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PREDICTION OF THE GROWTH INHIBITION OF *Bacillus stearothermophilus* var. *calidolactis* C 953 DEMONSTRATED AT AN EXAMPLE OF SELECTED ANTIMICROBIALS IN MILK

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Abstract: The size dependence of the inhibition zone on the concentration of selected antibiotics (procaine penicillin G, ampicillin, streptomycin), cleaning agents, disinfectants and preserving agents (NaOH, HCl, Ajatin, PUR, Jodonal M, H_2O_2) in the artificially contaminated milk has been investigated. Also, the degree of the hydrogen peroxide destruction affected by thermal treatment (100 °C/10 min) and by the combination of thermal treatment and storage (100 °C/10 min + 2 h/20 ± 2 °C) has been studied. By using the statistical software product SYSTAT the most convenient model was chosen: $y = a + bx + c \ln x$. The statistical significance of coefficients a, b, c and the model suitability were confirmed at the significance level $\alpha = 0.01$. This model serves to predict the size of the inhibition zone at different antimicrobials concentrations. The applied disk diffusion method with *B. stearothermophilus* var. *calidolactis* C 953 showed to be the most sensitive to beta-lactame antibiotics; the destruction of H_2O_2 was also well degradable in the chosen conditions.

antibiotics; disinfectants; cleaning and preserving agents; disk diffusion method

The EC Directive 85/397 of 1992 (EEC, 1992) stated four fundamental quality raw milk attributes, one of which are antibiotic residues. The detection limits of tests are a decisive criterion of this assessment are (Heeschen, Suhren, 1995).

In the recent time the application of rationalized methods for estimating the inhibition substances in milk such as Delvotest, BR-test, Intest, Penzym, Valio 101 and 102, FDT, SNAP and CHARM tests, etc. of a different provenience and sensitivity to antimicrobials and their mutual comparison, has become more and more frequent (Charm, Zomer, 1995; Suhren, 1993a, b; Reybroeck, 1995; Müller, Jones, 1993). The tests are suitable mainly for the routine analyses in central laboratories for grading of milk and

for a rapid checking of sanitation in dairy plants. In spite of these increasingly widespread testing systems resulting from subjective visual evaluations (hardly perceivable colour change) they continue to be the underlying and comparable standardized (classical) diffusion methods using *Bacillus stearothermophilus* var. *calidolactis* C 953 and distinguishing by a sensitivity to register the maximum residue limits (MRL_s) of antimicrobials.

The procedures of predictive microbiology which are applied nowadays in microbiological laboratories allow an objective evaluation and determination of the impact of environmental conditions on the growth of microorganisms, and moreover, they allow the modification of these conditions by means of the results adapted to mathematical relations (Valík, Görner, 1995).

The objective of this work was to find in the modelling form obtained by regression analysis the dependence of the inhibition growth (inhibition zone in mm) of the indicated strain on the concentration of selected antimicrobials which are commonly used in the veterinary and dairy practice. They included: antibiotics (procaine penicillin G, ampicillin, streptomycin, cleaning agents and disinfectans (NaOH, Ajatin, PUR, Jodonal M) and preserving agents (H_2O_2). In the latter case, the stability of H_2O_2 was also observed under various external conditions (without thermal treatment, after thermal treatment at 100 °C/10 min, and after thermal treatment combined with storage – 100 °C/10 min + 2 h/20 ± 2 °C).

MATERIAL AND METHODS

Bacterial Strain (Culture)

Bacillus stearothermophilus var. *calidolactis* C 953 (obtained in native form from the Institute of Veterinary Medicine, Bratislava, Slovak Republic) was used as a test organism. It was grown at 64 ± 1 °C in tryptone glucose agar (Difco). One day before use it was propagated in tryptone glucose extract broth (Difco). 18–24h inoculum contained of 10^8 cells/ml.

Antibiotics

Procaine penicillin G, ampicillin, streptomycin (Biotika Inc. Slovenská Ľupča, Slovak Republic).

Cleaning Agents, Disinfectants and Preserving Agents

NaOH, HCl (35–38%), Ajatin (quarter ammonium compounds), PUR, Jodonal M, H_2O_2 (28–32%).

The basic solutions served for the preparation of the concentration ranges of selected antimicrobials (Tables I–III) by diluting in the milk powder (MEDMILK Corp., Veľký Meder, Slovak Republic) after reconstitution by sterile diluted water (1 : 9). As a control the reconstituted dried milk without antimicrobials was used.

According to recommendations of IDF (1991) the pH value of reconstituted milk was adhered to over 6; the thermal inactivation of natural inhibitors during 5 min, at 80 °C was also performed.

