ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

POTRAVINÁŘSKÉ VĚDY

FOOD SCIENCES

2

OČNÍK 14
RAHA 1996
S 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.

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Potrav. Vědy, *14*, 1996 (2) : 81–89

COMPOSITION OF ESSENTIAL OILS IN HOPS AND HOP PRODUCTS

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Abstract: Hop essential oil constituents identification significantly contributes to the individual hop variety and hop product identification. The applied method consists of hop oil isolation using steam distillation and extraction, hop oil isolate separation into fractions followed by GC-MS under selected conditions. For hop oil separation into fractions, silica gel column chromatography was used. The results were evaluated by means of the principal component analysis. The GC-MS analysis of individual hop oil fractions proved a good separation power of the chromatografic arrangement used. The ratios of some substances, such as monoterpenes/sesquiterpenes, ß-farnesene/α-humulene, ß-farnesene/myrcene ratio were found to be of certain significance for the individual samples characterization.

hop essential oil; GC-MS analysis; principal component analysis

Hops and hop products are one of the basic sources in brewing technology. The most important hop constituents are hop resins and hop essential oils. The hop aroma of beer is influenced mostly by hop oil. The identification of hop essential oils contributes to the characterization of hop varieties and hop products. Using it's results it is possible to assess the oxidation degree and consequently the age of analysed hops or mode of its storage, in some cases even the way of hop processing. The impact of essential oil content on beer flavour can also be partially explained.

Hop essential oils can be separated from hop using solvent extraction (Lam et al., 1986; Stenroos et al., 1976) or headspace techniques (Murakami, 1989), which have the advantage of causing no thermal destruction and no hydrolysis of hop oils (Pickett et al., 1975). The used solvent must be, however, concentrated by evaporation and nonvolatile substances must be separated. Substances with higher boiling point than that of α-humulene cannot be usually detected using headspace analysis (Murakami,

1989). These comprise most of the oxidation products. Sufficient hop oil yields neccessary for their subsequent separation and identification can be obtained using steam distillation (Maier, 1988). This method, however, takes a lot of time (usually 4 hrs), but there exists a faster modification, which takes 1 hr (steam distillation) or 10 min (steam extraction), and which provides a good hop oil yields (Green et al., 1993).

Multivariation statistical analysis is available for evaluation of a large data collection (Meloun, 1994), e.g. the hop essential oil composition. This statistical method can simplify the relationships within the data collection. Novel methodology for unambiguous variety identification was developed. Bitter acids, hop essential oil and hop polyphenols were investigated. Principal component analysis (PCA) of both hop oil and hop polyphenols leads to an unambiguous hop variety identification (Keukeleire et al., 1994).

MATERIALS and METHODS

Hop Oil Isolation from Sample

100 g of natural hop was placed in a distlillation flask (4 l volume) together with 2 l of distilled water. The mixture was refluxed for 4 hrs. Separated hop oil was trapped into a collimator. Hop oil was spilled into a separatory funnel after the steam distillation and extracted with 30 ml of diethyl ether. The top organic phase was spilled into Erlenmayer flask and desiccated using anhydrous sodium sulphate. Then sodium sulphate was filtered out. The organic solvent was removed from samples by evaporatoration for 30 min. After weighing the sample was ready for chromatography.

Hop Essential Oil Separation into Individual Fractions

A 40 cm long glass column was filled with suspended silica gel (mesh 0.1 mm) together with n-pentane to form a 25 cm high layer. A mixture (2 ml) of isolated hop oil and n-pentane at a ratio 1 : 1 was injected onto the silica gel column, which was then filled with n-pentane. The hydrocarbon fraction of hop oil was obtained by using 80 ml of n-pentane for elution, then the oxidized fraction was obtained by using 80 ml of diethyl ether. The organic solvent was removed from both fractions by evaporatoration for 30 min. Fresh samples were then analysed by GC-MS.

Identification of Hop Essential Oil Constituents using Gas Chromatography

A gas chromatograph connected to a mass spectrometer was used for analysis of all samples.

Chromatographic Conditions

- C-MS system DX-303 Jeol (Japan) with data-station DA-5000
- column: SPB-5, Supelco (U.S.A.); stationary phase: 5% phenyl- and 95% methyl silicon; column length: 50 m; inner diameter: 0.32 mm; stationary phase film thickness: 1 μm
- injector temperature: 240 °C
- temperature program: initial temperature: 50 °C; then 4 °C/min gradient to 280 °C
- carrier gas: helium

Mass spectrometric Conditions

- column connected directly to an ion source at a temperature of 250 °C
- electron impact ionization process
- electron energy 70 eV

I. The list of analyzed samples

Sample number	Hop variety sign	Sample number	Hop variety sign
1	hybrid 4332	14	clone 72 (1993)
2	hybrid 4353	15	bitter hop hybrid
3	hybrid 4362	16	bitter hop hybrid
4	hybrid 4527	17	bitter hop hybrid
5	clone 72 (1994)	18	bitter hop hybrid
6	hybrid 4382	19	clone 72 (1993)
7	clone 72 (meristem)	20	clone 31 (1994)
8	hybrid 4240	21	Sířem variety (1994)
9	hybrid 3911	22	clone 72 (1994)
10	clone 72 (1994)	23	Sládek variety (1994)
11	bitter hop hybrid	24	Zlatan variety(1994)
12	bitter hop hybrid	25	Neo hop extract
13	bitter hop hybrid	26	Barth hop extract

Statistical Evaluation of Results

The results were evaluated by means of a principal component analysis. The principal component is a linear combination of the original variable (individual hop oil content in this case). Correlation among variables is a result of PCA and is determined by the so-called loadings of each variable in relationship to a relevant principal component. The more similar the loading values, the higher the positive correlation between the variables was observed. If the loading values have a different sign, the correlation between the variables is negative. When loading is raised to a second degree of magnitude, communality is obtained. The sum of all communalities for a definite principal component is called an eigenvalue, which determines the percentage of the total variance in the relevant principal component:

$$\text{percentage} = 100 \times \text{eigenvalue} / \text{number of variables}$$

RESULTS AND DISCUSSION

Hop Essential Oil Separation into Individual Fraction

Two samples (Nos. 25 and 26) were separated into a hydrocarbon fraction and into the oxidized one and identified by GC-MS.

The GC-MS analysis of the individual essential oil fractions proved a good separation power of the chromatographic arrangement used. Most of the nonpolar substances were eluted with n-pentane, whilst most of the polar (oxidized) ones were eluted with diethyl ether. There was one exception found in the case of a few oxidized sesquiterpenes overlapping into both fractions (Table II).

The sum of compounds found in both the polar and nonpolar fractions was found to be in a very good correlation with the total GC-MS analysis of the unseparated hop oil isolate.

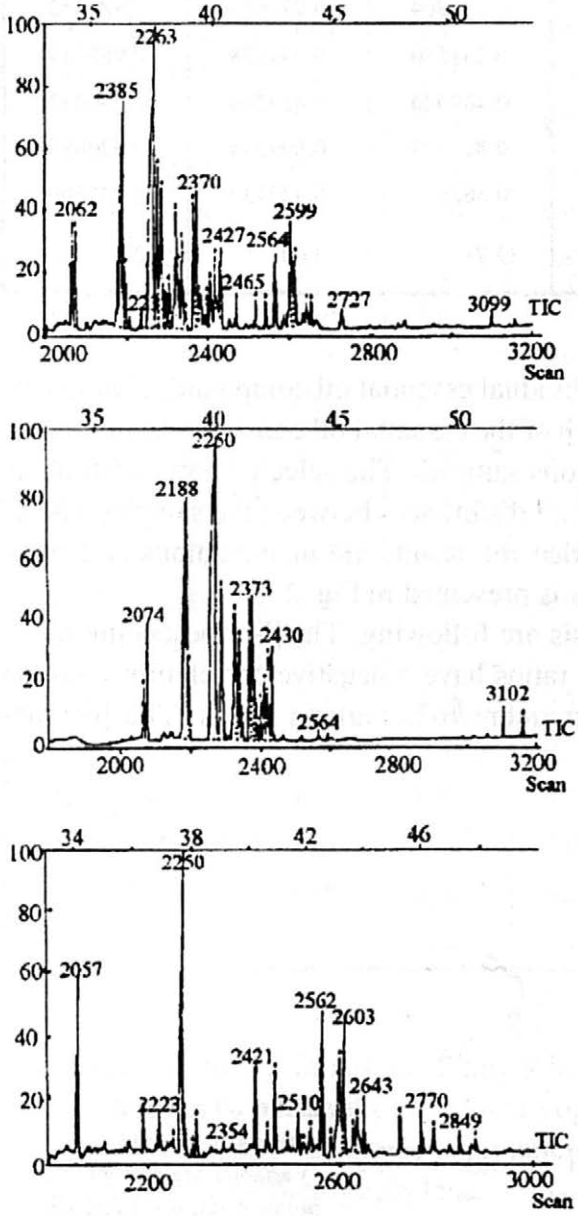
II. The number of substances identified in individual fractions of samples No.25 and 26

A sign of sample	No. 25	No. 26
Number of substances in hydrocarbon fraction	29	28
Number of substances in oxidized fraction	58	58
Number of substances in both fractions	10	3

The chromatogram of samle No. 26 is demonstrated in Fig 1 in comparison with the individual fractions.

Statistical Evaluation of Essential Oil Composition

The aim of PCA analysis of 24 natural hops was the determination of correlation of the individual properties (variables in PCA) of hops, which were



A. GC-chromatogram of the total hop essential oil
B. GC-chromatogram of the hydrocarbon fraction
C. GC-chromatogram of the oxidized fraction

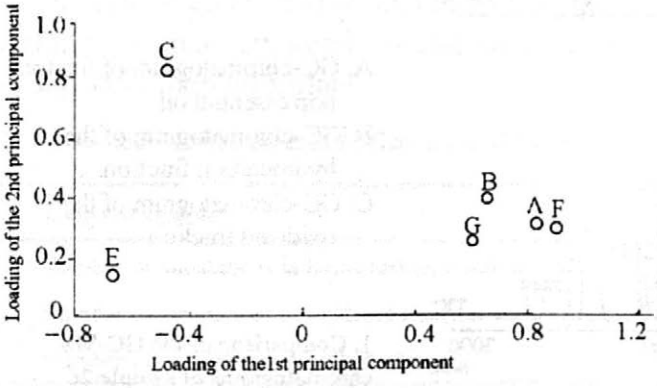
1. Comparison of the GC-MS chromatograms of sample 26

III. PCA analysis. Communalities of original hop variable (hop oil constitution)

Selected peak area ratios	Symbol in graph	Communality from the component		
		1st	2nd	3rd
Myrcene/α-humulene	A	0.691263	0.789478	0.970467
Myrcene/β-caryophylene	B	0.429697	0.588948	0.928932
β-farnesene/α-caryophylene	C	0.245804	0.973619	0.984452
β-farnesene/α-humulene	D	0.238590	0.984638	0.985219
β-farnesene/myrcene	E	0.469040	0.489200	0.730071
Monoterpenes/sesquiterpenes	F	0.811379	0.899244	0.920683
2-undecanone/α-humulene	G	0.362625	0.427137	0.944509
Percent of the explained total data variance	–	39.71	73.60	92.35

represented by ratios of some individual essential oil compounds (Table III). The effort was to determine which of the essential oil constituents are important for the characterization of hops samples. The selected hop constituents and their ratios did not comprise all differences between the samples, therefore it is not possible to assert that the results are unambiguous and complete. The graph of PCA analysis is presented in Fig. 2.

Conclusions from PCA analysis are following: The β-farnesene/myrcene and β-farnesene/β-caryophylene ratios have a negative correlation with the monoterpene/sesqiterpene and myrcene/α-humulene ratios. The β-farne-



2. Hop oil principal component analysis (Variables are presented as points A–G, see Table III)

IV. Quantitative individual samples essential oil analysis used as a base for PCA analysis

Sample	A	B	C	D	E	F	G
1	4.30	1.31	0.03	0.10	0.02	0.68	0.56
2	0.84	1.85	0.02	0.01	0.01	0.33	0.06
3	0.40	0.64	0.02	0.01	0.03	0.19	0.06
4	0.54	1.34	0.00	0.00	0.00	0.22	0.03
5	0.72	1.93	1.69	0.63	0.87	0.33	0.08
6	0.72	1.48	0.02	0.01	0.01	0.41	0.02
7	0.80	1.94	1.74	0.72	0.90	0.35	0.07
8	0.32	0.87	0.04	0.02	0.05	0.20	0.03
9	0.56	1.26	0.04	0.02	0.03	0.35	0.02
10	0.77	1.91	1.67	0.67	0.87	0.35	0.03
11	0.87	1.53	0.07	0.04	0.05	0.42	0.21
12	0.87	1.71	1.37	0.69	0.80	0.38	0.11
13	0.41	1.11	0.00	0.00	0.00	0.16	0.16
14	0.56	1.28	1.46	0.64	1.14	0.25	0.12
15	1.21	1.73	0.00	0.00	0.00	0.52	0.14
16	0.79	1.73	0.00	0.00	0.00	0.32	0.09
17	1.51	2.66	0.07	0.03	0.03	0.72	0.06
18	0.22	0.47	1.65	0.77	3.51	0.08	0.09
19	0.05	0.10	1.08	0.53	10.47	0.02	0.08
20	0.20	0.47	0.33	0.14	0.70	0.10	0.05
21	0.31	0.70	0.85	0.37	1.21	0.15	0.08
22	0.02	0.05	0.26	0.10	5.06	0.01	0.05
23	0.37	0.67	0.02	0.01	0.03	0.20	0.08
24	0.39	0.93	0.96	0.41	1.04	0.20	0.08

sene/myrcene and β-farnesene/β-caryophylene ratios have a weaker negative correlation with the myrcene/β-caryophylene and 2-undecanone/α-humulene ratios. The β-farnesene/α-humulene ratio correlates positively with that of β-farnesene/β-caryophylene.

As shown in Fig. 2 myrcene/β-caryophylene, myrcene/α-humulene, monoterpene/sesquiterpene and 2-undecanone/β-humulene ratios create a group of points, and these positively correlate with each other.

It seems that the content of β-farnesene, β-caryophylene, β-humulene, myrcene (the most abundant in hop oils) as well as the content of 2-undecanone are important for hop variety characterization.

For example, the 1st principal component explains the total variance very well in the case of the monoterpene/sesquiterpene ratio as shown in Table III (communality = 0.811379, i.e. about 81% of the total variance). The 2nd component explains e.g. the total variance of data in the case of the β-farnesene/β-caryophylene and β-farnesene/α-humulene ratios.

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Received November 23, 1995

Složení chmelových silic ve chmelu a chmelových výrobcích

Identifikace složek chmelových silic významně přispívá k identifikaci jednotlivých chmelových odrůd a chmelových produktů. Použitá metoda představuje izolaci chmelových silic destilací s vodní parou a extrakcí diethyletherem, dále separaci izolátu chmelových silic na jednotlivé frakce a následnou chromatografickou analý-

zu v systému GC-MS. Pro dělení silic na frakce bylo použito skleněné kolony naplněné silikagelem. Výsledky byly vyhodnoceny pomocí analýzy hlavních komponent. GC-MS analýza jednotlivých frakcí chmelových silic prokázala dobrou separační schopnost použité chromatografické sestavy. Značný význam pro charakterizaci jednotlivých vzorků mají poměry některých složek chmelových silic, zejména poměr monoterpeny/sesqiterpeny, ß-farnesen/α-humulen, ß-farnesen/myrcen a další.

chmelové silice; GC-MS analýzy; analýzy hlavních komponent

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RECENZE

Natural Toxic Compounds of Food Formation and Change during Processing and Storage

Přírodní toxické složky potravin Tvorba a změny během zpracování a skladování

J. Davídek (Ed.), Boca Raton, Ann Arbor, London, Tokyo, CRC Press 1995, 268 s.

Osmnáctičlenný autorský tým pod vedením prof. J. Davídka, DrSc., zpracoval mimořádně aktuální téma. V době, kdy stoupá ekologické uvědomění veřejnosti a tím také zájem o vliv toxických faktorů životního prostředí na kvalitu potravin, je velmi záslužné ukázat na problematiku přírodních toxických složek potravin, které se mohou někdy uplatnit významněji než toxické vlivy prostředí.

Kniha má dva oddíly, z nichž první je věnován vlastním přírodním toxickým látkám, které se tvoří během zpracování a skladování potravin.

Úvodní kapitola prvního oddílu podává přehled o složkách, které vyvolávají potravinovou intoleranci (P. Rauch, H. Rauchová). V samostatných částech jsou probírány jednak imunogenní složky (potravinové alergeny) především kravského mléka, zeleniny a obilovin, jednak ostatní složky (s neimunologickou odezvou), přičemž je především diskutována intolerance k laktóze a favismus.

Druhá kapitola je rozdělena na 11 částí a je věnována hlavním skupinám rostlinných i živočišných toxinů. Jedná se hlavně o alkaloidy (J. Velíšek, J. Hajšlová), saponiny (J. Velíšek), kyanogeny (M. Voldřich), glukosinoláty (J. Velíšek), rostlinné fenoly (J. Dostálová, J. Pokorný), lektiny – hemaglutininy (P. Kalač), toxické aminy a lathyrogeny (P. Kalač, J. Davídek), biogenní aminy (T. Davídek, J. Davídek), toxiny mořských živočichů (J. Dostálová, J. Pokorný), toxiny hub (J. Hajšlová), další toxické sloučeniny – především toxické prvky, fytáty, oxaláty, nitráty, nitrity (R. Koplík, J. Prugar, J. Davídek).

Každá probíraná skupina toxinů obsahuje nejen popis chemických vlastností, ale i toxických účinků, výskytu a jejich změn v průběhu zpracování a skladování příslušné potraviny.

Ve druhém oddíle publikace autoři podávají systematický přehled toxických a antinutričních látek, které se tvoří během zpracování a skladování potravin.

Samostatná kapitola je věnována toxickým látkám, které vznikají působením mikroorganismů (A. Príbela, T. Šinková), a to jednak bakteriálním toxinům, jednak mykotoxinům.

Toxické a antinutriční látky vznikající vlivem fyzikálních faktorů a chemickými reakcemi jsou rozvedeny v další kapitole, a to podle tří hlavních složek potravin, z kterých mohou vycházet: látky vznikající z proteinů (J. Pánek, J. Davídek, Z. Jehličková), ze sacharidů (J. Velíšek) a z lipidů (J. Pokorný, J. Velíšek).

V obou uvedených kapitolách se autoři neomezují pouze na mechanismus vzniku a na chemické vlastnosti popisovaných nežádoucích látek, ale diskutují i příslušná zdravotní rizika a možnosti jejich minimalizace.

Totéž platí i o poslední kapitole, která je věnována toxickým látkám vznikajícím vzájemnou reakcí mezi složkami potravin a potravinářskými aditivy, jmenovitě nitrososloučeninám (J. Čmolík, V. Kellner) a etylkarbamátu (J. Velíšek).

Početnému autorskému kolektivu se podařilo vytvořit na poměrně nevelkém stránkovém rozsahu obsažné dílo s vyváženým uspořádáním jednotlivých částí, které poskytuje skutečně komplexní pohled na uvedený problém.

Kniha bude nesporným přínosem pro široké spektrum odborníků zabývajících se z nejrůznějších hledisek kvalitou potravin.

Prof. Ing. Dušan Čurda, CSc.

Potrav. Vědy, *14*, 1996 (2) : 91–98

LOWER ORGANIC MONO-, DI- AND TRICARBOXYLIC ACIDS IN WINE

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Abstract: For the determination of lower organic acids in wine, the HPLC method on a column filled with ionex OSTION LGKS 0800 in an H^+ cycle was used. Organic acid content in Bohemian and Moravian wines was studied. Both the total acidity and the particular acid contents were compared. Generally, the Moravian wines were more acidic than the Bohemian ones, which was caused by the higher contents of tartaric and lactic acids in the Moravian wines. On the other hand the Bohemian wines showed a higher content of malic acid. The total acidity was also compared with respect to different kinds of wine.

carboxylic acids; wine; HPLC

Lower organic acids of wine are formed by biochemical processes in grapes or by the effect of microorganisms during fermentation.Tartaric and malic acids are the two major acids in grape juice and in wine. Organic acid concentrations change during fermentation and the aging of wine, and also other acids which were not contained in the grape juice originally can be formed, such as lactic or succinic acid. Citric and acetic acids belong to other important organic acids in wine, as well as fumaric and ascorbic acids in minor amounts (Linskens, Jackson, 1988).

