencie vertikálneho prenosu vírusu HTLV (lymphotropic T onkovírus spôsobujúci leukémiu) z matky na dieťa sa ukazuje tzv. frozen-and-thawed breast milk (zmrazenie a nasledovné rozmrazenie mlieka). Medzi dojčatami, ktorým dávkovali takto upravené mlieko, nevyskytla sa žiadna infekcia vírusom HTLV (Ando et al., 1989).

Dilema, či materinské mlieko treba tepelne ošetrovať alebo nie, zostane pravdepodobne naďalej nevyriešená, nakoľko spolu s nežiadúcimi mikroorganizmami sa natívne mlieko zbavuje termoinaktiváciou aj významných a nevyhnutných výživových a imunitných vlastností.

Mikrobiologické limity

Keďže surové materinské mlieko ako hlavná zložka výživy je často zodpovedné za ochorenie dojčaťa, je dominantnou úlohou aj legislatívne zabezpečenie požiadaviek jeho primeranej akosti (Voirin et al., 1990).

Pravdepodobne prvým vedeckým podkladom pre legislatívne zakotvenie mikrobiologických požiadaviek bola práca Wilimsona z roku 1978 (cit. Klen et al., 1987), ktorá určila nasledovné podmienky bezpečného spôsobu výživy aj u novorodencov hospitalizovaných na oddeleniach intenzívnej starostlivosti:

– celkový počet mikroorganizmov menej ako $2{,}5.10^3$ KTJ/ml,
– počet *Staphylococcus aureus* menej ako 1.10^2 KTJ/ml,

- neprítomnosť *Enterococcus faecalis*, ani baktérií z čeľade *Enterobacteriaceae* v 1 ml mlieka,
- neprítomnosť podmienene patogénnych mikroorganizmov,
- počet α hemolytických streptokokov menší ako počet *Staphylococcus epidermidis.*

Medzi zástancov používania neošetreného materinského mlieka patria hlavne škandinávske krajiny, kde sa realizuje zber na vysokej profesionálnej úrovni. Doteraz nezodpovedaná je aj otázka, či je patogenita mikroorganizmov matky pre jej vlastné dieťa rovnaká ako pre cudzie (Barret, 1988).

Výsledky mikrobiologických vyšetrení rôznych autorov sa navzájom odlišujú. V našich podmienkach sa akosť materinského mlieka hodnotí podľa ČSN 57 0101 (1963) a Smernice pre mikrobiologické vyšetrovanie požívatín (1992). Uvedené normy hodnotia tiež akosť výrobkov určených pre výživu novorodencov a vychádza sa z nich aj pri hodnotení materinského mlieka, ako aj dojčeneckej a detskej výživy na báze mlieka.

Z uvedeného literárneho prehľadu vyplýva, že problematika mikrobiológie materinského mlieka je stále vysoko aktuálna, a preto má veľký význam v jej výskume naďalej pokračovať.

Literatúra

AJUSI, J. D. – ONYANGO, F. E. – MUTANDA, L. N.:Bacteriology of unheated expressed breast milk stored at room temperature. East. Afr. Med. J., *66*, 1989: 381–387.

AL MAFADSA, S. M. – AL EISSA, Z. A. – SAEED, E. S. – KAMBAL, A. M.: Isolation of Brucella melitensis from human milk. J. Infect, *26*, 1993: 346–348.

ANDO, Y. – KAKIMOTO, K. – TANIGAWA, T. a kol.: Effect freeze-thawing milk on vertical HTLV-I transmission from seropositive mothers to children. Jpn. J. Cancer. Res., *80*, 1989: 405–409.

ATALAY, S. – BALABAN, N. – HELVACI, S.: Bacteriological comparison on colostrum and mature human milk. Microbiol. Bull., *23*,1989:127–132.

BARRET, J. T.: Textbook of Immunology. In: S. BIRCHER, 5, Missouri, C.V. (Eds.): Mosby Company, 1988:455.

BLAHUTOVÁ, M.: Kvalita materského mlieka z hľadiska mikrobiologického vyšetrenia. In: Mikrobiológia a epidemiológia – prednášky Purkyňovej spoločnosti, Martin, Jeseniova lekárska fakulta UK 1991:5.

BLASCHKE-HELLMENSEN, R. – HENKER, J. – FUTSHIK, M.: Result of mycologic studies of donated breast milk.Kinderarztl. Prax., *59*, 1991: 77–80.