Analytical Methods

Diffusion Disk Plate Method

Principle: The paper disk (Whatman l, diameter of 12 mm) soaked with the investigated sample is laid on the surface of the agar culture medium (glucose tryptone agar) with *B. stearothermophilus*. The incubation (64 ± 1 °C/4–5 h) during which the growth of the testing strain takes place, leads to turbidity of the agar, medium. If the investigated sample contains substance inhibiting the growth of the tested strain, transparent zones occur around the disk. Their size depends on the various conditions: concentration and the type of an antimicrobial substance, the density of inoculum and the thickness of agar layer, the activity of test organism, etc. In accordance with the STS 57 0331, inhibition zones (mean of three replicates measured by the ruler – from the disk border to the border of transparent zone) were compared to the size of the zones created by the checking penicillin solutions of known concentrations (posit.: ≥ 1 mm, which corresponds to penicillin concentration of 0.01 IU/ml). The choice of antimicrobials and concentration ranges (IU/ml, μg/ml, %) used for the test is shown in Table I.

Statistical Analysis

Regression analysis was carried out using the program SYSTAT (Varga, 1986, 1991, 1996).

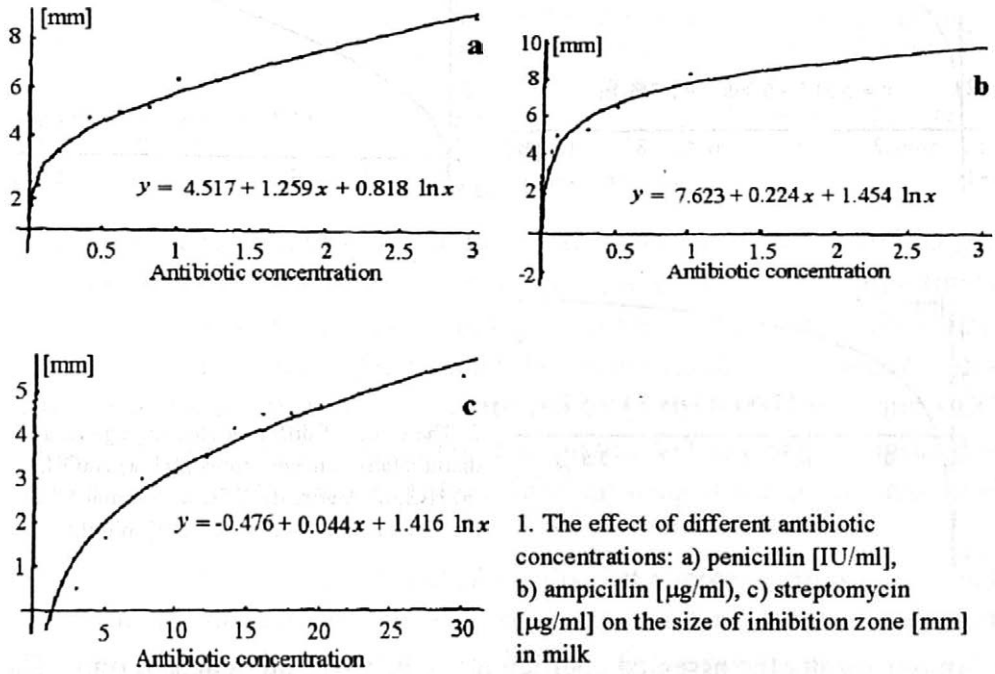
I. Concentration ranges of some chosen antimicrobials

PNC G [IU/ml]	0.003	0.005	0.01	0.02	0.04	0.06	0.08	0.1	0.2	0.4	0.6	0.8	1	3
AMP [μg/ml]	0.005	0.007	0.01	0.03	0.05	0.07	0.1	0.3	0.5	0.7	1	3		
SMC [μg/ml]	1	3	5	7.5	10	12	14	16	18	20	25	3		
NaOH [%]	0.2	0.4	0.6	0.8	1	2	4	6	8	10				
HC1 [%]	0.3	0.5	0.8	1	2	3		5	6	8	10			
Ajatin [%]	0.05	0.08	0.1	0.3	0.5	0.8	1	3	10					
PUR [%]	0.05	0.08	0.1	0.3	0.5	0.8	1	3	10					
Jodonal M [%]	0.1	0.3	0.5	0.8	1	3								
H_2O_2 [%] (untreated)	0.005	0.008	0.01	0.03	0.05	0.08	0.1	0.3	0.5	0.8				
H_2O_2 [%] (heat-treated 100 °C/10 min)	0.005	0.008	0.01	0.03	0.05	0.08	0.1	0.3	0.5	0.8				
H_2O_2 [%] (heat-treated and stored – 100 °C/ /10 min + 2 h/20 ± 2 °C)	0.005	0.008	0.01	0.03	0.05	0.08	0.1	0.3	0.5	0.8				