Tartaric acid is one of the most important organic acids in wine. High temperatures and a lot of sunshine during vegetation are necessary for its formation. It is formed probably from ascorbic acid in the young berries. The concentration of tartaric acid is reduced after fermentation due to the wine stone formation. Average concentrations of tartaric acid in wine vary from 1.5 to 5.0 g/l (Linskens, Jackson, 1988).

The second most important organic acid in wine is malic acid. Its concentration is reduced during grape aging. It is caused by its low stability and by its participation in respiration and metabolism. This acid is degraded into pyruvate, which, according to Rodopulo (Bujdoš, 1985), is then utilized

for the synthesis of other organic acids and carbohydrates. In wine it can be reduced to lactic acid by lactic bacteria. It is clear that only the homofermentative process is desirable. A heterofermentative process is not favourable because of acetic acid formation. The malic acid content in wine varies from 0.5 to 5.0 g/l (Linskens, Jackson, 1988). Higher concentrations of malic acid are unpleasant from the sensoric point of view giving wine a too rough character.

Lactic acid in wine occurs in its L-form mainly and is produced by bacteria (Linskens, Jackson, 1988). Its concentration in the grapes is very low, growing in the young wine, because this acid is formed from sugars as a by-product in alcohol fermentation. The greatest part of lactic acid is however formed in malolactic fermentation. Average content of lactic acid in wine varies from 0.5 to 5.0 g/l . Lactic acid has a nice, mild acidic character, and so it refines wine's acidity.

Citric acid occurs in grapes in small amounts only. It can be added to the sparkling wines because it forms complex systems with iron and thus prevents the metal haze formation. At the same time citric acid improves the character of wines that have a low total acidity. The content of citric acid in wine varies from 0.2 to 0.8 g/l, especially wines from botrytic grapes contain higher concentrations of this acid.

Other organic acids are also present in wine, especially acetic and succinic acids. These acids are formed mainly in wine, they are present in wine only in small quantities. Acetic acid can be formed in the case when the yeasts are under stress conditions, or it is formed by the action of acetic and lactic bacteria (Linskens, Jackson, 1988). It is produced by aerobic ethanol oxidation by the effect of the strain *Acetobacter*. *Lactobacillus* and *Leuconostoc* produce this acid from sugars or from citric acid. Succinic acid gets into wine as a secondary product of alcoholic fermentation (Schmitthenner, 1987). The average concentrations of acetic acid in wine are from 0.3 to 0.6 g/l and the content of succinic acid varies from 0.5 to 1.5 g/l. A higher content of acetic acid in wine is not favourable from the sensoric point of view because this acid gives wine an unpleasantly rough acidity.

All organic acids are very important for the wine character. They affect the taste and stability of wine. The total acidity is very important for protecting wine against microbiological contaminants (Minárik, 1985). Acidity also influences the course of grape juice fermentation, the rate of oxidation pro-

cesses, the solubility of tartatic acid and of its salts. Also the harvest time can be determined according to the acidity level. Another possibility how to use acidity determination is acidification and the deacidification of grape juice or wine.

Acidity determination in practice is often carried out by potentiometric titration (Linskens, Jackson, 1988), HPLC (Heidger, 1990; Marcé, Calull, 1990; Kupina et al., 1991; Fuleki et al., 1993) and capillary isotachophoresis (Kanianaký, 1990; Karovičová et al., 1990) are other modern methods used for this determination.

MATERIAL AND METHODS

Equipment

Separation of organic acids was carried out on a liquid chromatograph HPP 5001 connected with a dosing vent with a loop LCI 30 (Laboratorní přístroje, Prague). UV detector LCD 2082 (ECOM, Prague) was used for the determination. Measurement was carried out at a wavelength of 206 nm. Separation proceeded in a steel column 25 x 0.8 cm filled with ionex Ostion LGKS 0800 in an H^+ cycle (Tessek, Prague). The column was tempered to 55 °C in a glass case. An integrator Apex (DataApex, Prague) was used for the evaluation of results.

Chromatographic Conditions

The mobile phase was 0.005 M sulphuric acid. The separation was carried out by isocratic elution with a flow rate of 0.5 ml/min and the temperature was held constant at 55 °C. All samples were filtered through an acetate-cellulose filter (0.2 µm, Sartorius AG, Goettingen, Germany) before injection.

Standards

The standard solutions of the acids were prepared by dissolving the major carboxylic acids frequently found in wine (tartaric, malic, lactic, citric and acetic acid) (Lachema, Brno) in water to provide the concentration from 0.5 to 2.0 g/l. Injection volume was 20 µl.

Samples

21 different kinds of wine from wineries Víno Mikulov, Bohemia Sekt (Starý Plzenec) and some small-scale producers (Mělník wine region) were analysed. All wines originated from the vintage 1994.

Víno Mikulov – Welsch Riesling, Rhine Riesling, Müller-Thurgau, Pinot blanc, Gewürztraminer, Veltliner, Neuburger.

Bohemia Sekt (Starý Plzenec) – Welsch Riesling, Müller-Thurgau, Veltliner (2 samples), other wines imported from Romania and Austria.

Small-scale producers (Mělník) – Welsch Riesling (3 samples), Rhine Riesling, Müller-Thurgau, Pinot blanc, Veltliner (2 samples).

RESULTS AND DISCUSSION

High performance liquid chromatography enabled a simple determination of organic acids in wine. In our case it was anion-exchange chromatography. It is very accurate, rapid (time of one analysis was less than 20 minutes) and simple from the sample treatment point of view. The samples of white wines were filtered through a microfilter before injection.

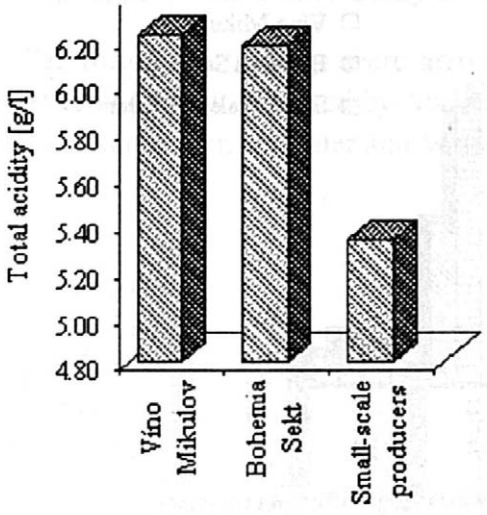
Different varietes of wines and wines from several producers were evaluated. The analyses showed that the organic acid spectra in the respective wines differ a lot. The results were compared from different points of view.

Comparison of the Content of all Determined Acids with respect to Different Producers

For the simplicity, the content of all determined acids will be designated as the total acidity. We should also include succinic acid into the total acidity, but we could not determine this acid by our method because of interference of an unknown substance with the succinic acid peak.

I. Average content of lower organic acids in wines from different producers

Wine	Content of particular organic acids [g/l]					
	citric	tartaric	malic	lactic	acetic	total
Víno Mikulov	0.29	2.50	1.29	1.91	0.23	6.22
Bohemia Sekt	0.51	1.71	1.91	1.76	0.30	6.17
Small-scale producers	0.42	1.59	2.10	1.05	0.17	5.33



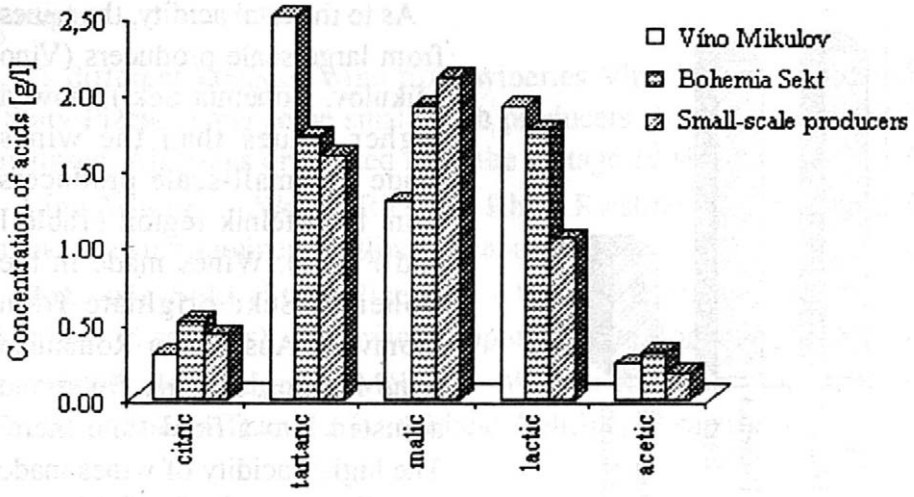
1. Average total acidity in wines from different producers

As to the total acidity, the wines from large-scale producers (Víno Mikulov, Bohemia Sekt) showed higher values than the wines made by small-scale producers from the Mělník region (Table I and Fig. 1). Wines made in the Bohemia Sekt originate from Moravian, Austrian or Romanian wine regions, and are fined and adjusted into a final state there. The higher acidity of wines made in Mikulov or in Starý Plzenec was caused mainly by a higher content of tartaric and lactic acids. The average concentration of tartaric acid in wines from Mikulov was 2.50 g/l, while wines made in the Mělník region contain only 1.59 g/l of this acid, on the average. The higher content of tartaric acid was caused by a warmer climate in the southern wine regions than in the northern Mělník region most probably.

Comparison of Individual Acid Contents with respect to the Different Producers

As we can see in Fig. 2, the highest content of tartaric acid was observed in wines from Mikulov, followed by wines from Starý Plzenec (wine made in Starý Plzenec were delivered there from Moravia, Austria or Romania), and the least concentration was measured in wines made in the Mělník region.

The malic acid content in the analysed wines differed a lot in our case. Among others, these variations were also caused by the fact that malolactic fermentation had not been finished in all wines yet. It showed that the wines from Mikulov had a lower content of malic acid than the wines made in the Starý Plzenec and Mělník region. The concentration of tartaric acid was in relationship with the malic acid content. The Bohemian wines showed higher concentrations of malic acid than the Moravian wines but at the same time the Bohemian wines contained a lower concentration of tartaric acid. The content of tartaric acid was at inverse ratio to the content of malic acid.



2. Average content of individual organic acids in wines from different producers

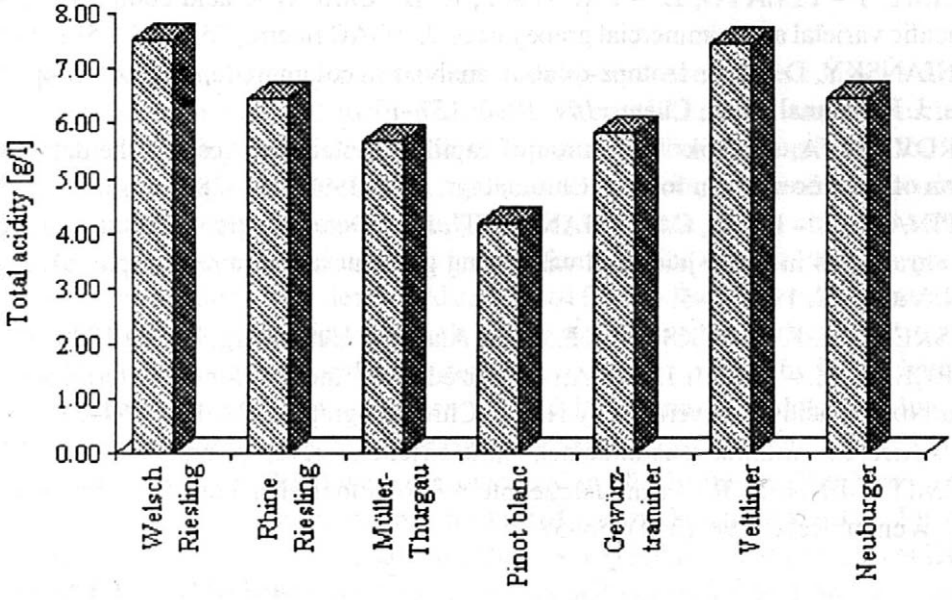
The relation between the concentration of malic and lactic acids was also studied in our experiments. After malolactic fermentation the concentration of lactic acid grows at the expense of the malic acid concentration. It is very favourable for the mild character of wine. The highest concentrations of lactic acid were found in the wines from Mikulov, the lowest ones in the wines made in the Mělník region.

II. Content of particular organic acids in wines made in Mikulov

Wine	Organic acids content (g/l)					
	citric	tartaric	malic	lactic	acetic	total acidity
Welsch Riesling	0.34	2.63	2.07	2.19	0.28	7.51
Rhine Riesling	0.26	2.24	1.77	2.01	0.17	6.45
Müller-Thurgau	0.17	1.60	0.70	2.98	0.24	5.67
Pinot blanc	0.02	1.97	0.46	1.31	0.41	4.17
Gewürztraminer	0.14	2.06	1.00	2.43	0.19	5.82
Veltliner	0.44	4.88	1.03	0.97	0.12	7.44
Neuburger	0.65	2.15	1.98	1.47	0.21	6.46
Average content	0.29	2.50	1.29	1.91	0.23	6.22

Comparison of the Total Acidity in Different Wine Varieties

The following findings were arrived at: Variety Pinot blanc showed the least total acidity, followed by Müller-Thurgau, Gewürztraminer, Rhine Riesling, Neuburger, Veltliner and Welsch Riesling (Table II and Fig. 3).



3. Total acidity of different wine varieties made in Mikulov

Although the absolute concentrations of each acid or the total acidity are of a great significance for the wine quality, the mutual ratio of all acids as well as of the other compounds contained in wine are more essential.

Comparison of the Total Acidity of Wines with respect to the Wine Regions

The varieties Welsch Riesling, Rhine Riesling and Veltliner were more acidic in the Moravian regions, the variety Müller-Thurgau showed a higher acidity in the Bohemian regions. Pinot blanc was of the same acidity in both regions, Moravian and Bohemian. The higher total acidity in the Moravian wines was caused by the higher content of tartaric and lactic acids mostly, in the Bohemian wines it was caused by malic acid. The contents of other acids differed only slightly.

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Received November 23, 1995

Nižší organické kyseliny v českých a moravských vínech

Ke stanovení základních organických kyselin v českých a moravských vínech byla použita metoda HPLC na koloně naplněné ionexem OSTION LGKS 0800 v H^+ cyklu. Byla srovnávána jak celková kyselost, tak obsah jednotlivých kyselin. Moravská vína vykazovala v průměru vyšší hodnoty kyselosti než vína česká, přičemž zvýšená kyselost byla způsobena především vyšším obsahem kyseliny vinné a mléčné. Na druhé straně česká vína obsahovala více kyseliny jablečné. Byla také porovnávána kyselost vín z hlediska různých odrůd.

organické kyseliny; víno; HPLC

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Potrav. Vědy, *14*, 1996 (2) : 99–108

OCCURRENCE OF PHTHALIC ACID ESTERS IN FOOD PACKAGING MATERIALS

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Abstract: Food packaging materials coming into immediate contact with the contents were analysed for phthalic acid esters (PAEs).The samples included retail packaging materials (plastic foil, paper, cardboard, aluminium foil with colour printing) used for various foods (sweets, wafers, meat and milk products, frozen foods, vegetables, dry ready-to-cook products, potato chips). The samples were collected in retail shops in Brno from June to August 1994. Simple and sensitive methods were developed and tested for the detection and determination of PAEs in the packaging materials. PAEs were extracted with chloroform or a 1 : 1 mixture of hexane and dichloromethane, and determined by HPLC with UV detection (224 nm) and a Separon SGX C 18 column. The detection limit for PAEs was 0.1 μg/g of the material. Di-2-ethylhexyl phthalate (DEHP) and dibutyl phthalate (DBP) were demonstrated in all 42 samples. Their concentrations are expressed in μg per one packing if its weight was lower than 2 g, or in μg per g in larger packings (salamis, cheeses, vegetables). The contents of DEHP and DBP varied between 57 and 3 097 μg and between 6 to 2 349 μg, respectively. The results are confronted with data on PAEs in packings and their migration into foods published abroad. A variability in the occurrence and concentrations of DEHP and DBP in retail packaging materials coming into direct contact with the food was apparent. The contamination of foods by PAEs can be effectively prevented by checks of food packaging materials.

phthalic acid esters; food packaging materials; analytical methods

Phthalic acid esters (PAEs) are widely used as plasticizers in the production of plastics (particularly PVC), as well as components of printing inks to improve the adhesion, flexibility and resistance of the printing. Di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), butylbenzyl phthalate (BBP), diethyl phthalate (DEP), and dicyclohexyl phthalate (DCHP) are most frequently used as the plasticizers. Phthalates are not bound chemically in the plastics and can consequently penetrate the materials, be leached into liquid environments and migrate from the outer printed side through the ma-

terial into the food. The presence of PAEs in packaging materials and their migration into packed foods have been confirmed by a number of authors (Castle et al., 1988; Page, Lacroix, 1988; Petersen, 1991; Nerín et al., 1993). The concentration of PAE in packed foods depends on many factors including the concentration of PAE in the material and printing, the powder consistence of the contents, the storage period and temperature, the fat content in the food, the contact area, etc. (Castle et al., 1988; Nerín et al., 1993).

Six PAEs have been included into the list of the most hazardous pollutants owing to their harmful effects on live organisms. Owing to the migration of toxic PAEs into packed foods, the phthalate-plasticized packaging materials (PVC) have been replaced by other polymers, such as polyethylene, oriented polypropylene or regenerated cellulose. Plasticizers are unlikely to be associated with the latter materials. Food manufacturers and processors order from many suppliers packings with various types of colour printings that can be subsequently modified and/or completed using various printing inks.

The monitoring of hazardous pollutants of the food chain does not yet include quality checks of food packaging materials that can become a significant source of food contamination. This paper describes methods of analysis for PAEs of packaging materials coming into direct contact with foods marketed in the Czech Republic.

MATERIALS AND METHODS

Materials

Packaging materials for the analysis of PAEs were collected in retail shops in Brno from June to August 1994. Foods preferred by children and packed into various types of printed materials were chosen (polypropylene-, polyamide- or polyvinilidenchloride – based materials, aluminium foil, paper). The packagings were cleaned before the analysis using methods dependent on their types and on the content of the packing (rinsing with water and drying, cleaning with cotton-wool soaked with ether, etc.).

Analysis

The method is suitable for the detection and determination of PAEs in plastic and other materials including printing inks.

Chemicals

Analytical standards: di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), butylbenzyl phthalate (BBP), diethyl phthalate (DEP). and dimethyl phthalate (DMP) were supplied by SUPELCO, Inc. (U.S.A.). Stock and working solutions were diluted with hexane and kept in a refrigerator.

Chloroform, acetone and dichloromethane were pesticide grade, n-hexane and methanol were LC grade.

Cleaning of glass and chemicals

Flasks and test tubes with ground stoppers were washed with water, heated in an oven at 390 °C for 4 hours and rinsed with acetone before use. Pipettes were thoroughly rinsed with acetone. Glass wool was extracted with chloroform. All organic solvents were distilled in a glass apparatus before use.

Instruments

Liquid chromatograph Varian 5000 (Varian, U.S.A.); injection loop 10 µl, Rheodyne 7125 (U.S.A.); chromatographic column Separon SGX C 18, grain size 5 µm (Tessek, Czech Republic); diode array detector Polychrom 9060 (Varian, U.S.A.); database CSW Version 1.0 (DataApex, Czech Republic); gas chromatograph Varian 3400 (Varian, U.S.A.); mass spectrometer Model Tracker (Finnigan MAT, U.S.A.); rotation vacuum evaporator.

Qualitative detection of PAEs

A sample (approximately 0.05 g) was dried in an oven at 70 °C for 40 min. The sample was weighed after cooling in a desiccator and put into a 100-ml flask. PAEs were eluted with chloroform at room temperature for 12 h. The content was shaken occasionally and the flask was put into a mechanical shaker for the last 30 min. The rest of the sample was taken out of the flask with forceps, rinsed with chloroform into the flask and dried in an oven at 70 °C for 70 min. The sample was weighed again after cooling in a desiccator and the extraction loss was calculated. The loss corresponds to the amount of the plasticizers in the sample:

$$\% \text{ (PAEs)} = \frac{m_1 - m_2}{m_1} 100$$

where: m_1 – sample weight before elution
m_2 – sample weight after elution

The chloroform eluate was dried and the residue was dissolved in 5 ml chloroform. 50 µl of the chloroform eluate were transferred into a test tube with ground stopper, chloroform was evaporated with a gentle stream of nitrogen and the residue was dissolved in 2 ml hexane for HPLC determination.