BURANASIN, P. – KUNAKORN, M. – PETCHCLAI, B. – RAKSAKAIT, K. a kol.: Detection of HIV type 1 proviral DNA in breast milk and colostrum of seropositive mothers. J. Med. Assoc. Thai., *76*, 1993: 41–45.
DEODAR, L. – JOSHI, S.: Microbiological study of breast milk with special reference to its storage in milk bank. J. Postgrad. Med., *37*, 1991: 14–16.
DUNN, D. T. – NEWELL, M. L. – ADES, A. E. – PECKHAM, C. S.: Risk of human immunodeficiency virus type 1 transmission through breastfeeding. Lancet., *340*, 1992: 585–588.
EL MOHANDES, A. E. – SCHATZ, V. – KEISER, J. F. – JACKSON, B. J.: Bacterial contaminants of collected and frozen human milk used in an intensive care nursery. Am. J. Infect. Control., *21*, 1993: 226–230.
HOOVER, D. G.: Bifidobacteria: Activity and potential benefits. Food Technology, *47*, 1993: 120–124.
ISHIBASHI, N. – SHIMAMURA, S.: Bifidobacteria: Research and development in Japan. Food Technology, *47*, 1993:126–135.
JUNKER, A. K. – THOMAS, E. E. – RADCLIFFE, A. – FORSYTH, R. B. – DAVIDSON, A. G. – RYMO, L.: Epstein-Barr virus shedding in breast milk. Am. J. Med. Sci., *302*, 1991: 220–227.
KLEN, R. – BLATTNÁ, J. – FIEDLEROVÁ, V. – ŠTRBÁK, V.: Ľudské mlieko, jeho zloženie a konzervácia. 1. vyd. Praha, Avicenum 1987: 92.
MARTINO, M. de – TOVO, P. A. – TOZZI, A. Z. – PEZZOTTI, P. – GALLI, L.: HIV-1 transmission through breastmilk. AIDS. Italian Register for HIV Infection in Children, AIDS, *6*, 1992:991–997.
MAY, J. T.: Microbial contaminants and antimicrobial properties of human milk. Microbiological Sciences, *5*, 1988:42–44.
MICHALIČKOVÁ, J.: Aktuálne problémy výživy dojčiat. Výž. a Zdr., 1993: 79–80.
MIKO, M.: Základy výživy. Bratislava, STU 1991: 306.
NEWBURG, D. S. – VISDCIDI, R. P. – RUFF, A. – ZOLKEN, R. H.: A human milk factor inhibits binding of HIV to CD4 receptor. Pediatr. Res., *31*, 1992: 22–28.
NWANKWO, M. U. – OFFOR, E. – OKOLO, A. A. – OMENE, J. A.: Bacterial growth in expressed breast milk. Ann. Trop. Pediatr., *8*, 1988: 92–95.
PERRE, P. van de – LEPAGE, P. – HOMSY, J. – DABIS, F.: Mother-to-infant transmission of HIV by breast milk. Clin. Infect. Dis., *15*, 1992:5 02–507.
PERRE, P. van de – SIMONON, A. – HITIMANA, D. G. – DABIS, F. a kol.: Infective and anti-infective properties of breastmilk from HIV-1 – infected women. Lancet, *341*, 1993: 930–931.
SIGMAN, M. – BURKE, K. I. – SWARNER, O. W. – SHAVLIK, G. W.: Effect of microwaving human milk:changes in IgA content and bacterial count. J. Am. Diet. Assoc., *89*, 1989: 690–692.
ŠVEJCAR, J.: Kojení – dar nejcennější. 2. vyd. Praha, ÚZV 1990: 16.

VOIRIN, J. – LE COUTOR, X. – BOUGLE, D. – MOUHADJER, M. – MICHEL, N. – OBLIN, I.: Development of maternal milk flora during continuous enteral feeding of premature infants. Pediatric, *45*, 1990: 725–729.
ZÁHRADNÍCKY, J. a kol.: Mikrobiológia a epidemiológia I. 4. vyd. Martin, Osveta 1991:609.
ZHANG, M. G.: The cross-section study of CMV isolation in infants urine and mothers breast milk. Chung. Hua. Liu. Hsing. Ping. Hsueh. Tsa. Chih., *11*, 1990: 226–228.
ČSN 57 0101: Mikrobiologické zkoušení mléka a mléčných výrobků. Praha 1963.
Smernica pre mikrobiologické vyšetrovanie požívatín, ČR, 1992.

More recent aspects of microbiological quality evaluation of maternal milk

The paper gives literary survey of newer knowledge from the field of microbiology of maternal milk in abroad and our country. Significance of monitoring of this territory actual situation is accented from the point of health, nutritional, technological and preventive view.

Kontaktní adresa:

Ing. Mária Greifová, CSc., Slovenská technická univerzita, Chemickotechnologická fakulta, Radlinského 9, 812 37 Bratislava, Slovenská republika, tel.: 07/395 260, fax: 07/493 198, e-mail: schmidt@checdel.chft.stuba.sk

Pokyny pro autory vědeckého časopisu Potravinářské vědy

Časopis uveřejňuje původní vědecké práce, krátká sdělení a v rubrice Přehledy kompilační články, tzn. práce, jejichž podkladem je studium literatury a které shrnují nejnovější poznatky v dané oblasti. Práce jsou uveřejňovány v češtině, slovenštině nebo angličtině. Rukopisy musí být doplněny krátkým a rozšířeným souhrnem.

Autor je plně odpovědný za původnost práce a za její věcnou i formální správnost. K práci musí být přiloženo prohlášení autora o tom, že práce nebyla publikována jinde.

O uveřejnění práce rozhoduje redakční rada časopisu, a to se zřetelem k lektorským posudkům, vědeckému významu a přínosu a kvalitě práce.