PNC G – procain penicillin G
AMP – ampicillin
SMC – streptomycin

RESULTS AND DISCUSSION

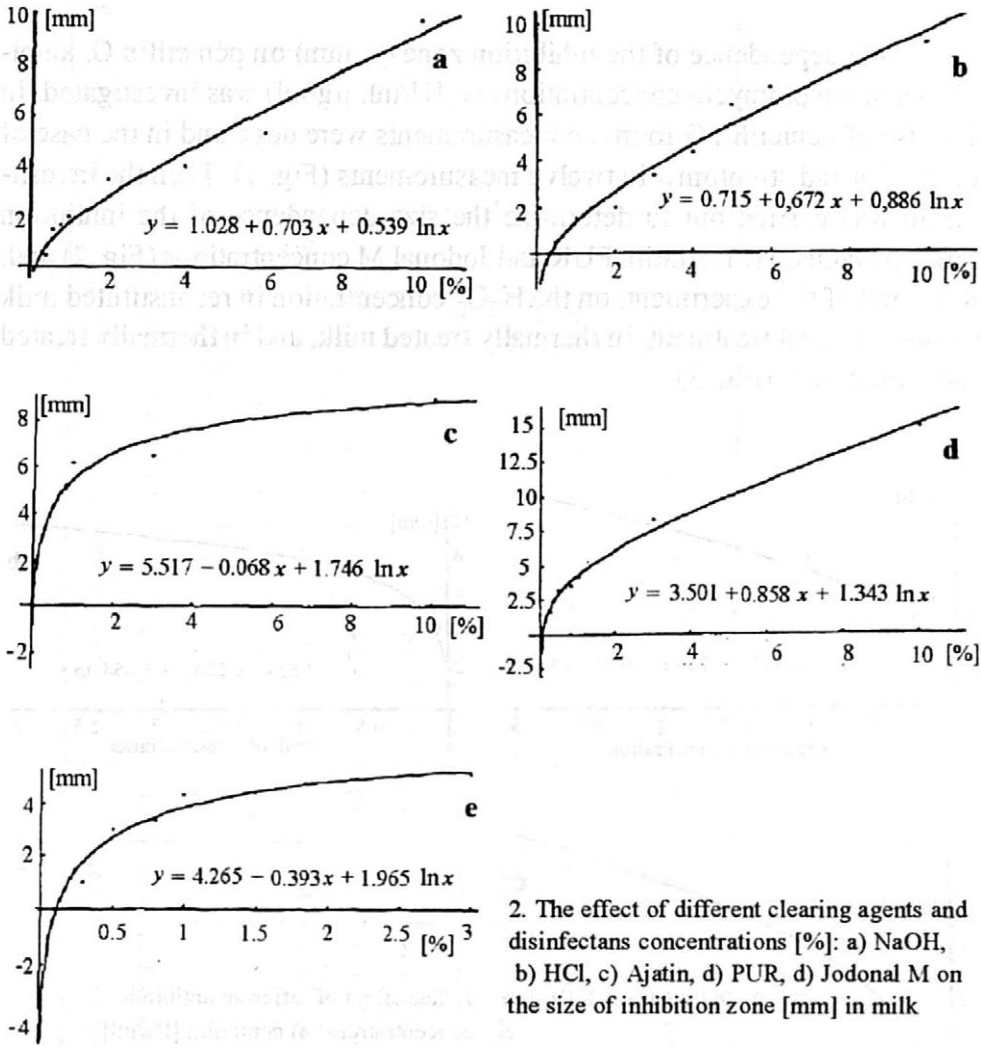
The size dependence of the inhibition zone (y, mm) on penicillin G, ampicillin and streptomycin concentrations (x, IU/ml, μg/ml) was investigated. In the case of penicillin G fourteen measurements were done and in the case of ampicillin and streptomycin twelve measurements (Fig. 1). Then the investigation was carried out to determine the size dependence of the inhibition zone on NaOH, HCl, Ajatin, PUR and Jodonal M concentrations (Fig. 2) and, at the end of the experiment, on the H_2O_2 concentration in reconstituted milk without thermal treatment, in thermally treated milk, and in thermally treated and stored milk (Fig. 3).