Quantitative determination of PAEs

A packaging or a sample thereof (if its weight exceeded 2 g) was cut into pieces and put into a 250-ml to 500-ml Erlenmeyer flask. PAEs were extracted with the 1 : 1 mixture of hexane and dichloromethane. The flask was shaken for 1 h and the liquid was decanted into a 100-ml flask through a funnel with a bunch of cotton-wool. The complete extraction procedure was repeated twice and the three extracts were pooled and evaporated in a water bath at 40 °C. The residue was dissolved in hexane for HPLC determination.

Purity checks of glassware were done along with each analysis to eliminate the risk of contamination by PAEs.

HPLC conditions

Column: Separon SGX C 18, grain size 5 µm, length 150 mm, diam. 3 mm
Detector: UV (diode array)
Wave length: 224 nm
Mobile phase: 0.3 % methanol in hexane
Flow rate:0.5 ml/min

The peak areas were measured and the concentrations of PAEs were derived from calibration curves constructed from at least four points within the concentration range of 0 to 100 mg PAE per kg. The response of the UV detector was linear within this range.

Parameters of the method

Regression coefficients for individual PAE calibration curves within the concentration range of 0 to 100 mg/kg were $r = 0.988$ to 1.000.

The detection limit for quantitative determination of PAEs was 0.1 µg per 1 g of the sample.

The identity of PAE was confirmed by mass spectrometry (capillary column DB – 5, 60 m x 0.25 mm; film 0.25 µm; injector temperature 200 °C; injec-

tion on column 2 μl; carrier gas He; working pressure 10^{-5} Torr; source temperature 200 °C; EI 70 eV; emission current 10 μA; interface 250 °C).

RESULTS AND DISCUSSION

A simple and sensitive method for qualitative detection and quantitative determination of PAEs in packaging and other materials, described in detail in the preceding part, has been developed and tested.

The set of samples included 42 packings of sweets, wafers, meat and milk products, frozen products, vegetables, potato chips and further foods preferred by children. DEHP and DBP were demonstrated in all the samples at concentrations ranging from < 10 to 1,000 μg per packing. The weights of individual packings were lower than 2 g (usually around 1 g) and therefore complete packings were analysed in most cases to eliminate differences resulting from various intensities and arrangements of printings. Partial samples were analysed only from larger packings, such as those for salamis, cheeses or potatoes, and the concentrations of PAEs are expressed in terms of μg per 1 g sample. The results of the analyses are presented in Table I. Although separate analyses of printed and unprinted parts of the packings were impossible in most cases, it is evident that printing inks are responsible for increased concentrations of PAEs.

Foreign authors have confirmed the migration of PAEs from packings and printing inks into foods and packings are regarded as the major source of food contamination by PAEs. Although polypropylene is manufactured without the addition of PAE plasticizer, Nerín et al. (1993) found in printed polypropylene packings 0.13 to 3.04 mg DBP and 0.31 to 2.50 mg DEHP per 1 g. Castle et al. (1988) investigated the migration of DBP from cellulose foil into foods. While the samples of the foil contained 0.7, 1.2 and 1.4 % DBP, its concentration in the packed chocolate was 30.6 and in meat pie 8.7 and 15.8 mg/kg. Page and Lacroix (1992) found in butter and margarine packings (aluminium foil-paper laminate) the following concentrations of PAEs: DBP – paper laminate 2.40–4.69, aluminium foil 10.4–17.2 μg /cm^2; DEHP – paper laminate 2.81–3.56, aluminium foil 11.0–16.1 μg/cm^2. The concentrations of DBP and DEHP in butter and margarine ranged between 2.4 and 10.6, and 2.3 and 11.9 mg/kg, respectively.

On 18th August 1994, the Chief Hygienist of the Czech Republic announced the following permissible concentrations of DEHP and DBP sum

I. Concentrations of DEHP and DBP in packaging materials

Product	Type of package	µg/packing (*µg/g of packing)	
		DEHP	DBP
Sweets and wafers			
Chocolate 1	plastic foil	291	1 071
Chocolate 2	plastic foil	348	747
Chocolate 3	plastic foil	174	885
Biscuits (vanilla)	plastic foil	2 712	141
Biscuits (cocoa)	plastic foil	2 619	36
Biscuits (chocolate)	plastic and Al foil, paper	180	2 349
Sponge-biscuits	plastic bag	351	1 062
Biscuits	plastic bag	2 754	1 212
Wafers (chocolate)	plastic foil	1 182	36
Wafers (nut – chocolate)	plastic foil	2 550	180
Wafers (cocoa)	plastic foil	198	1 308
Wafers	plastic and Al foil	2 154	57
Meat products			
Rolled pork	plastic foil	1 035	615
Liver salami 1	plastic foil	132	69
Liver salami 2	plastic foil	1 629	6
Salami 1 – foil	plastic foil	62.3*	40.3*
– foil with printing	plastic foil	78.2*	57.2*
Salami 2– foil	plastic foil	1 100*	608*
– foil with printing	plastic foil	1 100*	990*
Milk products			
Milk 1	cardboard box, plastic foil	1 038	63
Milk 2	plastic bag	273	59
Milk 3	plastic bag	549	303
Cheese (steamed, smoked)			
– foil	plastic foil	52.9*	354.2*
– foil with printing	plastic foil	215.1*	1.9*
Cheese (Eidam type 1)	plastic foil	55.6*	168.9*
Cheese (Eidam type 2)	plastic foil	104.9*	16.3*

Table I continues

Product	Type of package	µg/packing (*µg/g of packing)	
		DEHP	DBP
Frozen products			
Vegetables 1	plastic bag	70	720
Vegetables 2	plastic bag	690	780
Ice cream 1	plastic foil	3 027	2034
Ice cream 2	plastic foil	2 025	135
Ice cream 3	plastic foil, paper	102	87
Ice cream 4	plastic foil, paper	1 308	117
Ice cream 5	plastic foil, paper	93	48
Ice cream 6	plastic foil, paper	90	42
Sheet paste	plastic foil	90	36
Other foods			
Potato chips 1	plastic bag	2 994	45
Potato chips 2	plastic bag	114	1 509
Potato chips 3	plastic bag	3 195	93
Dry pancake mix	plastic bag	2 313	12
Rice pudding	cardboard box	2 829	693
Potatoes 1	plastic bag	27.8*	53.8*
Potatoes 2	plastic bag	40.2*	99.5*

valid until 31st December 1996: fruits, flour, leaf vegetables, alcoholic drinks, vine and dessert wines and foods in general (milk, red meat, poultry alcohol-free beverages, beer) 1.0 mg/kg; root vegetables and potatoes 0.7 mg/kg; other foods 4.0 mg/kg.

The concentrations in foods of DBP and DEHP reported by foreign authors from western countries were in many cases higher than those permitted in the Czech Republic. Thus Cocchieri (1986), who examined 200 Italian foods, found the highest concentrations of DBP in cheeses and potato chips (17.5 and 12.0 mg/kg, respectively) and of DEHP in salted meat (6.7 mg/kg). Sharman et al. (1994) surveyed the occurrence of DEHP and total PAEs in Norwegian, Spanish and British milk and milk products. Norwegian milk and cream contained 0.02–1.67 mg DEHP and 0.04–3.40 mg total PAEs per

1 kg. The concentrations of DEHP and total PAEs in Spanish milk and cream did not exceed 0.55 and 3.40 mg per 1 kg, respectively. Cheeses marketed in Great Britain contained 0.2–16.8 mg DEHP and 2.4–114.4 mg total PAEs per 1 kg, and cream and butter 0.2–7.4 mg DEHP and 2.2–55.6 mg total PAEs per 1 kg. Significant differences in the concentrations of PAEs were found between raw milk and milk products. Increased concentrations in the latter are apparently due to secondary contamination from the processing equipment and from food packings. The information on the current concentrations of PAEs in consumed foods in the Czech Republic is insufficient.

Elevated concentrations of PAEs in regularly consumed foods, such as milk and meat products, chocolate or frozen cream products, can considerably increase the total daily intake of the hazardous PAEs, particularly DEHP, DBP and BBP (Nerín et al., 1993).

In 1991, the Commission of the European Communities for Foods laid down tolerable daily intakes (TDI) for DEHP (0.025 mg per 1 kg body weight) and DBP (0.050 mg per 1 kg body weight). In children with body weights from 10 to 20 kg, these limits correspond to TDI of 0.25–0.50 mg DEHP and 0.50–1.0 mg DBP. In Great Britain, the tolerable daily intakes of 0.02 and 4.74 mg of DEHP and total PAEs, respectively, have been laid down (Ministry of Agriculture, Fisheries and Food, 1987; 1990).

Besides the major sources of PAEs mentioned above, foods and drinking water can be contaminated also from plastic components of processing equipment, PVC tubings and PAEs-containing paint coats and laminated surfaces of storage tanks (Petersen, 1991; Castle et al., 1990). Another important source that can jeopardize human health are PAEs-containing medical utensils, such as dialyzers, transfusion and infusion sets, connecting tubes and further materials that are often made of PVC containing up to 40 % DEHP (Dine et al., 1991; Khaliq et al., 1992).

The concentrations of DEHP and DBP in the tested samples varied within a wide range. The total PAEs content does not necessarily mean that the packaging is unsuitable for foods. Some types of packaging materials contain barrier layers preventing or at least reducing the migration of PAEs into the food.

Regarding the wide variety of local and imported products and the large number of manufacturers and suppliers of colour plastic and other packings, the risk of contamination of foods marketed in the Czech Republic by toxic PAEs at intolerable concentrations may exist. This risk can be prevented by

regular checks of food packaging materials. The migration of PAEs from the packaging and food analyses for PAEs will be the subjects of our further studies.

The paper presents results of the project A 093 95 0132 "Methods of detection and hygienic evaluation of residues of hazardous xenobiotics in the food chain" supported financially by the Ministry of Agriculture of the Czech Republic.

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Received March 16, 1995

Výskyt esterů kyseliny ftalové v obalech pro potraviny

Bylo provedeno stanovení esterů kyseliny ftalové (PAE) v potravinářských obalech, které jsou v přímém kontaktu s potravinou. Byly analyzovány obaly (plastové fólie, papír, papírový karton, hliníková fólie s barevnými potisky) z malospotřebitelských balení jednotlivých komodit (cukrovinky, oplatky, masné a mléčné výrobky, mražené výrobky, zelenina, smažené brambůrky). Vzorky byly odebrány ze spotřebitelské sítě v Brně v průběhu června až srpna 1994. Pro kvalitativní a kvantitativní stanovení PAE v obalových materiálech byly vypracovány a ověřeny jednoduché a citlivé analytické postupy. Extrakce PAE se provádí chloroformem nebo směsí hexan : dichlormethan (1 : 1), stanovení pomocí HPLC s UV detekcí při 224 nm na koloně Separon SGX C_{18}. Hexan s 0,3 % metanolu je použit jako mobilní fáze, průtok 0,5 ml za min. Limit detekce je pro jednotlivé PAE 0,1 μg na 1 g obalu. Identita PAE byla konfirmována pomocí plynové chromatografie – hmotnostní spektrometrie. Ve všech 42 vzorcích obalů byl prokázán di-2-ethylhexyl ftalát (DEHP) a dibutyl ftalát (DBP). Koncentrace jsou vyjádřeny v μg na obal (při hmotnosti obalu do 2 g) nebo v μg na 1 g obalu u větších balení (salámy, sýry, zelenina). Obsah ftalátů v obalech se pohyboval u DEHP od 57 do 3 027 μg, u DBP od 6 do 2 349 μg. Výsledky jsou diskutovány se zahraničními údaji o obsahu PAE v obalech a jejich migraci do potravin. Byla prokázaná variabilita ve výskytu a koncentracích DEHP a DBP v potravinářských obalech, které přímo kontaktují potravinu v malospotřebitelském balení. Kontrola obalů je vhodnou prevencí kontaminace potravin PAE.

estery kyseliny ftalové; potravinářské obaly; metody stanovení

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Potrav. Vědy, *14*, 1996 (2): 109–117

THE EFFECT OF BACTERIOCINS ON THE LACTOBACILLI GROWTH IN VACUUM PACKED MEAT PRODUCTS

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Abstract: Lactobacilli are usually prevailing microflora in vacuum packed meat products and in the internal of sausages of large diameter. Theirs growth under anaerobic conditions can be reduced by addition of bacteriocins. Pediocin was tested in vacuum packed frankfurters and the sausage Junior. The addition of pediocin caused the lower total and lactobacilli counts during the whole time of storage. The pH value and the composition of internal atmosphere were influenced, too.

bacteriocins; pediocin; meat products; shelf-life; vacuum; packaging

The shelf life of meat products can be improved by several ways. Storage stability of these products depends on the total preservation effect of various steps, treatments or conditions used within the production and storage. The final preservation effect is a combination of hurdles (B h u n i a et al., 1990). Such hurdles can be sterilisation or pasteurisation, reduction of redox potential (avoiding of oxygen), lowering of water activity of products by salting or drying, application of preservatives, reduction of pH value, storage at low temperature, etc. Very important factors affecting the shelf life of products are of course the initial count of micro-organisms, composition of microbial strains present in products and hygienic conditions of production.

A part of production of meat products is sliced and packed under vacuum. This type of products is always less stable than the whole sausages. The microbial recontamination is especially caused by the slicing. Considering the condition inside the package, not all from the wide range of microbial strains can grow. Under anaerobic conditions at low storage temperature the psychrophilic lactic acid bacteria create dominating part of micro-organisms (S t i l e s, H a s t i n g s, 1991). These conditions are also appropriate for the *Listeria monocytogenes* growth (K r ö c k e l, 1991). The lactic acid bacteria are most responsible for the deterioration of quality of the sliced vacuum

packed meat products due to the production of lactic acid and other metabolites (Ahn, Stiles, 1990).

The bacteriocins can be used as a possible "pure, not-chemical" treatment to improve the shelf-life of meat products (Buchanan, Klawitter, 1992).

Bacteriocins are polypeptides produced by bacteria and possessing antagonistic activity against other bacteria growth (Juven et al., 1991). The inhibitory effect of bacteriocins consists in their adsorption on the specific receptors of the cellular membrane followed by a series of lethal biochemical changes (Bhunia et al., 1990). They are tasteless and odourless, can be inactivated by digestive enzymes pepsin and trypsin (Lücke, Earnshaw, 1991). Since they are natural products, their use (or the use of their producers – starter cultures) as well as their consumption is harmless as proved in all toxicological studies (Ray, 1992).

From a wide spectrum of bacteriocins of various producers the pediocins are of special interest in meat processing. They are suitable for the use in vacuum packed meat products for their relatively low microbial count, low storage temperature and absence of oxygen (Ray, 1992).

Pediocin AcH is produced by *Pediococcus acidilactici* H (isolated from fermented salami). It has inhibitory effect against the large spectrum of grampositive bacteria, especially against lactobacilli, leukonostoc, *Staphylococcus aureus*, *Enterococcus* (Ray, 1992), *Clostridium perfringens*, *Listeria monocytogenes* (Juven et al., 1991; Medina et al., 1992; Buchanan, Klawitter, 1992), *Brochothrix thermosphacta* and *Bacillus cereus* (Juven et al., 1991). Gramnegative bacteria (*Escherichia, Salmonella, Yersinia* a *Aeromonas*) can become sensitive to pediocin if they are stressed by sublethal treatments, such as mild heating, freezing and others (Ray, 1992). The effect of bacteriocin depends on bacteria counts (Nielsen et al., 1990).

Pediocin may be used for control of microbial counts, incl. *Listeria monocytogenes*, also at higher temperature (Degman et al., 1992). The system of biocontrol represents thus an additive hurdle for listeria (Degman et al., 1992).

The molecule of pediocin is a polypeptide with the following sequence of 44 amino acids (the molecules of cysteine are bound by disulphide connections) (Stiles, 1994; Degnan et al., 1992):

NH_2-lys-tyr-tyr-gly-asn-gly-val-thr-cys-gly-lys-his-ser-cys-ser-val-asp-
-trp-gly-lys-ala-thr-thr-cys-ile-ile-asn-asn-gly-ala-met-ala-trp-ala-thr-gly-
-gly-his-gln-gly-asn-his-lys-cys-COOH

The pediocin is active in a wide interval of pH-values and it is stable for 60 min at 80 °C (Ray, 1992). It is destroyed by proteases (Juven et al., 1991; Bhunia et al., 1988).

MATERIAL AND METHODS

The effect of pediocin AcH was tested in industrial conditions of several Czech meat factories. It was investigated how the shelf-life of common meat products stored under different conditions is influenced by the addition of pediocin. The raw material composition and the process were the normally used ones.

Homogeneous smoked sausage Junior (Lyone-type) was produced by cuttering (the amount of sausage's mixture was 150 kg), 0.3% (w/w) of pediocin (commercial name ALTA 2341) were added. The studied samples and the control ones (without pediocin) were sliced and packed under vacuum in plastic film (Svitafan). The samples were stored under different temperature: at 4 °C (cooling storage) and 10 °C (elevated temperature).

In an other experiment the frankfurters were produced (150 kg sausage mixture), pediocin and/or ascorbic acid in different conbination were added. The addition of pediocin was the same as at the sausage above, i.e. 0.3% (w/w). The ascorbic acid was added in common amount of 300 mg/kg (w/w). After the usual heat treatment and cooling the samples were packed under vacuum in plastic film (Svitafan) and stored at 4 °C (cooling storage) or 20 °C (room temperature).

The microbiological methods included the estimation of the total counts and lactobacilli counts. The total counts were determined on the GTK agar after 24 h cultivation at 36 °C. The lactobacilli counts were determined using 48 h cultivation at 28 °C on the MRS medium.

The Acidimeter 333 with the combined glass and calomel electrode was used to measure of the pH value.

The composition of internal atmosphere was characterised by the carbon dioxide content. This value was measured using the gas chromatograph CHROM 4 with TCD detector and filing column PORAPACK Q.

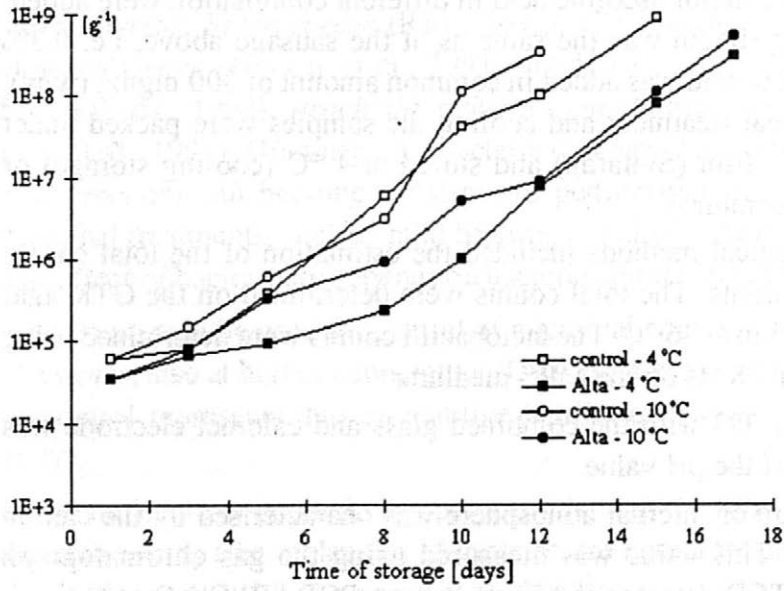
RESULTS AND DISCUSSION

The pediocin addition affected studied characteristics of the sausages. The obtained data are presented in the figures.

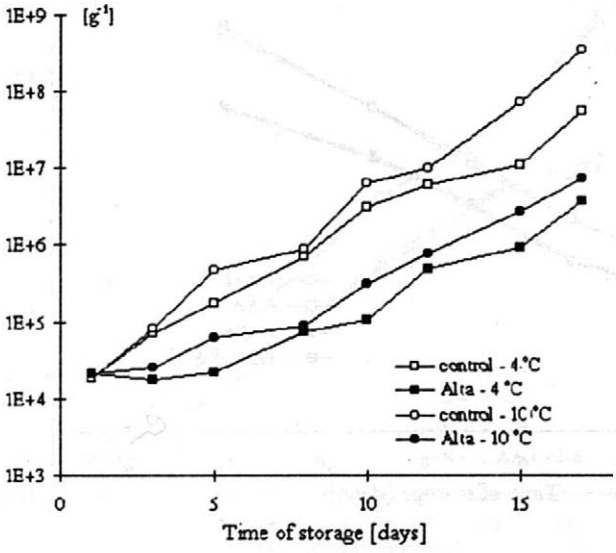
The shelf-life of smoked sliced sausage Junior became longer after pediocin addition. The increase of total micro-organisms counts was lower. During the all time of experiment the total micro-organisms counts in sausage containing pediocin were approx. 1 log lower than those in control samples (Fig.1).