Rozsah vědeckých prací nemá přesáhnout 10 stran psaných na stroji včetně tabulek, obrázků a grafů. V práci je nutné používat jednotky odpovídající soustavě měrových jednotek SI (ČSN 01 1300). Pokud je text práce zpracován na počítači, je vhodné zaslat do redakce i text na disketě s uvedením použitého editoru.

Vlastní úprava práce

Úprava rukopisu má odpovídat státní normě ČSN 88 0220 (formát A4, 30 řádek na stránku, 60 úhozů na řádku, mezi řádky dvojité mezery). Tabulky, grafy a fotografie se dodávají zvlášť, nepodlepují se. Na všechny přílohy musí být odkazy v textu.

Název práce (titul) nemá přesáhnout 85 úhozů. Je nutné vyvarovat se v něm obecných slov. Jsou vyloučeny podtitulky článků.

Krátký souhrn je informačním výběrem obsahu a závěru článku, nikoliv však jeho pouhým popisem. Musí vyjádřit všechno podstatné, co je obsaženo ve vědecké práci. Nemá překročit rozsah 170 slov. Je třeba, aby byl napsán celými větami, nikoliv heslovitě. Je uveřejňován ve stejném jazyce jako vědecká práce, má obsahovat základní číselné údaje včetně statistických hodnot.

Rozšířený souhrn je uveřejňován v angličtině, měly by v něm být v rozsahu cca dvou strojopisných stran komentovány výsledky práce, uvedeny odkazy na tabulky a obrázky, popř. na nejdůležitější literární citace. Je možné jej dodat česky, resp. slovensky jako podklad pro anglický překlad.

Úvod má obsahovat hlavní důvody, proč byla práce realizována a velmi stručnou formou má být popsán stav studované otázky.

Literární přehled má být krátký, je třeba uvádět pouze citace mající úzký vztah k problému. Doporučuje se co nejnižší počet citovaných autorů.

Metoda se popisuje pouze tehdy, je-li původní, jinak postačuje citovat autora metody a uvádět jen případné odchylky. Ve stejné kapitole se popisuje také pokusný materiál.

Výsledky je vhodnější dokumentovat graficky než tabelárně. V tabulkách je třeba shrnout statistické hodnocení naměřených hodnot. Tato část by neměla obsahovat teoretické závěry ani dedukce, ale pouze faktické nálezy.

Diskuse obsahuje zhodnocení práce, diskutuje se o možných nedostatcích a práce se konfrontuje s výsledky dříve publikovanými (požaduje se citovat jen ty autory, jejichž práce mají k publikované práci bližší vztah). Je přípustné spojení v jednu kapitolu spolu s výsledky.

Literatura musí odpovídat státní normě ČSN 01 0197. Citace se řadí abecedně podle jména prvních autorů. Odkazy na literaturu v textu uvádějí jméno autora a rok vydání. Do seznamu se zařadí jen práce citované v textu. Na práce v seznamu literatury musí být odkaz v textu.

Na zvláštním listě se uvádí **kontaktní adresa**, tj. plné jméno autora, akademické, vědecké a pedagogické tituly, podrobnou adresu pracoviště s PSČ, číslo telefonu, faxu a e-mail. adresu

Pokud autor používá v práci zkratek jakéhokoliv druhu, je nutné, aby byly alespoň jednou vysvětleny (vypsány), aby se předešlo omylům. V názvu práce a v souhrnu je vhodné zkratek nepoužívat.

Instructions for authors

Manuscripts in duplicate should be addressed to: RNDr. Marcela Braunová, Ústav zemědělských a potravinářských informací, Slezská 7, 120 56 Praha 2, Czech Republic.

Manuscript should be typed with a wide margin, double spaced on standard A4 paper. Articles on **floppy disks** are particularly welcome. Please indicate the editor programme used.

Text

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

Tables and Figures

Tables, figures and photos shall be enclosed separately. The text must contain references to all these annexes. Figures should be referred solely to the material essential for documentation and for the understandig of the text. Duplicated documentation of data in figures and tables is not acceptable. All illustrative material must be of publishing quality. Figures cannot be redrawn by the publisher. All figures should be numbered. Photographs should exhibit high contrast. Both line drawings and photographs are referred to as figures. Each figure should contain a concise, descriptive legend.

Offprints: Forty offprints of each paper are supplied free of charge to the author.

Authors have full responsibility for the contents of their papers. The board of editors of this journal will decide on paper publication, with respect to expert opinions, scientific importance, contribution and quality of the paper.

OBSAH – CONTENTS

Vědecký časopis POTRAVINÁŘSKÉ VĚDY ◆ Vydává Česká akademie zemědělských věd – Ústav zemědělských a potravinářských informací, Praha ◆ Redakce: Slezská 7, 120 56 Praha 2, tel.: 02/251 098, fax: 02/257 090, e-mail: braun@uzpi.agrec.cz ◆ Sazba a tisk: ÚZPI Praha ◆

Rozšiřuje Ústav zemědělských a potravinářských informací, referát odbytu, Slezská 7, 120 56 Praha 2