1. The effect of different antibiotic concentrations: a) penicillin [IU/ml], b) ampicillin [μg/ml), c) streptomycin [μg/ml] on the size of inhibition zone [mm] in milk

Using the statistical software product SYSTAT a variety of models complying with eleven dependence examined was tested and as the best model was chosen

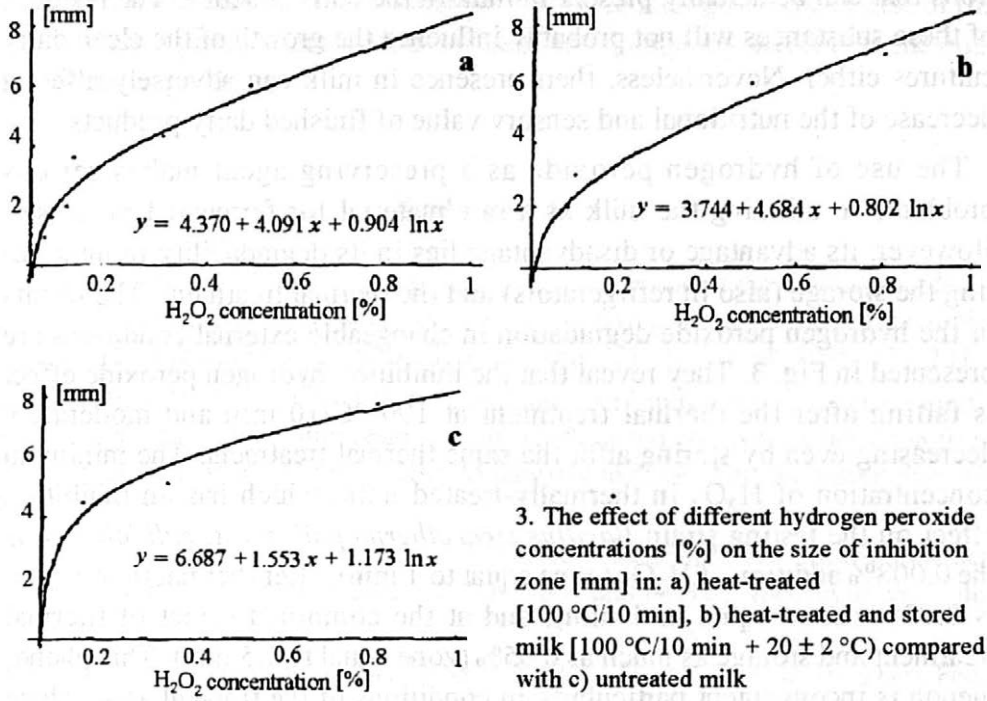
$$y = a + bx + c \ln x$$



2. The effect of different clearing agents and disinfectans concentrations [%]: a) NaOH, b) HCl, c) Ajatin, d) PUR, d) Jodonal M on the size of inhibition zone [mm] in milk

In our results the assessed coefficients *a*, *b*, *c* will be indicated only. The complete output from the computer can be obtained from the authors of the work.

In view of the applied diffusion method for sensitivity to investigated antimicrobials it follows from our experiments (corresponding to the existing findings) that the testing strain *B. stearothermophilus* var. *calidolactis* C 953 displays the highest sensitivity just to antibiotics of the beta-lactame



3. The effect of different hydrogen peroxide concentrations [%] on the size of inhibition zone [mm] in: a) heat-treated [100 °C/10 min], b) heat-treated and stored milk [100 °C/10 min + 20 ± 2 °C) compared with c) untreated milk

group (a detection limit for penicillin G at the concentration of 0.005 IU/ml; for ampicillin 0.007 μg/ml). Our results are comparable to detection limits shown in the literature (Ryšánek, Schlegelová, 1993); they also conform to limits of the highest admissible quantities of the residues of inhibition, substances in milk which are indicated by IDF (1991) and EEC (1992). Streptomycin as an antibiotic belonging to the group of aminoglycosides prevents the indicated strain from growing at the concentration of 3–4 μg/ml.

It followed from our experimental results that streptomycin was detected with a much higher intensity than it is shown in literature. The detection limit of 4.0–5.0 μg/ml achieved by the standardized disk assay was approximately twice lower than that described in literature (IDF indicates the detection limit as high as 13.0 μl/ml of milk).

Fig. 2 shows the detected inhibition concentrations of cleaning agents and disinfectants. Though the sensitivity of the diffusion disk method to their presence is incomparably higher than that of other tests, e.g. Penzym (Kurek et al., 1990), it does not registrer, however, much lower concentra-

tions that can be actually present in milk in the dairy practice. The residues of these substances will not probably influence the growth of the clean dairy cultures either. Nevertheless, their presence in milk can adversely affect a decrease of the nutritional and sensory value of finished dairy products.