The growth of lactobacilli was influenced in the similar way. The pediocin effect is even stronger, because it affects grampositive bacteria, especially lactic bacteria (Fig. 2). As lactobacilli produce lactic acid, the inhibition of them caused the smaller decrease of pH value in the samples containing pediocin.

As expected, the higher storage temperature (10 °C) caused the higher total and lactobacilli counts in comparison with the refrigerator conditions (4 °C) and the addition of pediocin was effective at both temperatures. Very important is the fact, that the shelf-life of pediocin-samples stored at 10 °C was even higher as that of control samples stored at 4 °C. Thus the bacteriocin can ensure the higher safety in the case of interruption of cooling condition during storage.



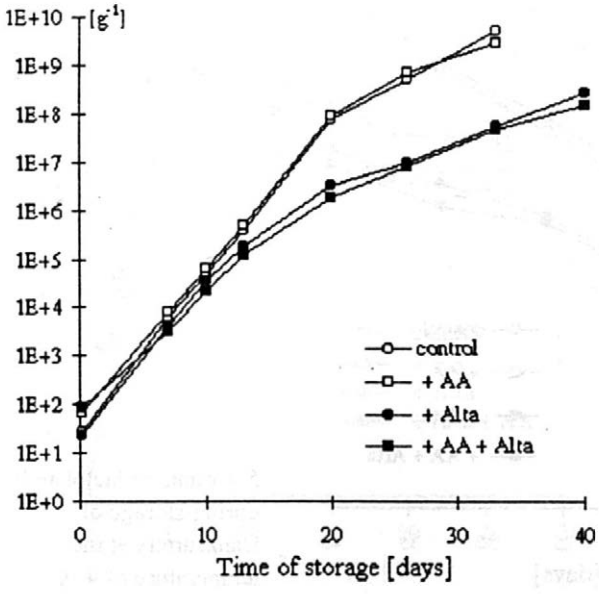
1. Total counts of micro-organisms during storage of sausage Junior



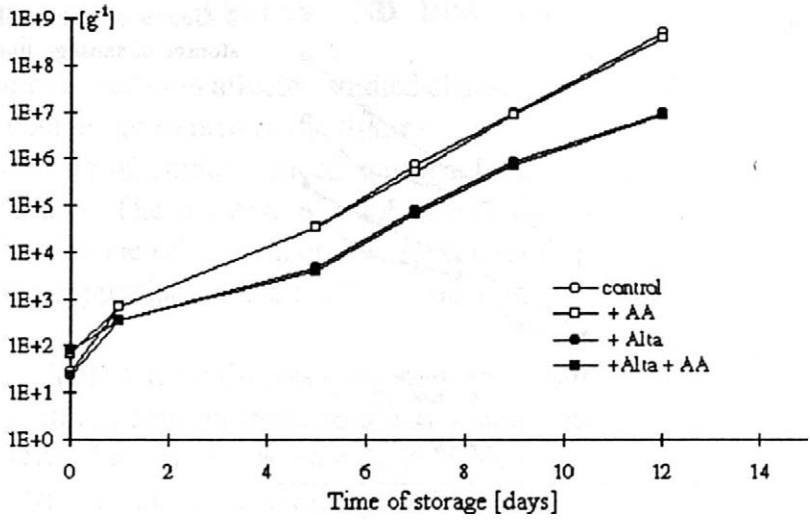
2. Counts of lactobacilli during storage of sausage Junior

The microbial growth was influenced by pediocin addition also in the frankfurters. At both temperatures the microbial counts were approx. 1 log lower in the samples, where pediocin had been added. (see Fig. 3 a 4). The growth of micro-organisms was faster at the temperature of 20 °C, as evident from Fig. 4. In the case the pediocin addition was not able to reduce the microbial growth under the values achieved at 4 °C without pediocin addition.

The pediocin influenced again especially the growth of lactobacilli. The increase of lactobacilli counts was significantly lower in the samples con-

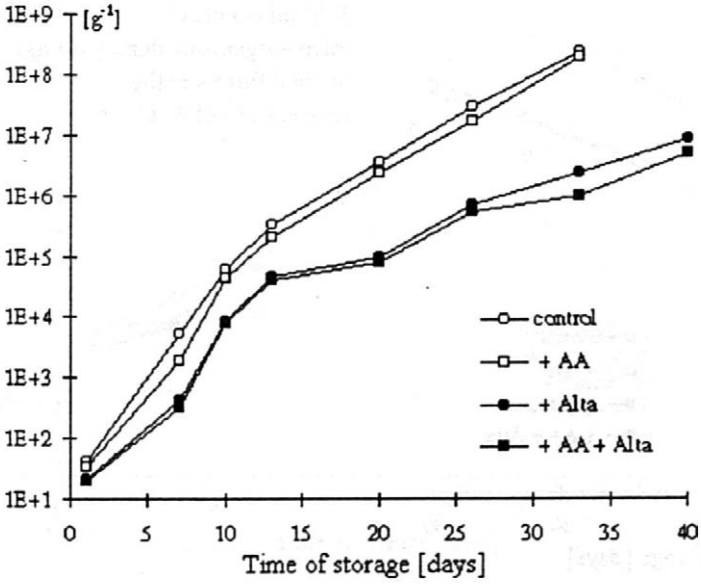


3. Total counts of micro-organisms during storage of frankfurters at the temperature of 4 °C

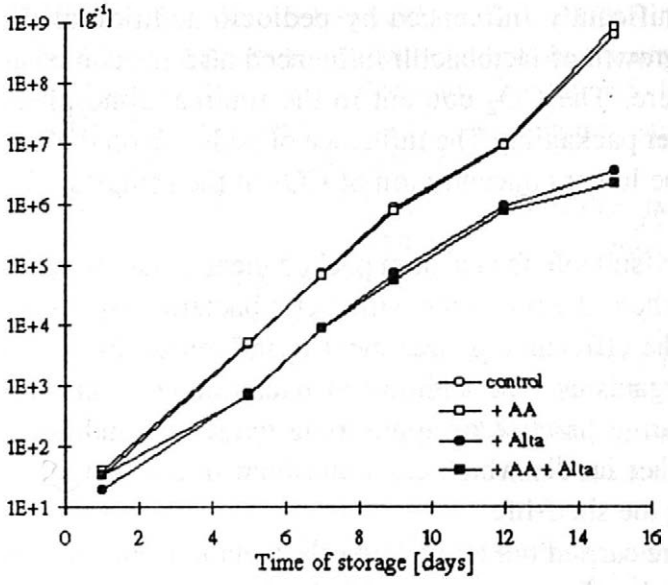


4. Total counts of micro-organisms during storage of frankfurters at the temperature of 20 °C

taining bacteriocin related to the control samples (Figs 5 and 6). Thus the frankfurters shelf-life became several days longer. This changes occurred at both temperatures. The count increasing was of course higher at higher temperature.



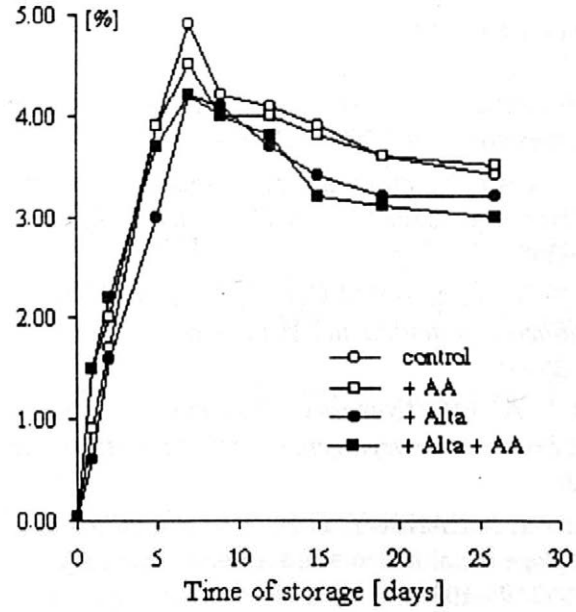
5. Counts of lactobacilli during storage of frankfurters at the temperature of 4 °C



6. Counts of lactobacilli during storage of frankfurters at the temperature of 20 °C

The shelf-life of sausages is influenced not only by temperature, but also by other factors, especially by the initial contamination. They will be studied in future experiments.

The addition of ascorbic acid (that was added to improve sausage colour) had no effect on the micro-organism growth (Figs 3–6).



7. The effect of pediocin addition on the composition of internal atmosphere

The pH value was significantly influenced by pediocin addition at the higher temperature. The growth of lactobacilli influenced also the composition of internal atmosphere. The CO_2 content in the internal atmosphere increased immediately after packaging. The influence of pediocin on the lactobacilli growth caused the lower concentration of CO_2 at the samples with bacteriocin addition (Fig. 7).

The pediocin can be very suitable for vacuum packed meat products and it can be used in all cases where the problems with lactic bacteria (especially lactobacilli) can occur. The efficiency of treatment is influenced by initial concentration of micro-organisms. The addition of bacteriocins cannot replace the good manufacturing practice or appropriate hygienic conditions, but it can be used as further hurdle which can contribute in the system of other hurdles to extending the shelf-life.

Further investigations are carried out to explain other relations and consequences in sausage production.

Conclusions

The application of bacteriocins cannot replace the following of high hygiene. The suitable application of bacteriocins can however reduce the growth of micro-organism (especially lactobacilli) in vacuum packed sausages and so extend the shelf-life of sausages in systems of hurdle effect.

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Received August 23, 1995

Vliv bakteriocinů na růst laktobacilů ve vakuově balených masných výrobcích

Laktobacily bývají obvykle dominující mikroflórou u vakuově balených masných výrobků i uvnitř salámů větších kalibrů. Zde se množí v anaerobních podmínkách za nízké teploty. Jednou z možností, jak snížit jejich četnosti a tím prodloužit údržnost masných výrobků, je aplikace bakteriocinů. V provozních podmínkách byla vyzkoušena účinnost pediocinu (komerční název Alta 2341), který byl přidán do vakuově balených výrobků (nakrájený homogenní měkký salám Junior a jemné párky). Ve všech případech vedla aplikace pediocinu ke zpomalení růstu mikroorganismů, celková četnost mikroorganismů se snížila zhruba o řád v celém průběhu skladování. Podobně i četnost laktobacilů v průběhu skladování byla nižší o jeden až o dva řády. Omezení růstu mikroorganismů se projevilo i změnami chemických vlastností masných výrobků: pH a složení vnitřní atmosféry.

bakteriociny; pediocin; masné výrobky; salámy; vakuum; balení

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Potrav. Vědy, *14*, 1996 (2) : 119–132

DETERMINATION OF SUCROSE IN SLICES, PULPS, RAW AND WHITE SUGARS BY NIR REFLECTANCE SPECTROSCOPY

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Abstract: Near infrared (NIR) reflectance spectroscopy was applied to the calibration procedure for the determination of sucrose content in slices, pulps, raw and white sugars. Calibration correlation between NIR and referent HPLC results gave correlation coefficient higher than 0.999 for slices, pulps and raw sugar. The relative standard error of calibration varried from 0.02 % (raw sugar) to 0.88 % (slices). In the case of white sugar the validity of calibration was not evaluated.

quantitative analysis; infrared NIR; sucrose; analysis of sugar factory products

Near infrared region (NIR) spectroscopy appears to be one of the most promising nondestructive analytical techniques for determining the composition of various food. It was applied to the rapid analysis of cocoa powder (Kaffka et al., 1982; Permanyer, Perez, 1989), chocolate (Dauberte et al., 1987; Čopíková et al., 1994), milk (Baer et al., 1983), and in the baking industry (Osborne, 1983; Suzuki et al., 1986). NIR transmittance spectroscopy was also used to determine the sugar content in natural fruit juices (Lanza, Li, 1984), in aqueous solutions (Dull, Giangiacomo, 1984; Giangiacomo, Dull, 1986), in soft drinks (Kemsley et al., 1992), and in molasses (Dumoulin et al., 1987). For sugar factory juices, NIR spectroscopy was tested as an on-line monitoring technique (Vaccari et al., 1990). Several papers were focused on the measurement of sucrose in multicomponent dry mixtures (Sverzut et al., 1987; Giangiacomo et al., 1981; Osborne et al., 1983) using NIR reflectance spectroscopy. Finally, the possibility of utilization of NIR technique for the analytical control of sugar products was described (Vaccari et al., 1987, 1988; Nomura et al., 1990).

Since the NIR technique is based on the correlation of a spectroscopic responces with the constituent concentration, the calibration of NIR instrument must be made on a set of known original samples. In this paper we examined the applicability of NIR spectroscopy working in reflectance mode to the quantitative analysis of pulps, slices, raw and white sugars.

MATERIAL AND METHODS

Material

All samples used were original industrial products supplied by the Sugar Factory at Modøany. The pulps and the slices were stored at 5 °C after drying for 3 h at 105 °C, and the raw and white sugars were stored without any pre-treatment. The presence of air moisture was excluded during storage. Sucrose, D-glucose and D-fructose were purchased (Lachema, Czech Republic). Betaine hydrochloride was purified by a preparative liquid chromatography using the strong cation exchanger Dowex W 50x8 (Fluka, Germany) in Ca^{2+} form with water as a mobile phase, m.p. 290 °C.

Sample Preparation for HPLC

Raw sugar as well as white sugar (1.0 g) was weighed into 100 ml volume flask, and made up to the mark with water at 20 °C. Samples of slice (1.5 g) or pulp (5.0 g) were stirred with water (50 ml) at ambient temperature for 15 min, and then filtered through a thin cotton net. After washing, the filtrates were collected and placed into 100 ml volumetric flask, alkaline lead(II)acetate powder was added, and the mixture was diluted to volume with water at 20 °C, mixed, and filtered. Prior to HPLC analysis, the solutions were deaerated by 20 min heating at a temperature 55–65 °C, and passed through a glass microfilter (2 μm, Tessek, Czech Republic).

HPLC

Ligand-exchange chromatography on a strong cation exchanger was used as described previously (Moravcová et al., 1993). Capacity factors of 0.33, 0.55, 0.69, and 1.00 were obtained for sucrose, D-glucose, D-fructose, and betaine, respectively. The sucrose content in the industrial samples was determined by the external calibration method for the peak heights

(Moravcová et al., 1993). The reproducibility of HPLC was expressed by the standard deviation (*SD*) and by the relative standard deviation (*RSD*) for ten replicate analyses of slice and raw sugar samples according to the following formulas:

$$SD = \left\{ \frac{1}{n-1} \left[\sum x_i^2 - \frac{1}{n} (\sum x_i)^2 \right] \right\}^{\frac{1}{2}} \quad [1]$$

where: x_i – sucrose concentration
n – number of analyses

$$RSD = \frac{100 \,.\, SD}{x_{av}} \quad [2]$$

where: x_{av} – average sucrose concentration

The *SD* value obtained for a slice was 0.4581 and *RSD* value was 1.49 %. For raw sugar, the *SD* value found was 0.6113, and the value of *RSD* was 0.63 %.

Sample Preparation for NIR Spectroscopy

Raw and white sugars were filled into a solid sample adaptor directly. Dry pulps and slices were crushed with a VM7-Minirasant grinder (OPS Přerov, Czech Republic) to obtain the particle size of the powder below 1 mm. The calibration set consisted of 10 samples of slices and of 20 samples each of pulps, raw and white sugars.

NIR Spectroscopy

The calibration was carried out on an Infralyzer 400 instrument (Technicon, U.S.A) equipped with 19 discrete narrowband filters (Table I), and interfaced to a 286/AT-PC IBM computer. The reflectance data were the average of two scans per sample.

The stepwise addition program selected the suitable wavelengths according to so-called *t*-value which allowed to reduce the number of filters needed. The excellent calibration is characterized by $t > 10$, if *t*-value is in a range from 4 to 10, the calibration seems to be acceptable, and value $t < 2$ indicates the unsatisfactory results:

I. The filter wavelengths of Infralyzer 400 instrument

Filter No	Wavelength [nm]	Filter No	Wavelength [nm]	Filter No	Wavelength [nm]
2	2 336	9	2 139	16	1 940
3	2 348	10	2 180	17	1 734
4	2 310	11	1 982	18	1 722
5	2 270	12	1 818	19	1 445
6	2 230	13	1 778	20	1 680
7	2 208	14	2 100		
8	2 190	15	1 759		

$$t\text{-value} = \frac{F_i}{SD_{F_i}} \qquad [3]$$

where: F_i – regression constant at given wavelength
SD_{F_i} – standard deviation of regression constant F_i

The multiple linear regression evaluated the regression constants of the calibration straight line:

$$\begin{aligned} Y_1 &= F_0 + F_1x_{1,1} + F_2x_{2,1} + \ldots + F_px_{p,1} \\ &\vdots \\ Y_n &= F_0 + F_1x_{1,n} + F_2x_{2,n} + \ldots + F_px_{p,n} \end{aligned} \qquad [4]$$

where: $Y_1, \ldots, Y_n$ – sucrose concentrations in calibration set
$F_0, .., F_n$ – regression constants
n – number of calibration samples
p – number of filters
x – optical density at selected wavelength expressed as log $(1/R)$
R – reflectance

The samples used for a calibration procedure must be a valid representation of the population, so this important condition was verified by HAT-statistics. These samples for which the computed HAT-criterion exceeds the value of 3 must be excluded. Another additional criterion applied to the cali-

bration samples was the ratio of a difference between sucrose content determined by HPLC and predicted by NIR (d_{ii}) to a standard error of estimation (*SEE*): The value of d_i /*SEE* ratio must be lower than 2.

$$SEE = \left[\frac{\sum (d_i - d_{av})^2}{p - n - 1} \right]^{\frac{1}{2}} \quad [5]$$

where: $d_{av} = (d_i/n)$

The relation between the NIR measurement and the reference HPLC method was evaluated by the correlation coefficient (r) according to equation [6] and also by the regression ratio R_p

$$r = \left[\frac{1 - SEE^2 (n - p - 1)}{SDR^2 \ (n - 1)} \right]^{\frac{1}{2}} \quad [6]$$

where: *SEE* – standard error of estimation (*SEE* eq. 5)
SDR – standard deviation of range defined by equation [7]

$$SDR = \left[\frac{\sum (x_j - x_{av})^2}{n} \right]^{\frac{1}{2}} \quad [7]$$

based on equation [9]. Finally, the calibration fit was evaluated by calculating the standard error of calibration *SEC* (eq. [8]), and the systematic error *BIAS* (eq. [10]):

$$R_p = \frac{r^2 (n - p - 1)}{(1 - r^2) p} \quad [8]$$

$$SEC = \left[\frac{\sum d_i^2}{n - p - 1} \right]^{\frac{1}{2}} \quad [9]$$

$$BIAS = \frac{\sum d_i}{n} \quad [10]$$

If the calibration curve fits a linear term ($r > 0.8$), the regression ratio R_p has a standard value from 100 to 1000, but it can reach up to 10 000. On the

considered as accidental and therefore unsatisfactory. From the practical point of wiev, the R_p value should be higher than 10.

RESULTS AND DISCUSSION

The reflectance data plotted as log(1/*R*) versus wavelength produce a curve comparable to an absorption spectrum. In natural products and other heterogeneous materials, the various constituents often have overlapping, coinciding or interfering bands, and a multiterm linear equation is usually necessary to describe any linear relationship between reflectance response and the concentration of an analyte. The linear regression procedure was first run with all wavelengths and correlation coefficient (*r*) as well as *t*-value were calculated. That wavelength giving a minimum *t*-value was subsequently eliminated, and the procedure was repeated until the best filter

II. The multiple linear regression for slices and pulps

Filter No.	Regression constants	*t*-Value	Filter No	Regression constants	*t*-Value
Slices[a]			8	–2 491.921	7.26
3	–9 849.875	15.76	9	6 153.442	13.62
4	10 699.707	16.41	10	–6 701.162	11.54
7	–44 712.798	16.55	11	–262.002	2.59
10	56 343.063	17.25	12	–3 454.098	16.66
14	–13 298.614	14.31	13	–14 998.036	13.62
16	367.72	7.54	14	–2 406.200	7.57
Pulps[b]			15	35 094.179	16.60
3	–1 118.073	4.00	16	–29.016	0.55
4	7 170.408	19.93	17	–5 613.146	6.76
5	–7 251.933	8.36	18	–8 762.250	6.90
6	1 578.458	3.68	19	–155.08	3.46
7	4 325.941	8.23	20	–1 059.348	7.62

[a] $F_0 = 270.81$, [b] $F_0 = -40.558$

III. The multiple linear regression for raw and white sugar

Filter No.	Raw sugar[a]		White sugar[b]	
	regression constants	*t*-value	regression constants	*t*-value
3	−1 484.746	1 5.00	−595.582	0.11
4	4 259.960	7 3.17	166.082	0.09
5	−1 641.471	1 1.40	−382.818	0.30
6	−1 029.230	3.11	832.48	0.56
7	3 215.390	7.66	1 381.007	0.13
8	−1 442.672	1 2.32	−586.75	0.13
9	–	–	−9.982	0.01
10	−82.944	0.74	-971.484	0.42
11	567.706	2.80	−726.899	0.15
12	1 034.217	9.82	252.515	0.20
13	−1 464.058	8.70	−485.416	0.09
14	−1 589.037	2 5.01	460.337	0.15
15	−4 767.298	5.04	548.725	0.18
16	−195.573	1.97	268.304	0.10
17	−1 430.585	3.46	−2 869.110	0.31
18	915.334	2.91	2 770.814	0.87
19	1 837.313	1 9.35	−83.370	0.03
20	3 172.995	8.45	26.736	0.02

[a] $F_0 = 178.804$, [b] $F_0 = 102.328$

combination was achieved. The final regression constants and *t*-value corresponding to the selected wavelength are summarized in Tables II and III.

The calibrations of slices, pulps and raw sugars were found to be acceptable or excellent according to *t*-value. In opposite, all wavelengths used for the white sugar calibrations gave *t*-value lower than 2 and therefore this calibration is not correct.

The validity of the concentration range of all calibration sets was proved by HAT-statistic and d_i/SEE ratio which both agreed in a satisfactory way with the values recommended (Tables IV–VI).

IV. Validity of slice and pulp calibration samples

Sample No.	Sucrose concentration [%, w/w]		Deviation d_i	d_i/SEE	HAT-criterion
	NIR	HPLC			
Slice					
1	70.155	70.19	−0.035	−0.09	1.48
2	68.904	69.57	−0.666	−1.64	0.46
3	75.237	74.91	0.327	0.81	0.95
4	71.636	71.39	0.246	0.61	1.42
5	22.922	22.96	−0.038	−0.09	1.43
6	32.567	32.17	0.397	0.98	0.63
7	27.789	27.75	0.039	0.10	1.47
8	29.067	29.69	−0.623	−1.54	0.58
9	30.651	30.83	−0.179	−0.44	1.32
10	31.303	30.77	0.533	1.31	0.25
Pulp					
1	9.632	9.65	−0.018	−0.77	1.02
2	9.436	9.43	0.006	0.24	1.05
3	8.739	8.70	0.039	1.67	0.89
4	19.260	19.28	−0.02	−0.85	1.01
5	5.646	5.48	−0.034	−1.48	0.93
6	4.576	4.56	0.019	0.80	1.02
7	7.825	7.80	0.025	1.08	0.98
8	3.715	3.72	−0.005	−0.19	1.05
9	4.355	4.34	0.015	0.64	1.03
10	4.431	4.44	−0.009	−0.37	1.05
11	19.534	19.53	0.014	0.59	1.03
12	18.364	18.36	0.004	0.19	1.05
13	11.257	11.26	−0.003	−0.11	1.06
14	10.035	10.06	−0.025	−1.07	0.99
15	1.74	1.79	−0.05	−2.18	0.78
16	3.468	3.45	0.018	0.78	1.02
17	4.505	4.52	−0.015	−0.64	1.03
18	1.263	1.26	0.003	0.13	1.05
19	2.619	2.58	0.039	1.69	0.89
20	7.556	7.56	—0.004	—0.18	1.05

V. Validity of raw sugar calibration samples

Sample No.	Sucrose concentration [%, w/w]		Deviation d_i	d_i /*SEE*	HAT-criterion
	NIR	HPLC			
1	98.493	98.5	–0.007	–0.4	1.08
2	99.396	99.4	–0.004	–0.22	1.11
3	98.210	98.2	0.01	0.56	1.09
4	99.197	99.2	–0.003	–0.19	1.12
5	97.474	97.5	–0.026	–1.43	0.80
6	97.868	97.9	–0.032	–1.77	0.92
7	98.814	98.8	0.014	0.76	0.97
8	98.410	98.4	0.01	0.55	1.10
9	97.219	97.2	0.019	1.07	1.04
10	98.101	98.1	0.001	0.05	1.04
11	97.701	97.7	0.001	0.04	0.95
12	96.815	96.8	0.015	0.85	1.07
13	95.318	95.3	0.018	0.98	0.87
14	98.080	98.1	–0.02	–1.12	1.03
15	97.906	97.9	0.006	0.34	1.10
16	98.390	98.4	–0.01	–0.53	1.00
17	98.992	99.0	–0.008	–0.44	1.11
18	98.735	98.7	0.035	0.095	0.83
19	95.867	95.9	–0.033	–1.82	0.83
20	96.314	96.3	0.014	0.77	0.94

A comparison of the NIR and standard HPLC methods to estimate sucrose concentration in slices, pulps, and raw sugar showed that these two methods gave extremely close results with regression coefficient exceeding 0.999 (Table VII). For white sugar, the correlation coefficient was substantially lower but still comparable with those reported previously (Sverzut et al., 1987; Giangiacomo et al., 1981). These results indicate clearly the linear relation between log(1/*R*) and sucrose concentration and no another transformation of data to the first or second derivative form is nedeed. However, the

VI. Validity of white sugar calibration samples

Sample No.	Sucrose concentration [%, w/w]		Deviation d_i	d_i /*SEE*	HAT-criterion
	NIR	HPLC			
1	99.901	99.93	–0.029	–0.67	1.03
2	99.716	99.69	0.026	0.58	1.04
3	99.920	99.90	0.02	0.46	1.04
4	99.737	99.75	–0.013	–0.3	1.05
5	99.867	99.78	0.087	1.97	0.83
6	99.905	99.92	–0.015	–0.34	1.05
7	99.816	99.80	0.016	0.37	1.05
8	99.833	99.91	–0.077	–1.74	0.88
9	99.900	99.95	–0.05	–1.14	0.98
10	99.881	99.96	–0.079	–1.79	0.87
11	100.006	99.98	0.026	0.56	1.04
12	100.011	99.98	0.031	0.70	1.03
13	99.812	99.78	0.032	0.72	1.03
14	100.005	99.97	0.035	0.79	1.02
15	99.793	99.84	–0.047	–1.06	0.99
16	99.637	99.61	0.027	0.61	1.03
17	99.790	99.82	–0.03	–0.67	1.03
18	99.847	99.83	0.017	0.38	1.05
19	99.827	99.86	–0.033	–0.75	1.02
20	99.726	99.67	0.056	1.27	0.96

regression ratio R_p seems to be the more important criterion than regression coefficient itself. The R_p value for slices, pulps, and raw sugar (Table VII) confirms again the linear fit between NIR and HPLC results but the very low regression ratio calculated for white sugar unambiguously determines no correlation between NIR and HPLC methods. This conclusion is also supported by uncorrect *t*-value (Table II) found for the white sugar and discussed above. The accuracy of the reference HPLC method could be considered as the major reason. The standard deviation of the replicate analysis of raw sugar was established to be 0.61 (see Material and Me-

VII. Statistic evaluation of NIR calibration

Criterion[a]	Slices	Pulps	Raw Sugar	White Sugar
n	10	20	20	20
p	6	18	17	18
x_{min} [%, w/w]	22.96	1.26	95.30	99.61
x_{max} [%, w/w]	74.91	19.53	99.40	99.98
x_{av} [%, w/w]	46.02	7.90	97.87	97.85
SDR	20.987	5.598	1.096	0.109
SEE	0.405	0.023	0.018	0.044
SEC	0.702	0.101	0.056	0.192
BIAS	0.0001	–0.0001	0	0
r	0.9998	1.0000	0.9999	0.9154
R_p	1 490.01	3 258.92	428.41	0.287
100 SEE/x_{av} [%]	0.88	0.29	0.02	0.04
100 SEC/x_{av} [%]	1.52	1.30	0.05	0.20

[a] For definitions of symbols see Material and Methods

thods); if a similar value can be supposed for white sugar the scatter will be aproximately 0.3. In such a case, all calibration samples having sucrose concentration from 99.61 to 99.98% will occupy the interval 99.85 ± 0.3 determined by the scatter of replicate analysis.

The standard errors (*SEE* and *SEC*, Table VII) are comparable with literature data (Sverzut et al., 1987; Osborne, 1988; Alfaro et al., 1990). The highest *SEE* as well as *SEC* were obtained for the calibration of slices probably due to the greatest concentration range of sucrose which varried from 22.96 to 74.91%. Interestingly, the raw sugar can be analyzed directly because the moisture content does not tend to mask the signal of sucrose and the particle size of raw sugar is rather uniform.

Validation of the Infralyzer 400 calibration is in the progress.

Conclusion

The results demonstrate applicability of the NIR reflectance spectroscopy to the determination of sucrose in pulp, slice and raw sugar produced in

sugar factories. The samples are analyzed with a little pre-treatment and subsequent analysis is fast with accuracy comparable with referent method.

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Received November 23, 1995

Stanovení sacharózy ve sladkých a vyslazených řízcích, v surovém a rafinovaném cukru pomocí infračervené spektroskopie v blízké oblasti

Spektrometr Infralyzer 400 pracující v reflektančním režimu byl kalibrován pro stanovení sacharózy ve sladkých a vyslazených řízcích a v surovém i rafinovaném cukru mnohonásobnou lineární korelací mezi výsledky NIR metody a srovnávací vysokoúčinné kapalinové chromatografie na silném měniči kationtů. Vysoké hodnoty regresních konstant a odpovídající hodnoty dalších statistických kritérií potvrzují lineární závislost optické mohutnosti vyjádřené jako log (1/*R*) na obsahu sacharózy. Relativní standardní chyba kalibrace (*SEC*) byla 0,88 % pro sladké řízky s koncentrací sacharózy 22,96–74,91 %, 0,29 % pro vyslazené řízky s koncentrací sacharózy 1,26 až 19,53 % a konečně 0,02 % pro surový cukr, pro který byla koncentrace sa-

charózy 95,30–99,40 %. Tyto hodnoty jsou srovnatelné s referenční metodou HPLC, která měla *SEC* = 0,24 % pro rozmezí sacharózy od 12,52 do 65,52 %. V případě rafinovaného cukru nebyla nalezena uspokojivá korelace mezi výsledky NIR metody a HPLC pravděpodobně díky tomu, že zvolená srovnávací metoda nebyla dostatečně přesná. Pro velice úzké rozmezí koncentrací sacharózy v rafinovaném cukru (99,61 až 99,98 %) byla směrodatná odchylka opakované analýzy HPLC metodou 0,61 %. Surový a rafinovaný cukr byly měřeny přímo, neboť přítomná vlhkost stanovení nerušila, ale sladké a vyslazené řízky byly před analýzou vysušeny a rozemlety.

Výsledky ukazují vhodnost NIR spektrometrie ke stanovení sacharózy v cukrovarnických meziproduktech (sladké a vyslazené řízky) a surovém cukru a mohou být použity k zavádění modernějších analytických postupů do cukrovarnické praxe.

kvantitativní analýza; infračervená spektrometrie; NIR spektrometrie; sacharóza; analýza cukrovarnických meziproduktů a produktů

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Potrav. Vědy, *14*, 1996 (2) : 133–140

URČOVANIE PREDPOKLADANEJ TRVANLIVOSTI PASTERIZOVANÉHO MLIEKA

Prediction of the Shelf-life of Pasteurized Milk

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Abstract: Procedure developed by Griffiths and Phillips (1988b) which allows to predict shelf-life of pasteurized milk at different storage temperatures was verified in this study. The shelf-life of commercial whole and skim pasteurized milks was evaluated and compared with calculated shelf-life after storage at 4 and 10 °C. Pre-storage at 15 and 20 °C for 24 hours was used for obtaining pasteurized milk samples of various bacterial quality. Obtained results indicate that the better the microbial quality, the larger the differences between calculated and determined shelf-life, but the discrepancies were proportional for all storage conditions. The verified method may need modification in order to predict accurately shelf-life of high quality pasteurized milks, which after pre-incubations give very low plate count (0–10 colonies) on selective media.

shelf-life; pasteurized milk; storage temperature

Abstrakt: V práci sme overovali model určovania predpokladanej trvanlivosti pasterizovaného mlieka pri rôznych teplotách skladovania, ktorý publikovali Griffiths a Phillips (1988b), založený na hodnotení počtu mikroorganizmov mlieka preinkubovaného pri štyroch teplotách na troch selektívnych pôdach a na agare s odstredeným mliekom. Vypočítaná trvanlivosť bola porovnaná so skutočnou trvanlivosťou jednotlivých vzoriek pasterizovaného mlieka pri teplote skladovania 4 a 10 °C. Zistili sme, že podľa overovaného postupu sa dá pomerne presne určiť predpokladaná trvanlivosť pasterizovaného mlieka s celkovým počtom mikroorganizmov okolo 10^5 cfu/ml. Tento postup by však bolo potrebné modifikovať pre pasterizované mlieko s vysokou mikrobiologickou kvalitou, ktorá dáva na jednotlivých selektívnych pôdach veľmi nízke počty kolónií (1 až 10).

trvanlivosť; pasterizované mlieko; teplota skladovania

V posledných rokoch nastali významné zmeny vo zvozu surového mlieka z poľnohospodárskych podnikov a v jeho následnom skladovaní a spracovávaní. Väčšina surového mlieka sa v súčasnosti rýchlo schladí ihneď po nadojení a je skladovaná pri teplotách pod 7 °C až do zvozu do mliekárne. V dôsledku týchto skutočností sa zmenila mikroflóra mlieka a prevládajúcimi baktériami v mlieku sú psychrotrofné gramnegatívne mikroorganizmy. Mnoho autorov vyšetrovalo povahu týchto psychrotrofných baktérií, sledovali aktivitu ich degradatívnych enzýmov a boli uskutočnené pokusy týkajúce sa vzťahu medzi rozsahom kontaminácie mlieka psychrotrofnými baktériami a zhoršením kvality vyrábaného výrobku (Muir, Phillips, 1984).

Zvlášť v posledných rokoch bolo vyvinutých veľa testov a skúšok na hodnotenie kvality a predpokladanej trvanlivosti mliečnych výrobkov, ktorých princíp sa pohybuje od jednoduchého hodnotenia mikroorganizmov až po komplexné stanovenie ich metabolitov (Bishop, White, 1986). Súčasné prediktívne modely sú väčšinou založené na mikrobiologických kritériách, pretože inštrumentálne metódy určené na meranie zmien ukazovateľov kazenia nie sú ešte stále dostatočne citlivé alebo spoľahlivé. Z praktických dôvodov potrebujeme také funkcie, ktoré plne vyhovujú experimentálnym a vypočítaným údajom rastových pomerov v rozsahu suboptimálnych rastových teplôt psychrotrofných baktérií. V prípade pasterizovaného mlieka to budú teploty okolo 4 a 12 °C (Stepaniak et al., 1995). Nakoľko teplota je jedným z najvýznamnejších faktorov ovplyvňujúcich trvanlivosť pasterizovaných mliečnych výrobkov (Griffiths, Phillips, 1988a), cieľom našej práce bolo overiť postup určovania trvanlivosti pasterizovaného mlieka pri rôznych teplotách skladovania, ktorý uverejnili Griffiths a Phillips (1988b).

MATERIÁL A METÓDY

Pri overovaní modelu predpovedania alebo určenia trvanlivosti pasterizovaného mlieka na základe hodnotenia počiatočného stavu psychrotrofných mikroorganizmov, ktorý opísali Griffiths a Phillips (1988b), sme vyšetrili celkom 30 vzoriek mlieka, z toho 15 vzoriek plnotučného a 15 vzoriek nízkotučného pasterizovaného mlieka získaných postupným nakupovaním v obchodnej sieti.

Z každej vzorky sme odobrali štyrikrát po 10 ml mlieka do sterilných skúmaviek so závitovým uzáverom, ktoré boli inkubované 25 hodín vo vodných kúpeľoch pri teplotách 12, 15, 18 a 21 °C. Zároveň sme každú vzorku rozdelili na dve časti, pričom jednu časť sme uschovali pri teplote 4 °C a druhú pri teplote 10 °C. Týmto spôsobom sme spracovali 14 vzoriek (po 7 vzoriek z obidvoch druhov mliek) ihneď po ich dodaní do laboratória, 8 vzoriek (po štyri vzorky z obidvoch druhov mliek) po predchádzajúcom skladovaní (tzv. predskladovaní) 25 hodín pri teplote 15 °C a 8 vzoriek (po štyri vzorky z obidvoch druhov mliek) po 24hodinovom predskladovaní pri teplote 20 °C. Predskladovaním vzoriek pasterizovaného mlieka pri 15 a 20 °C sme získali vzorky pasterizovaného mlieka s rôznou mikrobiologickou hodnotou.

Hodnotenie sledovaných psychrotrofných baktérií

Po príslušnej inkubácii, ktorá umožňuje lepšie hodnotenie jednotlivých druhov mikroorganizmov, sme hodnotili ich počty rozterom 0,2 ml inokula troch po sebe idúcich riedení (10^0–10^{-2}) na predsušený povrch príslušných selektívnych pôd nasledovným spôsobom: Celkový počet psychrotrofných mikroorganizmov sme stanovovali z mlieka inkubovaného 25 hodín pri teplote 12 °C na agare s odstredeným mliekom, pripraveným z agarového základu pre bakteriológiu (Bacto-agar, Difco) s prídavkom sterilného odstredeného mlieka v množstve 10 %; počet mikroorganizmov *Pseudomonas* spp. z mlieka inkubovaného 25 hodín pri teplote 15 °C na agare s odstredeným mliekom (10%) s prídavkom cetrimidín-fucidincephaloridínu (*Pseudomonas* C-F-C Supplement, Oxoid); počet grampozitívnych kokov z mlieka inkubovaného 25 hodín pri teplote 18 °C na agare s odstredeným mliekom (10%) obohatenom prípravkom Staph./Strep. Selective Supplement (CNA Supplement, Oxoid, obsahuje síran kolistínu a kyselinu nalidixínovú) a konečne počet mikroorganizmov *Bacillus* spp. z mlieka inkubovaného 25 hodín pri teplote 21 °C na agare s odstredeným mliekom (10%) s prídavkom polymyxínu B (*Bacillus cereus* Selective Supplement, Oxoid). Všetky inokulované selektívne pôdy sme inkubovali 25 hodín pri teplote 21 °C.

Výpočet predpokladanej trvanlivosti

Predpokladanú trvanlivosť v hodinách (t) pre jednotlivé vzorky sme vypočítali podľa dvoch rovníc:

a) jednoduchá rovnica

$$t = \frac{1}{[0{,}00621 \,.\, (T - (269{,}55 - 0{,}74\,(\mathrm{CFC}_{15}) - 0{,}11(\mathrm{CFC}_{15})^2))]^2} \qquad [1]$$

kde: T – teplota skladovania mlieka v °K (°K = °C + 273,16)

CFC_{15} – $\log_{10}$ počtu stanoveného z mlieka inkubovaného 25 hodín pri teplote 15 °C na agare s odstredeným mliekom (10%) obsahujúcim *Pseudomonas* C-F-C Supplement

b) komplexná rovnica

$$t = \frac{1}{[0{,}00621(T - (267{,}62 - 0{,}597(\mathrm{MA}_{12}) + 0{,}419(\mathrm{STREP}_{18}) + 0{,}469\,(\mathrm{PMX}_{21}) - 1{,}086\,(\mathrm{CFC}_{15})))]^2}$$

kde: T – teplota skladovania mlieka v °K

MA_{12} – $\log_{10}$ počtu stanoveného z mlieka inkubovaného 25 hodín pri teplote 12 °C na agare s odstredeným mliekom (10%)

STREP_{18} – $\log_{10}$ počtu stanoveného z mlieka inkubovaného 25 hodín pri teplote 18 °C 25 na agare s odstredeným mliekom (10%) obsahujúcim Staph./ /Strep. Selective Supplement

PMX_{21} – $\log_{10}$ počtu stanoveného z mlieka inkubovaného 25 hodín pri teplote 21 °C 25 na agare s odstredeným mliekom (10%) obsahujúcim *Bacillus cereus* Selective Supplement

CFC_{15} – $\log_{10}$ počtu stanoveného z mlieka inkubovaného 25 hodín pri teplote 15 °C na agare s odstredeným mliekom (10%) obsahujúcim *Pseudomonas* C-F-C Supplement

V deň pred, v deň a po dni vypočítanej trvanlivosti jednotlivých vzoriek plnotučného a nízkotučného pasterizovaného mlieka a neskôr v pravidelných intervaloch sme stanovovali CPM, až kým nedosiahol hodnotu 5.10^7 cfu na 1 ml (log 7,5), alebo kým nedošlo k vzniku zmien charakteristických pre kazenie sa mlieka aj bez dosiahnutia uvedeného limitujúceho počtu.