The use of hydrogen peroxide as a preserving agent makes serious problems in utilizing the milk as a raw material for fermented processes. However, its advantage or disadvantage lies in its degradability in milk during the storage (also in refrigerators) and the thermal treatment. The results of the hydrogen peroxide degradation in changeable external conditions are presented in Fig. 3. They reveal that the inhibition hydrogen peroxide effect is falling after the thermal treatment at 100 °C/10 min and moderately decreasing even by storing after the same thermal treatment. The minimum concentration of H_2O_2 in thermally-treated milk, which has an inhibition effect on the testing strain *Bacillus stearothermophilus* var. *calidolactis*, is the 0.008% addition of H_2O_2 (zone equal to 1 mm), after thermal treatment it is 0.03% (zone equal to 1 mm), and at the combined effect of thermal treatment and storage as much as 0.05% (zone equal to 1.5 mm). This phenomenon is inconvenient particularly in conditions of the tropical zone where the hydrogen peroxide is accepted as a preserving agent to prevent the souring of milk; its addition is useless during the decomposition at high temperatures. On the other hand, in conditions of the temperate zone this is a convenient property, inasmuch as the use of hydrogen peroxide as a preserving agent is not permitted there.

The indicated procedure applied to a short list of antimicrobials has permitted us to evaluate the range of the inhibition action of chosen concentrations on the tested strain *B. stearothermophilus* var. *calidolactis* C 953; the applied method has shown to be sufficiently sensitive to retaining the maximum admissible amounts of antibiotic residues in milk in accordance with the requirements of IDF (1991) and EEC (1992).

Advantage of antimicrobials inhibition prediction by using the statistical model in our experiments is undoubted. By extending the antimicrobials range (antibiotics, sulphonamides and other medicaments, further disinfectants and preserving agents, etc.) it will be possible to impartially evaluate also the other impacts of external as well as internal environmental conditions (medium, pH, the presence of natural inhibitors, etc.), and them to

determine the undesirable consequences of these changes in the course of the fermentation process and thereby to be able to positively affect also the quality of dairy products.

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Predikcia inhibície rastu *Bacillus stearothermophilus* var. *calidolactis* C 953 demonštrovaná na príklade vybraných antimikrobiálnych látok v mlieku

Pomocou diskovej difúznej metódy (DDM) s *Bacillus stearothermophilus* var. *calidolactis* C 953 bola skúmaná závislosť veľkosti inhibičnej zóny od koncentrácie vybraných antibiotík (procain penicilín G, ampicilín, streptomycín), čistiacich, dezinfekčných a konzervačných prostriedkov (NaOH, HCl, Ajatin, PUR, Jodonal M, H_2O_2) v umelo kontaminovanom mlieku. Zisťoval sa tiež stupeň deštrukcie peroxidu vodíka vplyvom tepelného ošetrenia (100 °C/10 min) a kombinovaním tepelného ošetrenia a skladovania (100 °C/10 min + 2 h/24 ± 2 °C). S použitím štatistického softwarového produktu SYSTAT bol vybraný najvhodnejší model: $y = a + bx + c \ln x$. Štatistická významnosť koeficientov a, b, c a vhodnosť modelu sa potvrdili na hladine významnosti $\alpha = 0{,}01$. Tento model slúži na predikciu veľkosti inhibičnej zóny pri rôznych koncentráciách antimikrobiálnych látok. Použitá DDM sa ukázala najcitlivejšia pre beta-laktámové antibiotiká; deštrukcia H_2O_2 bola tiež pri zvolených podmienkach dobre detekovateľná.

antibiotiká; dezinfekčné, čistiace a konzervačné činidlá; disková difúzna metóda

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STABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN SOME EXTRACTION SOLVENTS*

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Abstract: Stability (losses) of priority PAH in some solvents used for their determination in food materials was investigated under conditions which may occur in routine analysis. The most significant changes were observed in chloroform solutions left in volumetric flasks at ambient temperature on indirect daylight. Appreciable losses of benzo(a)pyrene, benzo(g,h,i)perylene, and pyrene were noticed, whereas fluoranthene, chrysene and benzo(b)fluoranthene seem to be much more stable. Moreover, other unidentified chromatographic peaks (HPLC/FLD) emerged in chloroform solutions after 3 days. Extraction mixtures hexane – acetone (3 : 2, v/v) and cyclohexane – isopropanol (7 : 3, v/v), suggested as chloroform substitution in analytical procedure, appeared to be more convenient for the storage of PAH's extracts. The lowest stability was observed for benzo(a)pyrene and benzo(g,h,i)perylene; nevertheless no additional chromatographic peaks were observed in these solvents. Another significant source of the analytical error may be an evaporation of solvent from standard solution vials in autosampler and/or analyte degradation.

polycyclic aromatic hydrocarbons; determination; stability; extraction; HPLC; PAH; degradation

Polycyclic aromatic hydrocarbons (PAH) are the most spread group of chemical carcinogens. Therefore, they are considered a top priority in the environmental, toxicological, and food research, and their levels in some food commodities are limited by strict regulation measures.