Príprava selektívnych pôd na hodnotenie sledovaných skupín mikroorganizmov, ako aj rovnice použité na výpočet predpokladanej trvanlivosti boli prevzaté z práce, ktorú uverejnili Griffiths a Phillips (1988b).

I. Určovanie predpokladanej trvanlivosti pasterizovaného mlieka pri r ôznych teplotách skladovania v dňoch – Prediction of shelf-life of pasteurized milk at different storage temperatures in days

Pasterizované mlieko[1]	Teplota predskladovania[2]	Vypočítaná trvanlivosť podľa[3]				Skutočná trvanlivosť[4]		Konečný CPM[5] [log cf.ml^{-1}]	
		jednoduchej rovnice[6]		komplexnej rovnice[7]					
		4 °C	10 °C	4 °C	10 °C	4 °C	10 °C	4 °C	10 °C
Plnotučné[8]		15,60	5,14	11,70	4,28	31,00	11,00	7,6253	7,8407
Odstredené[9]		18,93	5,86	13,55	4,79	32,57	10,71	7,2944	7,6512
Plnotučné	15 °C	8,36	3,30	6,73	2,93	15,75	5,25	8,0610	7,7986
Odstredené		18,93	5,86	13,57	4,74	26,25	8,50	6,8388	7,5024
Plnotučné	20 °C	6,15	2,76	4,94	2,42	13,75	4,75	7,9719	8,2287
Odstredené		15,40	5,01	9,89	3,88	16,25	6,00	8,3594	7,0606

[1]pasteurized milk; [2]pre-storage temperature; [3]the shelf-life calculated according to; [4]real shelf-life; [5]final Standard Plate Count; [6]simple equation; [7]complex equation; [8]whole; [9]skim

VÝSLEDKY A DISKUSIA

Priemerné hodnoty vypočítanej trvanlivosti podľa jednoduchej i komplexnej rovnice, ako aj priemerná skutočná trvanlivosť vzoriek pasterizovaného mlieka vyjadrená v dňoch sú uvedené v tab. I. Prirodzený logaritmus priemerných hodnôt sledovaných skupín mikroorganizmov na selektívnych pôdach je uvedený v tab. II. Po štatistickom vyhodnotení rozdielov (Studentov *t*-test) medzi trvanlivosťou vypočítanou podľa jednoduchej a podľa komplexnej rovnice sme zistili, že čím je pasterizované mlieko s horšou mikrobiologickou kvalitou, tým sú rozdiely medzi vypočítanou trvanlivosťou podľa obidvoch rovníc a tiež medzi vypočítanou a skutočnou trvanlivosťou menšie (tab. III). Z výsledkov týkajúcich sa porovnávania vypočítanej a skutočnej trvanlivosti vidieť, že v prípade pasterizovaného mlieka s vysokou mikrobiálnou kvalitou skutočná trvanlivosť je pri dodržaní teploty skladovania 4 alebo 10 °C až dvakrát dlhšia než je predpokladaná trvanlivosť. Napr. predpokladaná trvanlivosť plnotučného pasterizovaného mlieka pri teplote skladovania 4 °C vypočítaná podľa jednoduchej rovnice berúcej do úvahy len hodnotenie pseudomonád je 15,60 dní, zatiaľ čo skutočná trvanli-

II. Priemerné hodnoty (log cfu.ml^{-1}) sledovaných skupín mikroorganizmov zistených na selektívnych médiách – Average values (log cfu.ml^{-1}) of monitored microbial groups determined on selective media

Mikroorganizmus[1]			15 °C		20 °C	
	plnotučné[2]	odstredené[3]	plnotučné	odstredené	plnotučné	odstredené
MA	1,6020	0,8129	5,7038	5,6989	6,8460	5,8513
CFC	3,0000	0,0000	5,5602	0,0000	6,2771	4,8926
Strep.	0,0000	0,0000	0,0000	0,0000	1,4771	2,0969
PMXB	3,2041	3,1613	6,5729	5,9897	6,7678	6,7684

MA – agar s odstredeným mliekom (10%) – milk agar with skim milk (10%)

CFC – agar s odstredeným mliekom (10%) obsahujúci C-F-C Supplement – milk agar (10%) containing C-F-C Supplement

Strep. – agar s odstredeným mliekom (10%) obsahujúci Staph./Strep. Selective supplement – milk agar (10%) containing Staph./Strep. Selective Supplement

PMXB – agar s odstredeným mliekom (10%) obsahujúci *Bacilluss cereus* Selective Supplement – milk agar (10%) containing *Bacillus cereus* Selective Supplement

[1]microorganism; [2]whole; [3]skim

III. Hladina významnosti, na ktorej sa porovnávané súbory štatisticky významne líšia – Significance level at which the compared sets are statistically different

Pasterizované mlieko[1]	Teplota predskladnenia[4]	4 °C	10 °C	4 °C		10 °C	
		j. r.–k. r.	j. r–k. r.	j. r.–sk. t.	k. r.–sk. t.	j. r.–sk. t.	k. r.–sk. t.
Plnotučné[2]		0,2	0,2	0,05	0,02	0,05	0,05
Odstredené[3]		0,002	0,002	0,01	0,002	0,002	0,002
Plnotučné	15 °C	N	N	0,2	0,1	0,2	0,05
Odstredené		0,1	0,1	0,2	0,05	0,002	0,002
Plnotučné	20 °C	N	N	0,1	0,05	0,05	0,01
Odstredené		N	N	N	0,2	N	0,2

j. r. = predpokladaná trvanlivosť vypočítaná jednoduchou rovnicou pri danej teplote – prediction of shelf-life according to simple equation

k. r. = predpokladaná trvanlivosť vypočítaná komplexnou rovnicou pri danej teplote – prediction of shelf-life according to complex equation

sk. t. = skutočná trvanlivosť – real shelf-life

N = rozdiel medzi porovnávanými súbormi nie je štatisticky významný – not statistically different

[1]pasteurized milk; [2]whole; [3]skim; [4]pre-storage temperature

vosť dosahovala priemerne 31 dní. Podobne pri teplote skladovania 10 °C bola tým istým spôsobom vypočítaná trvanlivosť v dĺžke 5,14 dní, kým skutočná trvanlivosť predstavovala až 11 dní.

Pri určovaní predpokladanej trvanlivosti postupom, ktorý berie do úvahy komplexné hodnotenie mikroorganizmov (komplexná rovnica), rozdiel medzi dosiahnutou a predpokladanou trvanlivosťou je ešte výraznejší. To znamená, že overovaný model značne podhodnocuje trvanlivosť pasterizovaného mlieka s vysokou mikrobiálnou kvalitou.

Naproti tomu pri hodnotení predpokladanej trvanlivosti pasterizovaného mlieka horšej mikrobiologickej kvality, ktoré sme získali tzv. predskladovaním pri teplote 15 alebo 20 °C po dobu 24 hodín vidieť, že rozdiely medzi predpokladanou a skutočnou trvanlivosťou sú zanedbateľné. Z tab. I je zrejmé, že u odstredeného mlieka predskladovaného pri teplote 20 °C je rozdiel medzi skutočne dosiahnutou trvanlivosťou a trvanlivosťou vypočítanou podľa obidvoch rovníc pre obidve skladovacie teploty (4 a 10 °C) nie väčší ako 1 deň (t.j. 24 hodín).

Na základe získaných výsledkov pri overovaní postupu na určovanie predpokladanej trvanlivosti pasterizovaného mlieka sme dospeli k záveru, že tento postup je potrebné modifikovať pre pasterizované mlieko s vysokou mikrobiologickou kvalitou, ktoré dáva na jednotlivých selektívnych pôdach veľmi nízke počty (0–10 kolónií) (Gašincová et al., 1994).

Vývoj prediktívnych metód na určovanie potenciálnej trvanlivosti mliekárenských výrobkov je dôležitou výskumnou aktivitou mliekárenského výskumu. Aplikácia prediktívnej mikrobiológie by mohla zaručiť ekonomickejšiu výrobu, výrobu menšieho množstva neštandardných výrobkov a mohla by poskytnúť viac informácií týkajúcich sa rozhodnutia, aký výrobok má byť vyrobený zo surového mlieka (Stepaniak, 1991).

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Došlo 16. 3. 1995

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Potrav. Vědy, *14*, 1996 (2) : 141–150

MINIMALIZÁCIA OBALOVÝCH ODPADOV

Minimization of Packaging Waste

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Abstract: One of the means of maintaining utility features of a product is packaging which substantially adds to creation of image of the given product. Therefore, the packages have been gradually added to the most important products of everyday consumption and the volume of their production has been increasing. Innovation trends in packaging technique have substantially developed. While in the 60s, which are considered the golden age of packaging technique, attractiveness and functional features in addition to essential protective function of packaging were of primary importance, in the 70s after energy crisis and in the 80s at the beginning of ecological crisis, emphasis started to be put on energetic and ecological aspects. Enormous increase in production and consumption of plastic covers (since 1977 by 200 %) leading to the increase of the volume of solid waste, which is usually difficult to degrade, enforced the gradual introduction of very strict regulations. In 1987 EC countries introduce the directive concerning covers, which should decrease the energy consumption in their production, increase the percentage of returnable packages and introduce efficient recycling. In the paper they include specific requirements of EU about the packing materials. In their effort to minimize package wastes numerous countries have elaborated various systems for their reduction. The article describes and analyses in detail German "dual system", Swedish deposit system and French system "Eco-Emballages" leading to minimization of wastes.

packaging; packaging technique; ecology; recycling; waste dumps; waste incineration; minimization of waste; deposit; deposit system; dual system.

Abstrakt: Jedným z prostriedkov uchovania úžitkových vlastností výrobku je obal, ktorý výrazne prispieva i k vytváraniu image daného výrobku. Preto sa postupne obaly zaradili k najdôležitejším výrobkom dennej spotreby, rastie ich objem výroby. Inovačné trendy obalovej techniky prešli dlhým vývojom. Zatiaľ čo v 60. rokoch, ktoré sú považované za zlatý vek obalovej techniky, bola vedľa základnej ochrannej funkcie prvoradá príťažlivosť a funkčnosť obalu, po energetickej kríze v 70. rokoch a začiatku ekologickej krízy v 80. rokoch sa začína

klásť dôraz na energetické a ekologické aspekty. Obrovské zvýšenie výroby a spotreby plastových obalov (od roku 1977 o 200 %), vedúce k nárastu množstva tuhého odpadu, naviac väčšinou málo degradabilného, si vynútilo postupné prijatie veľmi tvrdých opatrení. V krajinách ES v roku 1987 vstúpila do platnosti smernica o obaloch, ktorá má okrem iného znížiť spotrebu energie pri ich výrobe, zvýšiť percento návratných obalov a zaviesť dôkladnú recykláciu. V článku sú zahrnuté špecifické smernice o obalových materiáloch EÚ. V snahe minimalizovať obalové odpady si mnohé krajiny vypracovali rôzne systémy na ich redukciu. Článok popisuje a podrobne rozoberá nemecký „duálny systém", švédsky depozitný systém a francúzsky systém „Eco-Emballages" vedúce k minimalizácii odpadov.

obaly; obalová technika; ekológia; recyklácia; skládkovanie; spaľovanie odpadu; minimalizácia odpadu; depozit; depozitný systém; duálny systém

Ľudia ako spotrebitelia potravinárskych produktov majú určité požiadavky na obaly, ktoré spotrebúvajú. Požadujú, aby bol obal bezpečný, hygienicky nezávadný a schopný udržať kvalitu tovaru bez použitia čo najmenšieho množstva konzervačných látok. V poslednom čase sa čoraz dôležitejším stáva i to, že spotrebitelia súčasne vyžadujú, aby boli obaly prijateľné aj z hľadiska životného prostredia. Najmä vo vyspelých krajinách sa čoraz častejšie stretávame s pojmom „zelená revolúcia", ktorý úzko súvisí s rastúcim spotrebiteľským tlakom na používanie obalov, ktoré neohrozujú životné prostredie (Köbunner, 1992).

Pri hodnotení obalu z hľadiska jeho vplyvu na životné prostredie treba zvážiť všetky vplyvy od získania a spracovania nerastných surovín, cez spotrebu energie v priebehu výroby a distribúcie obalov, emisie do ovzdušia a vody až po spracovanie odpadu rôznymi spôsobmi (recykláciou, skládkami, spaľovaním a pod.). Len takouto súhrnnou analýzou môžeme dospieť k celkovým vplyvom rozličných obalových systémov na životné prostredie.

Vo vyspelých krajinách môžu byť nové obaly dané do obehu jedine vtedy, ak je možné buď ich viacnásobné využitie, alebo je ich ľahké znovu zhodnotiť, t. j. vopred musí byť vybudovaný systém recyklácie. Vo väčšine štátov existujú pre spôsob zhodnocovania obalov právne podklady.

Dôležitý prínos k otázke problému „Hospodársky rast versus ekológia" priniesla Maastrichtská dohoda o Európskej únii zo 7. 2. 1992, ktorá zakot-

vuje „Podporu neustáleho a životné prostredie neohrozujúceho hospodárskeho nárastu založeného na trvalom rozvoji v súlade s potrebami životného prostredia".

Zatiaľčo európsky súdny dvor v predchádzajúcej judikatúre vychádzal z toho, že odpady treba v zásade považovať za tovar, nová judikatúra – založená na poznaní, že budúcim opatreniam Spoločenstva na základe Maastrichtskej zmluvy v dôsledku právnej normy 130 R (ochrana životného prostredia) už nehrozí nebezpečenstvo, že zúčastnené štáty budú blokovať proces ich odsúhlasovania – dospela k názoru, že princípy voľného obehu tovaru sa dajú čoraz menej použiť pre odpady. Napríklad podľa rozhodnutia zo dňa 9. 7. 1993, RS 2/93 „Odpad nie je tovarom. Opatrenia Spoločenstva týkajúce sa odpadového hospodárstva podliehajú ochrane životného prostredia".

Spomínaná Direktíva Európskej únie má za cieľ harmonizovať národné opatrenia týkajúce sa obalového hospodárstva a odpadového hospodárstva v oblasti obalov, aby sa znížil ich dopad na životné prostredie, prispieť ku kompetencii a fungovaniu vnútorného obchodu a vyhnúť sa prekážkam v obchodovaní skomolením a obmedzením kompetencie únie.

Na dosiahnutie týchto cieľov Direktíva ustanovuje úlohy a hlavné požiadavky, ktoré balenie musí spĺňať a predkladá opatrenia na prevenciu pri výrobe obalového odpadu a na zabezpečenie operácií na návrat, opätovné použitie a obnovu v spojitosti s balením a obalovým odpadom, aby sa zabezpečilo zdravie obyvateľstva a ochrana životného prostredia.

Direktíva sa týka celého balenia v obchode v Únii a celého obalového odpadu bez ohľadu na to, či je použité alebo vzniklo na úrovni priemyslu, obchodu, úradov, predajní, služieb alebo domácností, bez ohľadu na použitý materiál a bez ohľadu na to, či ide o primárne, sekundárne alebo terciárne balenie.

Vysoký podiel obalov v tuhom domovom odpade bol v uplynulých rokoch široko diskutovaný v rámci Európskej únie a výsledkom asi štvorročného procesu rokovania je Smernica Európskeho parlamentu a Rady z 20. 12. 1994 č. 94/62/EC, o obaloch a obalovom odpade (ďalej len smernice o obaloch). Táto smernica o.i. stanovuje niektoré vlastnosti obalov, pokiaľ ide o obsah toxických látok, označenie obalov a hlavne stanoví ciele pre zhodnocovanie obalového odpadu a recykláciu jednotlivých typov obalového odpadu.

Smernica stanovuje, že:

- 50–65 % (váhovo) obalového odpadu bude zhodnotené (priame spaľovanie sa nepovažuje za zhodnocovanie, využitie ako paliva áno);
- 25–45 % obalového odpadu bude recyklovaných a pri tom najmenej 15 % každého obalového materiálu.

Americká Agentúra na ochranu životného prostredia (Environmental Protection Agency – EPA) identifikovala štyri možné varianty minimalizácie odpadu:

1. redukcia zdrojov,
2. recyklácia,
3. spaľovanie odpadu spojené s úsporou energie,
4. skládkovanie.

Tieto možnosti sú zoradené podľa priority, no v skutočnosti sa využívajú v rôznych kombináciách v závislosti od miestnych podmienok.

Redukcia zdrojov spočíva v minimalizácii odpadu prostredníctvom spotreby menšieho množstva materiálov a energie, t.j. v novom riešení obalov s redukovaným využitím niektorých materiálov (napr. hliníka).

Recyklácia je ďalším spôsobom redukcie množstva odpadu, ktorá však musí byť aj ekonomicky odôvodnená. Recyklácia akéhokoľvek odpadu by sa mala uskutočňovať len vtedy, ak hodnota recyklovaných zdrojov prevyšuje vplyv na životné prostredie a náklady spojené so zberom a spracovaním tohto odpadu (Nentwig, 1991).

Spaľovanie pevného odpadu by sa malo uskutočňovať len vtedy, ak dochádza k redukcii objemu pevného odpadu o viac ako 70 %, pričom by sa mali využívať moderne vybavené pece a zariadenia neznečisťujúce ovzdušie. Vo Švédsku napr. spaľovanie odpadu poskytuje významné množstvo elektrickej energie a pary pre systém priameho vykurovania. Týmto spôsobom dochádza k redukcii spotreby nenahraditeľného pevného paliva.

V súčasnosti stojí mnoho krajín pred vážnym problémom, ako sa vysporiadať s obalovým odpadom, pretože skládky majú obmedzenú kapacitu a skládkovanie odpadu sa javí ako najjednoduchší spôsob spracovania odpadu. Mnohé skládky sa uzatvárajú, pretože nevyhovujú čoraz náročnejším požiadavkám ochrany životného prostredia. Jednou z možností je i lepšie využitie recyklácie obalového odpadu.

Systém recyklácie a opätovného využitia obalov možno z hľadiska maloobchodnej jednotky rozdeliť takto:

1. Systém recyklácie bez depozitu, pričom sa zber uskutočňuje mimo maloobchodnej jednotky. Tento systém sa u nás využíva nedostatočne a existujú obrovské rezervy v jeho uplatnení.
2. Systém recyklácie s depozitom, so zberom v maloobchodnej jednotke. Využíva sa vo veľkom vo vyspelých krajinách, napr. recyklácia hliníkových plechoviek na nápoje.
3. Systém znovupoužitia s depozitom, so zberom v maloobchodnej jednotke. Napr. u nás využívaný systém zálohovania sklenených fliaš a ich sústreďovanie v maloobchodnej jednotke.

Ak je depozit vhodne stanovený, môže byť základom vysokej návratnosti obalov. Pre maloobchodníka sú však často výhodnejšie nenávratné jednorazové obaly, pretože návratný systém je náročný na skladový priestor, spotrebu práce, a to bez tomu zodpovedajúceho zisku. Samozrejme, že je to aj otázkou veľkosti predajne. Vo veľmi malých predajniach môže byť návratný systém veľkým problémom, zatiaľ čo vo veľkopredajniach sa tento môže dobre realizovať. To znamená, že v podstate existuje rozpor medzi prvoradými záujmami maloobchodu a cieľmi v ochrane životného prostredia. Aby sme vylúčili tento rozpor, je nevyhnutné podporovať recykláciu materiálov mimo maloobchodnej jednotky. Recyklácia so zberom v maloobchodnej jednotke si takisto vyžaduje priestor, ale obyčajne nie až v takom rozsahu, pretože je často možné recyklovateľný materiál stlačiť (napr. hliníkové plechovky) alebo sústreďovať vo forme črepín (sklenené obaly).

V našich súčasných podmienkach by bolo možné využiť v podstate dve formy separácie domového odpadu priamo v domácnostiach, alebo separáciu uskutočniť až po zvoze domového odpadu. Druhá alternatíva je menej výhodná, nakoľko je nákladnejšia. V nadväznosti na separáciu odpadu by bolo však nutné vypracovať príslušné technológie spracovania vyseparovaných obalov.

V nasledujúcej časti sa sústredíme na niektoré príklady podpory minimalizácie obalových odpadov:

1. Nemecký Duálny systém.
2. Švédsky systém recyklácie hliníkových plechoviek, t.j. švédsky depozitný systém hliníkových nápojových obalov.
3. Francúzsky model označovaný ako ECO Emballages (eko obaly).

Nemecký Duálny systém

Na základe rozhodnutia nemeckej vlády o regulácii redukcie obalových odpadov sa v roku 1990 začal uplatňovať Duálny systém (zákon o odpadoch je v Nemecku platný od roku 1986).

Duálny systém znamená zber, spracovanie a recykláciu obalových materiálov, ktoré sa využívajú za účelom predaja tovarov. Tento systém sa realizuje paralelne s existujúcim systémom riadenia komunálneho odpadu. Je samostatný, nespadá pod federálnu právnu úpravu. Materiály zbierané týmto systémom sú separované do troch skupín:

– recyklovateľné materiály,
– materiály, ktoré sú potom spaľované („termálna recyklácia"),
– nerecyklovateľný zvyšok.

Do Duálneho systému nepatrí systém návratných obalov. Cieľom Duálneho systému je poskytnúť systém zberu recyklovateľných materiálov, a to predovšetkým priamo v domácnostiach. Plánovanie a realizácia Duálneho systému je organizovaná združením Duales System Deutschland GmbH, založeným v septembri 1990 95 podnikmi z oblasti obchodu, priemyslu (výrobcovia obalov) a dodávatelia surovín. V súčasnosti je v združení asi 200 podnikov.

Duálna firma je zodpovedná za:

1. zber obalov od spotrebiteľov, triedenie a odovzdanie (bezplatné) priemyselným podnikom na spracovanie a recykláciu materiálov,
2. akceptácia obalov do Duálneho systému – výrobca sa zaväzuje, že materiál môže byť a bude recyklovaný – akceptovaný obal je označený „zelenou bodkou",
3. financovanie zberu, triedenie a označovanie, ako aj poskytovanie informácií o systéme.

Duálny systém je financovaný prostredníctvom „zelenej bodky" (grüne Punkt) (Kováčiková, 1992), ktorá označuje všetky obaly patriace do tohto systému. Podnik, ktorý predáva balené produkty, môže toto označenie použiť len vtedy, keď zaplatí určitý poplatok. Pritom nie je nevyhnutné, aby sa podnik stal členom združenia Duálneho systému. „Zelenú bodku" môže získať nemecká i zahraničná firma. Tým, že sa určitá predajňa stane členom združenia, zaručuje, že bude väčšinou predávať produkty s obalmi označenými „zelenou bodkou". Toto označenie je pre spotrebiteľa symbolom odlišujúcim obaly, ktoré sú súčasťou zberu v rámci Duálneho systému, od

ostatných obalov. Spotrebiteľ totiž predstavuje rozhodujúci prvok v realizácii Duálneho systému. Na jednej strane je to práve spotrebiteľ, ktorý vyhľadáva produkt v určitom obale. Len vtedy, keď si väčšina spotrebiteľov bude vyberať tovary označené „zelenou bodkou" a odmietať tovary s neoznačeným obalom, bude sa viac firiem snažiť získať tento symbol. Financovanie Duálneho systému je podporované práve týmto spôsobom. Na druhej strane úspech Duálneho systému veľmi závisí od motivácie spotrebiteľov, aby prijali systém zberu recyklovateľných materiálov a poskytovali správne roztriedené materiály.

Združenie Duálneho systému súčasne uzavrelo dohody na vysokej úrovni s rôznymi priemyselnými odvetviami využívajúcimi druhotné suroviny pri svojej produkcii. Tieto odvetvia zaručujú, že budú akceptovať roztriedený druhotný materiál.

Financovanie Duálneho systému sa teda uskutočňuje prostredníctvom poplatkov za získanie „zelenej bodky". Tento poplatok však pokrýva len náklady na zber a triedenie materiálu. Náklady na recykláciu hradí priemysel a tieto sú zahrnuté do ceny rozličných druhov obalov, čo sa odrazí v raste cien tovaru. Zisk je nulový. Podnikom spracovávajúcim druhotné suroviny sa neplatí žiadny poplatok.

Duálny systém sa začal najskôr uplatňovať len v niektorých oblastiach, predovšetkým v husto osídlených a v oblastiach s vážnymi ekologickými problémami. Do konca roku 1995 sa plánuje vytvoriť celoštátny systém zberu so súčasným zákazom používania nerecyklovateľných obalov.

Treba však poukázať aj na nedostatky Duálneho systému, ktoré spôsobujú, že nemožno jednoznačne kladne odpovedať na otázku, či bude Duálny systém podporovať minimalizáciu obalového odpadu, a to z viacerých dôvodov:

- poplatok za jeden obal je príliš nízky,
- spotrebiteľ bude musieť nakoniec tento poplatok zaplatiť, čo sa odrazí v raste cien produktov,
- Duálny systém neobsahuje požiadavky na výrobný proces.

Švédsky depozitný systém hliníkových obalov

Vo Švédsku sa tento depozitný systém využíva od roku 1984. Za koordináciu a riadenie depozitného systému je zodpovedná firma AB Svenska Retur-

pack, ktorej vlastníkmi sú výrobcovia nealkoholických nápojov, pivovary, obchodné firmy a firmy vyrábajúce plechovky.

Do depozitného systému patria hliníkové plechovky od piva a nealkoholických nápojov všetkých veľkostí. Obaly výrobkov určených na export nie sú zahrnuté do tohto systému.

Depozitný systém spočíva v tom, že výrobca plechoviek alebo dovozca nápojov ukladá depozit na všetky návratné plechovky, ktoré sú distribuované v krajine a tento uhradí firme Returpack. Depozit potom prechádza podobne ako návratná plechovka všetkými štádiami od pivovarov, resp. výrobcov nealkoholických nápojov, cez maloobchod ku konečnému spotrebiteľovi. Maloobchodná predajňa zahŕňa depozit na návratné hliníkové plechovky do celkovej ceny tovaru, ktorú platí spotrebiteľ. Spotrebiteľovi je depozit vrátený vtedy, ak vráti plechovku na určené miesto, ktorým je obyčajne maloobchod. Plechovky sa z maloobchodu dostávajú opäť do skladov výrobcov nápojov, pričom tieto zaplatia depozit maloobchodu a výrobcovia zasa dostávajú príslušnú sumu od firmy Returpack. Plechovky sa nakoniec dopravujú do podnikov uskutočňujúcich výrobu nových hliníkových materiálov, ktorý tieto dodávajú výrobcom plechoviek. Dopravné náklady výrobcov nápojov a maloobchodných predajní kryje Returpack (Packaging and the environment ...., 1991).

Švédsky výbor na ochranu životného prostredia zaviedol jednotný symbol pre všetky produkty, ktoré sú recyklovateľné. V prípade návratných plechoviek je tento symbol doplnený švédskym textom „Returpack", t.j. návratná plechovka. Tento symbol musí byť umiestnený na obale tak, aby bol ľahko viditeľný. Jeho farba a umiestnenie sa môže líšiť v závislosti od požiadaviek výrobcu.

Dôležitou súčasťou celého procesu realizácie depozitného systému je kontrola. Zárukou depozitného systému sú v podstate vysoké sumy peňazí, ktoré sú v obehu vo forme depozitu i samotnej hodnoty hliníkových obalov. Kontrola a bezpečnosť sú zdôraznené v pravidlách pre maloobchodné predajne a tiež v dohodách, ktoré Returpack uzatvára s pivovarmi a výrobcami nealkoholických nápojov.

Pravidlá, ktorými sa musia riadiť maloobchodné predajne zabezpečujúce realizáciu výrobkov, ktorých obaly sú zaradené do depozitného systému, obsahujú povinnosť maloobchodnej predajne akceptovať všetky hliníkové nápojové obaly nezávisle od značky výrobku a povinnosť uhradiť spotrebiteľovi

pri vrátení depozit. Príjem plechoviek môže byť manuálny alebo mechanizovaný. Automatické zariadenia na kompresiu použitých plechoviek musia byť schválené firmou Returpack ako aj plastové obaly (vrecia), v ktorých sú plechovky skladované. Tieto pravidlá upravujú takisto skladovanie a odovzdanie plechoviek distribútorom, t.j. pivovarom, resp. výrobcom nealkonápojov, ktoré majú povolenie firmy Returpack. Účasť maloobchodnej jednotky v depozitnom systéme je dobrovoľná, ale stanovenie depozitu je povinnosťou, tento musí byť stanovený jednotne pre všetky hliníkové plechovky.

Dohody, ktoré uzatvára Returpack s výrobcami nápojov upravujú podmienky zberu, kontroly, skladovania a dodávky týchto obalov. Zber návratných plechoviek z maloobchodu uskutočňujú obyčajne výrobcovia nápojov súčasne s novými dodávkami tovaru do maloobchodnej jednotky.

Na záver by sme chceli ešte poukázať na výsledky výskumov, ktoré sa uskutočnili v USA a ktoré poukazujú na to, že nápojové obaly predstavujú priemerne len 5–7 % z celkového odpadu. Preto sa depozitný systém, ktorý sa snaží riešiť problém s odpadom, nejaví efektívne, a to z toho dôvodu, že nezahŕňa zvyšných 95 % odpadu. Ide o problém súvisiaci so správaním sa spotrebiteľov.

Oveľa efektívnejším spôsobom redukcie množstva nápojového odpadu sa javí využitie vzdelávacích programov prostredníctvom médií. O tom svedčí aj štúdia zostavená Inštitútom pre aplikovaný výskum v Kalifornii. Táto štúdia skúmala využitie vzdelávacích programov v deviatich štátoch USA a ukazuje, že náklady na tieto programy sú 10-krát nižšie ako sú náklady na zavedenie depozitného systému s cieľom redukovať obalový odpad z nápojov a súčasne vyžadujú 19-krát nižšie náklady, ak vezmeme do úvahy redukciu celého odpadu.

Francúzsky model

Tento model označovaný Eco-Emballages (eko obaly) (Novák, 1995) je liberálny voči jednotlivým spôsobom zhodnocovania obalov a nemá striktne stanovené ciele. Jeho finančné zdroje sú celoštátne, ale realizácia zhodnocovacích programov pre obalový odpad prebieha postupne v spolupráci s oblasťami a obcami, ktoré sa k nej prihlásia. Pri vnútornom pohľade zapadá francúzsky systém do celkovej stratégie zlepšovania nakladania s tuhým domovým odpadom.

Toto nám vlastne potvrdzuje, že minimalizácia odpadu sa musí realizovať kombináciou jednotlivých možných spôsobov s prihliadnutím na miestne podmienky a možnosti. Treba vhodne spájať využitie recyklácie materiálov s vývojom nových obalov, informovanosťou spotrebiteľov, so snahou redukovať nerecyklovateľné obaly na minimum.

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Došlo 27. 3. 1995

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Potrav. Vědy, *14*, 1996 (2) : 151–160

REVIEWS

METHODS OF SO_2 DETERMINATION IN FOODS

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Sulphites belong to chemical compounds added to various plant products, partially processed vegetables and fruits, final food products as well as beverages to prevent, or inhibit the activity of microorganisms and their growth.

Sulphites along with benzoic, sorbic and propionic acids, and the methyl, ethyl and propyl esters of p-hydroxybenzoic acid (parabens) represent the most commonly used preservatives in the food industry. The most widely used preservatives in fruit juices and soft drinks, apart from sulphur dioxide, are sorbic and benzoic acids either individually or as mixtures (Saag, 1988).

"Sulphites" or "sulphiting agents" are terms generally applied to a variety of sulphur-based compounds. The permitted sulphiting agents used by the food industry are listed in Table I. Sulphites have been added to foods as preservative agents for centuries. The use of sulphiting agents or S [IV] oxoanion compounds has been traced to antiquity when SO_2 (from the fumes of burning sulphur) was used by the Egyptians and Romans to cleanse and disinfect wine vessels (Taylor, Bush, 1986).

I. The list of preservatives with their EC code numbers which can be used on food labels as alternatives to the specific names of the preservatives (Official Journal of EC)

Code	Compound	Code	Compound
E 220	Sulphur dioxide	E 224	Potassium metabisulphite
E 221	Sodium sulphite	E 226	Calcium sulphite
E 222	Sodium hydrogen sulphite	E 227	Calcium hydrogen sulphite
E 223	Sodium metabisulphite	E 228	Potassium hydrogen sulphite

The practice of adding sulphites to wine has continued to the present day, and food technologists have developed many of additional applications for these compounds (Walker, 1985). These substances are being applied to maintain the outward appearance of freshness in salad bar vegetables, assure a desirable texture and

colour in instant potatoes, and prevent the unappetizing, but apparently harmless "black spots" formation on shrimp. Sulphites have many functional uses in foods because they are effective bleaching agents, antimicrobials, oxygen scavengers, reducing agents, and enzyme inhibitors.

When added to a food matrix, some of the sulphiting agents bind to the molecules of the food (aldehydes, ketones, sugars, anthocyanins and other pigments, tannins, etc.) and some then do. The portion of the sulphiting agent that does not bind with the food is called "free sulphite". This "free sulphite" is a mixture of SO_2, bisulphite ion, and sulphite ion in dynamic chemical equilibrium. The percentage of each of the three chemical species in the matrix depends upon the pH (acidity) of the food (Wedzicha, 1984). Two forms of bound sulphite have been observed, reversibly bound and irreversibly bound sulphite. Under certain conditions some of the bound sulphite molecules will dissociate (or break apart) and form free sulphite. The portion that dissociates is called reversibly bound sulphite. The portion that does not dissociate is referred to as the irreversibly bound sulphite (Fazio, Warner, 1990). Although sulphites had had a GRAS status (Generally Recognized As Safe) for a long time, recently the use of sulphites in foods has become an issue of concern to regulatory agencies because sulphites have been implicated as initiators of asthmatic reactions. Asthmatic attack can be serious, and the ingestion of foods containing sulphite has been alleged to have caused several deaths in recent years. For these reasons, the practice of adding sulphites to foods that are to be sold or served raw to the public was banned by revocation of the GRAS status for use on fruits and vegetables intended to be served or sold raw to the consumer, except for potatoes and grapes. Also the Food and Drug Administration (FDA) issued the rule on 9 July 1988, which required sulphite declaration on the label of any food containing detectable (10 ppm) amounts of sulphite as well as regulations relevant to the use of $S0_2$ as a fumigant for table grapes.

Sample Preparation

A lot of analytical procedures have been followed to quantify the sulphiting agents, expressed as SO_2 in foods. With regard to the presence of many interference compounds in foods, mainly indirect procedures have been used for SO_2 determination when the most used step for SO_2 separation is its separation from an acidified sample. While free SO_2 can be determined under acid conditions by aspirating a sample by air flowing (Cardwell, 1993), heating to bolling point brings about the release of both free and bound SO_2. For release of bound SO_2 can also be used as the first step an addition of base, i.e. NaOH (Burguera, Burguera, 1988; Bartroli et al., 1991; Falcone, Maxwell, 1992).

Since sulphite is usually labile and can be lost during isolations and separations, its content may be stabilized by reaction with formaldehyde to form a stable deriva-

tive hydroxymethylsulphonate [HMS] (Warner et al., 1990). Similarly, a highly alkaline medium is more suitable with regard to the formation of the doubly charged sulphite anion that is more stable than bisulphide – the dominant sulphite species found at the pH levels of normal foods (Fazio, Warner, 1990).

Traditional Procedures of Determination

The Monier-Wiliams method is the best known indirect procedure, traditionally adopted as the official method in many countries. The sample is dissolved or suspended in a solution of ca 4M hydrochloric acid and refluxed for 100 min while a nitrogen stream is passed through the solution. The liberated SO_2 is swept through a water-cooled condenser to a 3% hydrogen peroxide trap where the SO_2 is oxidized to sulphuric acid for quantitation by titration with base. Sulphuric acid content relates stoichiometrically to SO_2 that distilled from a sample. The modification, when H_2SO_4 reacts with barium to give insoluble barium sulphate is also well-known.

Rothenfusser method (Davídek et al., 1977) is based on the precipitation of benzidine with H_2SO_4, when 1 g benzidine-sulphate corresponds to 0.234 g SO_2. The mass of barium, or benzidine sulphates is determined gravimetrically after separation and drying.

The advantage of the Monier-Williams procedure and its modifications is that these procedures require 50–100 g test portion, because a representative test portion can be taken without extensive comminution, which invariable leads to some loss of very labile sulphite. Rankine method uses aqueous methanol and phosphoric acid in a two-stage analysis developed to separate free and combined sulphites in wine. After the addition of phosphoric acid, nitrogen sweeps out the SO_2 derived from the free sulphite. The sample is then heated to bolling to drive out SO_2 derived from reversibly bound sulphite. SO_2 is determined after its oxidation with hydrogen peroxide, as mentioned in the Monier-Wiliams method (Rankine, Pocock, 1970). Although some carbonyl-bound forms of sulphite, i.e. α-hydroxysulphonates, are relatively stable at low pH levels, this approach has been a successful procedure for separating free and bound sulphites in wine. Later, considerable improvements were made to the original Rankine method (Fujita et al., 1979).

Modified Rankine method replaced the aspiration by an *i*njection system contributed a great deal to the simplification of procedure, being accompanied with an increase in reproducibility. For injection was used air, because the use of an inert gas gave little increase in recovery rate. Free SO_2 was expelled from the sample by bubbling at 0 °C for 30 min. It was confirmed that no bound SO_2 was dissociated under these conditions. The phosphoric acid concentration had an important role in the liberation of sulphite. When 25% phosphoric acid was used, more than 99 % of

free SO_2 was expelled by cold bubbling and more than 99% of bound SO_2 was recovered by heating afterwards for 10 min.

A principle of Ripper titration method is based on the titration of samples with iodine solution. While free SO_2 is determined directly, total SO_2 is determined iodometrically 15 min after alkalization with NaOH. But, this method is not recommended by the Association of Official Analytical Chemists (AOAC) with regard to poor precision and a large systematic error (Wiliams, 1992).

Gas Chromatography

A combination of head-space technique and gas chromatography was applied to the separate determination of free and bound sulphites in foods (Hamano, 1979). Tartaric acid was shown to be an excellent extractant for selective extraction of free sulphite in the presence of bound sulphite, whilst an alkaline extractant containing potassium-sodium tartarate and ferrous sulphite (deoxidant) liberated free and bound sulphite completely. Sulphite was released from each solution adding 10% phosphoric acid to filtered sample in a tightly stoppered tube. The mixture was shaken, kept for 10 min at 0 °C, then again shaken vigorously for 10 s. Immediately after, 1 ml of head-space gas was withdrawn into a gas-tight syringe and injected into a gas chromatograph at these conditions: glass column filled with APS-1000) column temperature 70 °C, temperature of flame photometric detector 200 °C. Sulphite was determined by means of a calibration curve, and bound sulphite was calculated by the difference between total and free sulphites. Recoveries of 10-20 ppm of free sulphite and 100-200 ppm of bound sulphite from dried pineapple, dried apricot, white and red wines were 92.5-104.4 and 93.5-98.0%, respectively, the detection limit was 0.5 ppm. Comparing results of the Monier-Wiliams method to the modified Rankine and gas chromatography methods it was found that both methods were valid for the separate determination of free and bound sulphites in most foods, and these methods could be preferred for routine analysis because of their simplicity and speed (Mitsuhatsi, 1979).

A gas chromatography method for the determinatlon of free and bound SO_2 in pale and dark beers was compared to the Monier-Wiliams distillation and colorimetric methods (Moreno, Vega, 1989). Bound SO_2 was decomplexed in alkaline medium in the presence of mercuric chloride and both free and total SO_2 were determined, using headspace technique at these chromatographic conditions: glass column (2 m x 4 mm, i.d.) packed with Carbopak B HT 1000. Injector temperature was 100 °C, and column temperature was isothermal 45 °C. N_2 was used as a mobile phase with a flow rate of 50 ml per min and for the determination a flame photometric detector. As followed from the results, the gas chromatography determination was very suitable for determination with regard to high correlation with both reference methods as well as good reproducibility and rapidity.