Analytical methods suitable for routine analysis of PAH in food were discussed in the last publication (Cejpek et al., 1995) and the HPLC with fluorescence detection after clean-up of chloroform extracts by GPC was recommended. Simple chloroform extraction enhanced by sonication yields

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satisfactory recoveries even for materials with high protein content, where alkaline hydrolysis was considered to be necessary previously (Stijve, Hischenhuber, 1987; Perfetti et al., 1992). Chloroform extracts are ready for clean-up by GPC on soft or rigid gels.

On the other hand, the use of both toxic and ozone depleting substances, such as chlorinated hydrocarbons, is subjected to serious criticism and the alternative analytical procedures avoiding these solvents should be applied.

Solutions of PAH both in acetonitrile and toluene were prepared by Vaessen et. al. (1988) as prospective reference materials for foods. Ampoules were analysed 0, 3, 6, 9 and 12 months after storage at –20 °C and +20 °C respectively, in darkness. Results showed that solutions are stable and that no contamination occurred during storage. Storage of PAH collected on solid supports (air samples) appears to be reliable for preserving integrity of such samples for periods up to 118 days at room temperature, if stored away from light (Kloster et al., 1992). Nevertheless, the official AOAC 973.30 method (AOAC, 1990) recommends to perform analysis as far as possible under subdued light and to store all solutions in low actinic flasks because these compounds are susceptible to photooxidation.

In our study, the stability of PAH in some of extraction solvents (and mixtures) suggested to replace chloroform – either for the extraction or as a mobile phase for GPC – was studied in model experiments on standard solutions.

MATERIAL AND METHODS

Chemicals

Chloroform and acetone (p.a., Lachema Brno, Czech Republic) were purified by re-distillation in glass before use. Methanol, acetonitrile (Merck, gradient grade), n-hexane, isopropanol, cyclohexane (Merck, for organic trace analysis) were used as received. All glassware was washed with detergent and rinsed with distilled water and acetone before use.

Reference materials

Standard solution of 16 priority PAHs (PAH – Mix 9) in acetonitrile was supplied by Dr. Ehrenstorfer GmbH, Germany (c = 10 μg/ml each):

acenaphthene [Ace], acenaphthylene [Acy], anthracene [Ant], ben-zo(a)anthracene [B(a)A], benzo(a)pyrene [B(a)P], benzo(b)fluoranthene [B(b)F], benzo(g,h,i)perylene [B(g,h,i)P], benzo(k)fluoranthene [B(k)F], chrysene [Chr], dibenzo(a,h)anthracene [DB(a,h)A], fluoranthene [Flt], fluorene [Flu], indeno(1,2,3-c,d)pyrene [I(c,d)P], naphthalene [Nap], phenanthrene [Phe] and pyrene [Pyr]. Working solutions were prepared by dilution of standard solution with extraction solvents (mixtures) to concentration 50 ng/ml.

Methods

Working solutions in 25 ml volumetric flask with glass stoppers were stored on a laboratory shelf (indirect daylight) at ambient temperature 22 ± 2 °C.

2 x 0.5 ml sample aliquotes were taken from tested solutions every day, concentrated by gentle stream of nitrogen near to dryness and dissolved in 0.5 ml acetonitrile. HPLC with fluorimetric detection (FLD) was employed for determination of individual PAHs. Freshly prepared working solution of PAH in acetonitrile was used as a reference (standard) solution. All analyses were performed at least in duplicate.

To test the concentration changes in vials placed into autosampler carrousel, 1 ml amber vials containing the standard solution in acetonitrile were re-analysed within few days.

HPLC determination

The HPLC/FLD system (Hewlett–Packard 1050 ternary gradient Pump, HP 1050 Autosampler, HP 1046 A Fluorescence Detector, and analytical column MERCK LiChroCART 250–4, 5 μm), were used under following conditions: gradient elution [A – methanol : acetonitrile : water (50 : 25 : 25, v/v/v), B – acetonitrile; 0 min – 100% A, 1 min – 100% A, 22 min – 100% B], injection volume 20 μl, column temperature 40 °C. PAH were detected and quantified at time programmed excitation/emission wavelengths given in Table I.