High Pressure Liquid Chromatography

Roughly ten years ago, an ion pair high pressure liquid chromatography (HPLC) was introduced for the determination of sulphites in foods (Sullivan, Smith, 1985). The method combined the chemical approach of the Monier-Wiliams technique for liberating the sulphite from the matrix with ion chromatography. As found, the ion chromatographic procedure offered several important advantages over the Monier-Wiliams method, e.g. a lower detection limit (1 ppm) in compare to the Monier-Wiliams method. The analysis time was reduced from 2–3 hr to 25 min and results were not affected by the presence of volatile acids, or even organic sulphur compounds.

The removal of interferences from matrix may also be carried out by headspace technique with following HPLC analysis what makes possible to determine low ppm levels of SO_2 in both liquid and solid foods (Lawrence, Chada, 1987).

After HPLC separation, various detection systems are used for sulphite detection; an electrochemical detection is preferred to detection in UV region (Anderson et al., 1986; Kim et al., 1981; Wiliams et al., 1992) with regard to better selectivity, although direct UV detection (Pizzoferrato et al., 1990}, or even the detection after post-column colorimetric reaction (Warner et al., 1990) can also be used. Fluorimetric detectíon is also possible because a reaction of the formaldehyde-bisulphite complex with 5-amino fluorescein gives a nonfluorescent product. The sulphite is measured indirectly by its suppression of the fluorescence of the reagent. Shandera a Jackson (1993) used for SO_2 detection a differential refractometer after separation on sulphonated styrene-divinyl benzene HPLC column using o-phosphoric acid as mobile a phase.

Some chromatographic conditions of separation as well as sample preparation techniques are shown in Table II.

Flow Injection Analysis

Flow injection analysis (FIA) is now widely used for the determination of various compounds and it is based on the reproducible injection of samples into a flowing stream of a carrier or a reagent solution.

A rapid, relilable method (60 samples/h) for the simultaneous determination of free and total SO_2 in wine has been developed employing a third-generation continuous flow analyzer. The methodology was based on the formation of a coloured compound between analyte, formaldehyde, and p-rosaniline (Dasguta et al., 1980). The principle of the determination lies in drawing up the sample (wine or standard) by the autosampler and splitting with a half going to channel 1 (free SO_2) and the other half going to channel 2 (total SO_2). For the determination of total SO_2 the sample is first made basic with NaOH to liberate the bound SO_2. After this

II. HPLC systems used for determination of sulphites

Type of sample	Sample preparation	Stationary phase	Mobile phase	Detector	Reference
Fruit juices, dried bread, white wine	Monier-Williams distillation	ion-exchange polystyrene with $-NH_3^+$ and $-NR_3^+$ groups	0.0024M Na_2CO_3 0.003M $NaHCO_3$ flow rate 2 ml/min.	conductivity detector	Sulinan, Smith, 1985
Lemon juice, instant mashed potatoes, beer	extraction with Polytron for 1 min. pH 2.0 for free and pH = 8.9 for total sulfite	anion–exclusion	8mM H_2SO_4	electro chemical detection	Kim et al., 1987
White wine	extraction procedure as in [21]	anion exchange resin	0.04% H_3PO_4	electro-chemical detection	Willams et al., 1992

point, the chemistry for both the free and total SO_2 determinatlon is identical. The sample is made acidic with H_2SO_4 to convert all forms of free SO_2 to gaseous SO_2, a portion of which diffuses across a gas-permeable membrane (which separates colour pigments in liquid samples from the colour development section of the manifold) into a stream of 1% H_2SO_4. This stream is mixed with a solution of formaldehyde, and finally this intermediate adduct reacts with p-rosaniline. The resulting colored complex is measured at 550 nm (Falcone, Maxwell, 1992). Continuous flow analysis measured 75-100% of free SO_2 compared to the Ripper iodometric method. Recoveries from sulphite spiked wine samples ranged from 62 to 104 % , with an average precision of 4.5%. p-rosaniline, which is commercially unavailable ln a pure state and thus must be purified, was substituted by p-amino-azobenzene and the red coloured complex was measured at 520 nm (Bartroli et al., 1991).

Similarly, spectrophotometric detection can also be done after the decolourization of malachite green (Sullivan et al., 1990). A chemiluminiscence detection based on the oxidation of the disulphitomercurate complex with cerium (IV) in an acidic solution in the presence of riboflavin sulphate is also possible (Burguera, Burguera, 1988). However, all spectrofotometric detections require the addition of an extra line in the manifold for the colouring reagent as well as commercial gas diffusion modules that are expensive and membranes have limited life time.

To simplify the SO_2 determination by FIA, a new method was described for alcoholic beverages. A dual electrode electrochemical detector eliminates interferences by their reduction at an upstream coulometric electrode (-0.26 V) before the reductive detection of SO_2 at the amperometric electrode (-0.41 V) (Cardwell et al., 1993). This method offers significant advantages over other flow injection procedures:

1. The single channel FIA system is easier to assemble and operate than the more complex manifolds containing a gas diffusion module;
2. The FIA system does not require any modification for the determination of free and total SO_2 in samples;
3. The use of chemical reagents is minimal;
4. The method is also applicable to red wines and maintains the high sample throughput of 30/h with good precision.

Capillary Isotachophoresis

This technique offers a valuable analytical tool with regard to a minimum of sample pre-treatment and relatively short analysis time. Reijenga et al. (1982) determined sulphites in red and white wines. The only preparation step was based on sample diluting with formaldehyde to prevent oxidation of sulphites during the determination. The operational system used as leading electrolyte 0.01M chloride solution with counter ion β-alanine at pH = 2.90 With additive 0.05% Mowiol and 0.2% HEC. Terminating electrolyte was 0.005 sodium propionate. Permitted concentrations of sulphides in the range $0.1–3.1.10^{-3}$ M was easy possible to detect by conductivity detector.

Enzyme Methods

Sulphite oxidase (EC 1.8.3.1.) is readily available and has been used to develop the methodology for sulphites in food. The described procedures are based upon relatively nonspecific UV and oxygen measurements, but a considerable degree of selectivity is realized because of enzyme specificity. Available documentation suggests that the methods are limited to 50 ppm SO_2 (Fazio, Warner, 1990).

The commercially available kit requires approximately 45 min per test. The sulphite in the test portion is enzymatically oxidized by oxygen to form sulphate and hydrogen peroxide, which reacts with reduced nicotinamide-adenine dinucleotite (NADH) in the presence of NADH-peroxidase (Beutler, 1984). The decrease of NADH is proportional to sulphite concentration. Since the NADH level is monitored by measurement of absorbance at 340 nm, the food extract must be purified for interference substances. But, because of poor sensitivity, it is not possible to use the enzyme kit for foods which contain less than 50 ppm sulphite.

Recently, biosensors for the determination of SO_2 have been developed; they may be used as immobilized whole cells, organelles, or enzymes with an oxygen electrode or a platinum electrode. A sulphite-sensing electrode with immobilized sulphite oxidase on the tip was tested (Smith, 1987). The decrease in oxygen due to the enzymatic oxidation was proportional to the sulphite in the test solution. Good correlation with the Monier-Wiliams method was reached with concentrations over 75 ppm SO_2. However, those biosensors were affected by oxygen concentration and temperature.

On the other hand, a pH sensitive glass electrode that is temperature-compensated is not influenced by temperature and oxygen concentration, and it is also more frequently used and cheaper than an oxygen electrode. For these reasons, a biosensor based on the glass electrode was developed to determine free SO_2 in wine (Nakamura et al., 1993). The biosensor consists of a microbial membrane of *Thiobacillus thiooxidants* JCM7814 and a flat glass electrode. A porous gas-permeable membrane is incorporated in the biosensor system to separate free SO_2 from bound SO_2 and to avoid the buffer action of the sample solution. This biosensor gave a linear relationship between pH decrease and concentration of SO_2 up to 50 ppm with a response time of 20 min. The detection limit was 5 ppm, and lifetime of the microbial membrane was ca 30 days at 4 °C. The biosensor could specifically determine free SO_2 in wine with relative standard deviations of 7.4 % for red wines, or 5.0 % for white wines, respectivelly.

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Official Journal of the European Communities. No. 172/27, 1994.

Metódy stanovenia SO_2 v potravinách

Práca sa zaoberá prehľadom metód stanovenia oxidu siričitého ako konzervačnej látky v potravinách. Diskutované sú možnosti stanovenia tradičnými postupmi (Moniers-Wiliamsova, Ropperova a Rankinova metóda), ale aj stanovenia pomocou plynovej chromatografie, vysokotlakovej kvapalinovej chromatografie, prietokovej injekčnej anazýzy a enzýmových metód, vrátane úpravy vzorky na samotné stanovenie.

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Potrav. Vědy, *14*, 1996 (2) : 161–162

ŽIVOTNÍ JUBILEA

Prof. Ing. Ivo Ingr, DrSc., šedesátníkem

Devatenáctého února se dožívá význačného životního jubilea prof. Ing. Ivo Ingr, DrSc. Je to až neuvěřitelné, jak ten čas utíká. Není tomu tak dávno, co ještě navštěvoval vysokou školu, a již oslavuje své šedesátiny.

Narodil se v Kelčanech na Hodonínsku a absolvoval Střední průmyslovou školu konzervárenskou v Bzenci a poté Vysokou školu chemicko-technologickou, Fakultu potravinářské a biochemické technologie v Praze. Studium ukončil ve specializaci konzervace potravin a technologie masa obhajobou diplomové práce na téma Technologicko-ekonomické zhodnocení linek na porcování a balení masa.

Po absolvování vysoké školy v roce 1961 nastoupil do bývalého Jihomoravského průmyslu masného v Brně jako pracovník technické kontroly, poté vedoucí laboratoří, a na tomto pracovišti zakončil svoji technickou kariéru jako vedoucí provozu. Po získání praxe byl na základě konkurzního řízení přijat do Výzkumného ústavu veterinárního v Brně-Medlánkách jako odborný pracovník na oddělení hygieny a technologie potravin živočišného původu. Zde také vypracoval kandidátskou disertační práci na téma Vztahy mezi topografií, složením a stabilitou depotních tuků prasat, kterou obhájil v roce 1971 na VŠCHT v Praze. O dva roky později předložil habiltační práci Zhodnocení metod objektivního posuzování čerstvosti a kažení masa se zřetelem na možnost využití analýzy porfyrinu. V roce 1976 přechází na Vysokou školu zemědělskou v Brně – nejprve jako odborný asistent, v roce 1977 byl jmenován a ustanoven docentem pro obor technologie živočišných produktů. V roce 1983 předložil a obhájil na VŠZ v Praze doktorskou disertační práci Kvalitativní aspekty výroby vepřového masa a získal hodnost doktora zemědělsko-lesnických věd. O dva roky později byl jmenován profesorem.

Prof. Ingr je velmi dobrým vysokoškolským učitelem. Zajišťuje výuku z technologie masa a základů konzervace potravin pro obor Technologie potravin a technologii masa a vajec a hodnocení a zpracování ryb na oboru Zootechnika.

Své vynikající organizační schopnosti využil v řadě akademických funkcí. V současné době pracuje jako proděkan agronomické fakulty Mendelovy zemědělské a lesnické univerzity v Brně.

Prof. Ingr je i mimořádně pilný ve vědecké oblasti. Důkazem toho je kolem 300 odborných a vědeckých prací uveřejněných jak v domácích, tak i v zahraničních časopisech. Je také spoluautorem dvou knižních publikací a 20 vysokoškolských skript. Působí rovněž jako předseda poradního sboru odborného časopisu Maso a je

členem redakční rady našeho časopisu Potravinářské vědy, dále časopisu Výživa a potraviny a sborníku Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis.

Z jeho rozsáhlých odborných a vědeckých aktivit vyjímám jeho členství v České akademii zemědělských věd, kde je místopředsedou odboru Výživa obyvatelstva a jakost potravin. Je dále členem představenstva a členem výkonného výboru Společnosti pro výživu a členem její jihomoravské pobočky, místopředsedou výboru odborné skupiny pro potravinářskou a agrikulturní chemii České chemické společnosti, kde každoročně organizuje semináře zaměřené na jakost potravin a potravinářských surovin. Při těchto akcích, respektive v jejich předvečer, se prof. Ingr uplatňuje i jako dobrý společník, rád si zazpívá a zamuzicíruje s moravskou amatérskou kapelou. Bylo by možné uvést i jeho další aktivity, především jeho členství v různých vědeckých radách, domnívám se však, že vzhledem k jeho rozsáhlé činnosti by tento výčet nebyl stejně úplný.

Vážený příteli, dovol mi, abych Ti jménem přátel popřál k Tvému životnímu jubileu především pevné zdraví, osobní spokojenost a také trochu toho lidského štěstíčka jak v dalším životě, tak i v odborné a společenské práci. Šedesát let je věk, kdy již stojí za to alespoň na chviličku se ohlédnout, pozdravit se s kamarády, zavzpomínat a opět se rychle vrhnout do práce. V tomto věku má již sochař to své dílo z velké části rozpracované, schází jen těch několik mistrných úderů a dílo je hotové. Tedy Ivo, ať se Ti to dílo podaří.

Prof. Ing. Jiří Davidek, DrSc.

Potrav. Vědy, *14*, 1996 (2): 163–164

NEKROLOG

K úmrtí profesora Ing. Milana Bareše, DrSc.

Profesor Bareš se narodil 13. března 1937 v Košicích. Dětství prožil v severních Čechách. Pro svůj vyhraňující se zájem o chemii začal studovat na Střední průmyslové škole chemické v Lovosicích, kterou absolvoval v roce 1956. Odtud pak byla přímá cesta na Vysokou školu chemicko-technologickou v Praze. Studium na Ústavu technologie mléka a tuků úspěšně ukončil v roce 1961.

Po vojenské službě nastoupil do Výzkumného ústavu tukového průmyslu – pracoviště pro detergenty v Rakovníku. Záhy se však vrátil na Vysokou školu chemicko-technologickou v Praze jako řádný aspirant. Jak se ukázalo, byl tento návrat trvalý a studium detergentů již zůstal prof. Bareš věrný. Kandidátskou disertační práci obhájil v roce 1966, přičemž již od roku 1964 působil v Ústavu technologie mléka a tuků jako odborný asistent.

Činnost profesora Bareše jako pedagoga a vědeckého pracovníka byla velmi bohatá a všestranná. Všichni jej máme v paměti jako talentovaného odborníka a mimořádně pilného člověka, pro kterého bylo typické využití každého dne pro práci, které zasvětil celý svůj život, pro výchovu nových inženýrů i pro soustavné vlastní sebevzdělávání. Je však třeba připomenout i lidský rozměr jeho osobnosti. Měl hluboké porozumění pro své spolupracovníky, studenty a své žáky.

Jeho životním posláním se stalo studium vlastností povrchově aktivních látek a detergentů, které se původně odvozovaly od studia tukových látek. Bylo jen logické, že jeho první práce byly zaměřeny na přípravu tenzidů na bázi tukových látek. Je velmi těžké (a nutně bude i neúplné) postihnou celou odbornou práci prof. Bareše. Jeho činnost zasahovala do oblasti analytické i fyzikální chemie a technologie až po studium aplikačních vlastností tenzidů a detergentů.

Chtěli bychom připomenout alespoň některé zásadní milníky vědecké činnosti profesora Bareše. Svoji vědeckou dráhu začal na problematice syntézy esterů mastných kyselin se sacharózou. Více než 20 let práce věnoval syntézám, analytice oxyethylenovaných tenzidů a jejich následným aplikacím. Z oblasti analytiky tenzidů stačí připomenout jeho světově prioritní metodu stanovení mýdel ve skupině aniontaktivních tenzidů metodou dvoufázové titrace v kyselé oblasti. Významný (a v důsledcích i pro technickou praxi) byl jeho přístup k řešení řady problémů za použití polymerních látek, ať již jako heterogenních katalyzátorů, nosičů katalyzátorů, a v poslední době jako komponent pro prací prostředky. V této oblasti zasáhl prof. Bareš významným a originálním způsobem do technické realizace reakcí katalyzovaných imobilizovanými enzymy na polymerních matricích. Asi vůbec jedním z nej-

viditelnějších výsledků jeho práce při aplikaci polymerů byla stabilizace desorpce parfémových kompozic pro mýdla a prací prostředky. Tyto práce byly oceněny státní cenou a dostalo se mu i mezinárodního uznání. Výsledky jeho vědecké činnosti jsou shrnuty v řadě původních vědeckých prací, ve sbornících konferencí a ve více než 50 udělených patentech a autorských osvědčeních. Je málo autorů, kteří se mohou pochlubit tak vysokým stupněm realizace výsledků vědeckotechnické činnosti v praxi, jako byl právě profesor Bareš. V posledních letech života věnoval zvýšenou pozornost oblasti vývoje kosmetických výrobků. Bylo by současně omezené, kdybychom zúžili odbornou činnosti prof. Bareše jen na oblast tenzidů a detergentů. Významným způsobem zasahoval do oblasti chemie a technologie tuků, a to jak do oleochemie, tak do oblasti potravinářské.

Je možné považovat prof. Bareše za jednoho ze zakladatelů sice poměrně úzkého, ale významného vědního oboru o povrchově aktivních látkách a detergentech u nás. Lze bez nadsázky říci, že jeho jméno je v tomto oboru u nás všeobecně uznávanou autoritou a pojmem.

V oboru technologie tuků a detergentů se v důsledku řady okolností habilitoval až v roce 1983, v roce 1988 byl jmenován řádným profesorem na VŠCHT v Praze, čemuž předcházela obhajoba doktorské disertační práce. Jeho pedagogické působení na VŠCHT zahrnuje dlouhý časový úsek více než 30 let. Z perspektivy těchto let je možné konstatovat, že nehledě na jeho pedagogické zařazení, velmi záhy po nástupu na VŠCHT jej všichni znali jako erudovaného odborníka v oboru, který si zvolil, i jako výborného pedagoga. Všichni, kdo navštěvovali jeho přednášky, budou jistě souhlasit, že byly nejen srozumitelné, zajímavé, na úrovni současného poznání, ale že předznamenávaly budoucí vývoj v oboru. Jako vysokoškolský učitel a odborník ovlivnil odbornou úroveň i myšlení generací studentů. Svým osobním příkladem, zapáleností pro obor, který měl rád, ovlivnil hodně budoucích absolventů tak, že i jim se stala problematika tenzidů a detergentů celoživotní náplní. Tak se řada jeho student nejen uplatnila v průmyslu výroby tenzidů, detergentů a kosmetických výrobků, ale z mnohých vyrostli další významní odborníci, kteří s prof. Barešem většinou zůstávali v blízkém odborném a osobním kontaktu po celý život.

Na Ústavu technologie mléka a tuků zastával řadu let funkci tajemníka, na Fakultě potravinářské a biochemické technologie působil po dvě funkční období jako proděkan, jako člen a později jako předseda komise pro státní závěrečné zkoušky. Mimo fakultu byl aktivně činný v odborné skupině pro tuky, detergenty a kosmetickou chemii Československé společnosti chemické, kde působil po řadu let jako předseda pracovní skupiny pro detergenty. Profesor Bareš byl mezinárodně uznávaným odborníkem, což je možné mj. doložit tím, že v minulém roce byl vyzván k účasti na celosvětovém kongresu o tenzidech ve Španělsku jako čestný předseda jedné ze sekcí.

Profesor Bareš odešel. Zanechal po sobě velké celoživotní dílo a výzvu svým následovníkům. My všichni, kdo jsme jej znali, budeme na něj vždy s láskou a s úctou vzpomínat.

Doc. Ing. Vladimír Filip, CSc.

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.

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Opravenka

Prosíme čtenáře, aby si v přehledném článku J. Houšová: Přehled a charakteristika publikovaných modelů mikrovlnného ohřevu potravin, uveřejněném v čísle 6/1995, opravili rovnici [5] na straně 487:

$$\alpha = \frac{2\pi f}{c}\sqrt{\frac{k'}{2}\left(\sqrt{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2}-1\right)} \qquad [5]$$

kde: f – frekvence mikrovln

c – rychlost světla

k' – relativní dielektrická konstanta ($\varepsilon'/\varepsilon_0$)

$\varepsilon', \varepsilon''$ – dielektrická konstanta a dielektrický ztrátový faktor materiálu