RESULTS AND DISCUSSION

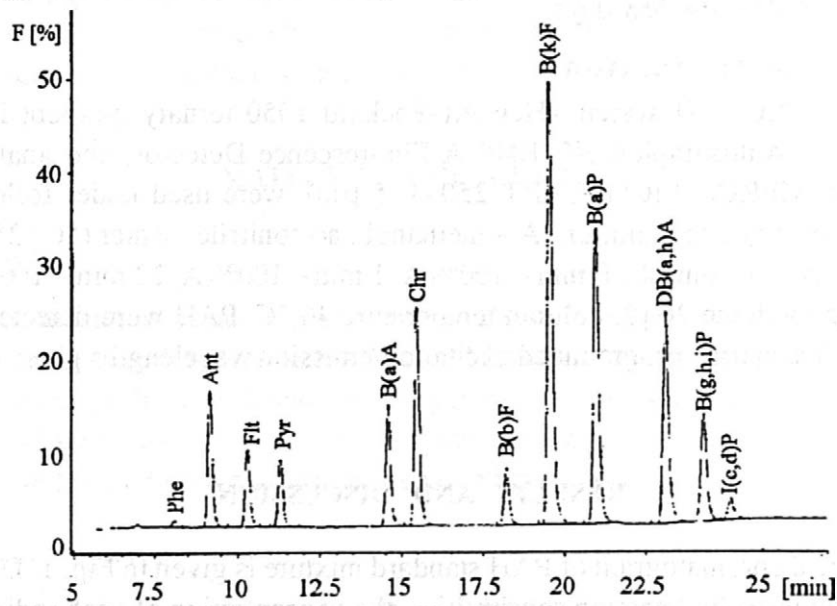
Typical chromatogram of PAH standard mixture is given in Fig. 1. Despite of differences in detection sensitivities, the concentration of each individual PAH is more than 5 times above the practical limit of quantification, even for

I. Excitation and emission wavelengths used for PAH detection by programable fluorimetric detector

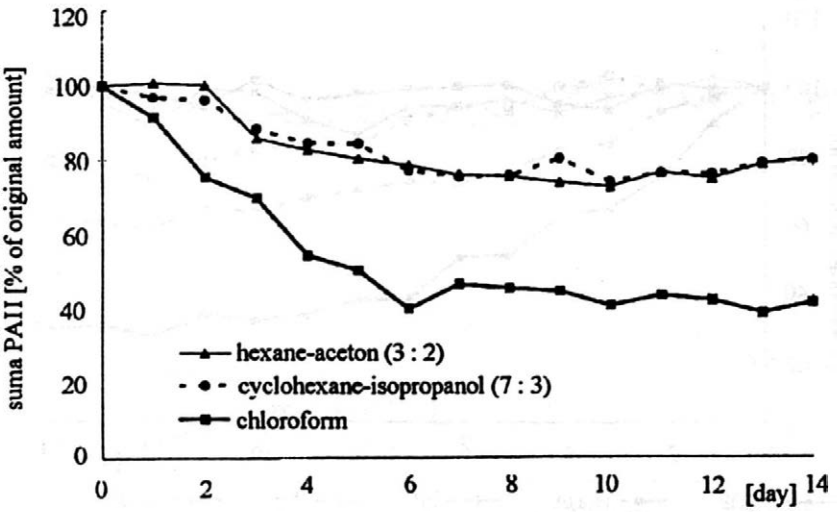
Detected analytes	Time [min]	Excitation [nm]	Emission [nm]
Phe, Ant. Flt, Pyr	4.0	232	420
B(a)A, Chr	13.5	264	384
B(b)F, B(k)F, B(a)P	17.0	295	405
DB(a,h)A, B(g,h,i)P, I(c,d)P	24.5	300	500

phenanthrene or indeno(1,2,3-c,d) pyrene. Stability of all PAH expressed as "suma PAH" seems to be significantly lower in chloroform than that in other solvents/mixtures (Fig. 2).

However, there are considerable differences between individual compounds belonging to this group. Whereas the photostability of pyrene, benzo(a)pyrene, benzo(a)anthracene, (Fig. 3) benzo(g,h,i)perylene and dibenzo(a,h)anthracene (Figs. 6 and 7) in chloroform is relatively low, fluoranthene, chrysene and benzo(b)fluoranthene were quite stable for two weeks on daylight. Considerable losses of benzo(a)pyrene, benzo(a)anthracene, pyrene were observed as early as after 24 h in chloroform.

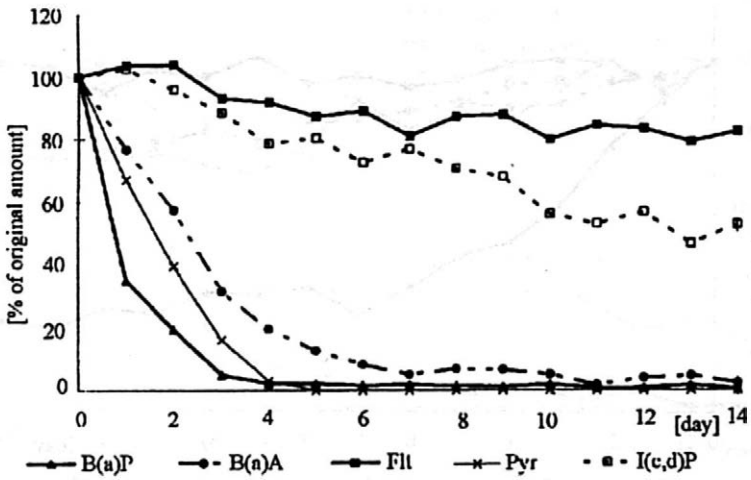


1. Chromatogram of standard mixture (PAH-MIX IX), 50 ng/ml each compound

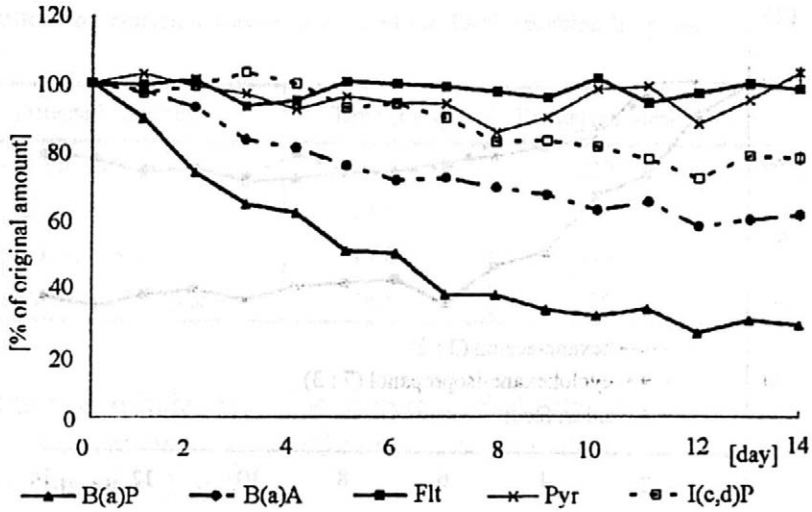


2. Stability of PAH solvents/mixtures (expressed as "suma PAH")

Chromatogram of PAH stored in chloroform after 3 day exposition to daylight is given in Fig. 8. Small unidentified peaks not present in the freshly prepared chloroform solution emerged simultaneously with losses of parent compounds. It could be assumed that unknown degradation products of PAH were detected, because such peaks were not noticed in pure chloroform exposed to sunlight. No additional chromatographic peaks were observed in other solvents despite of certain PAH losses.

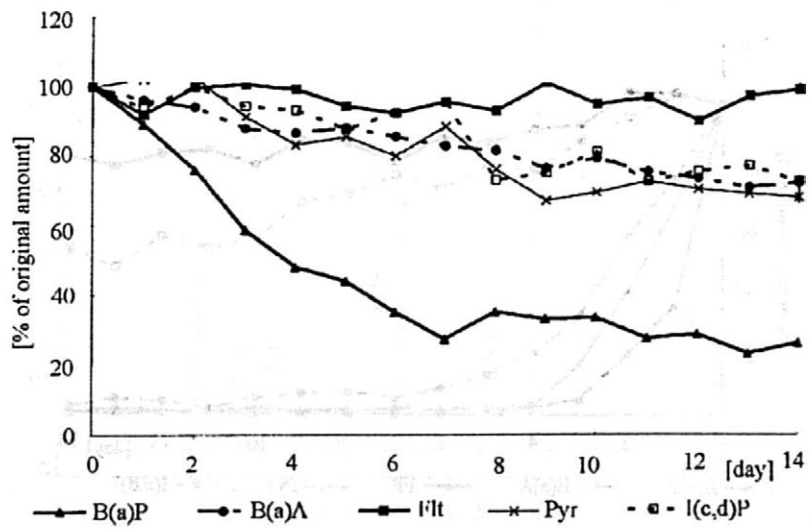


3. Stability of individual PAH in chloroform solution

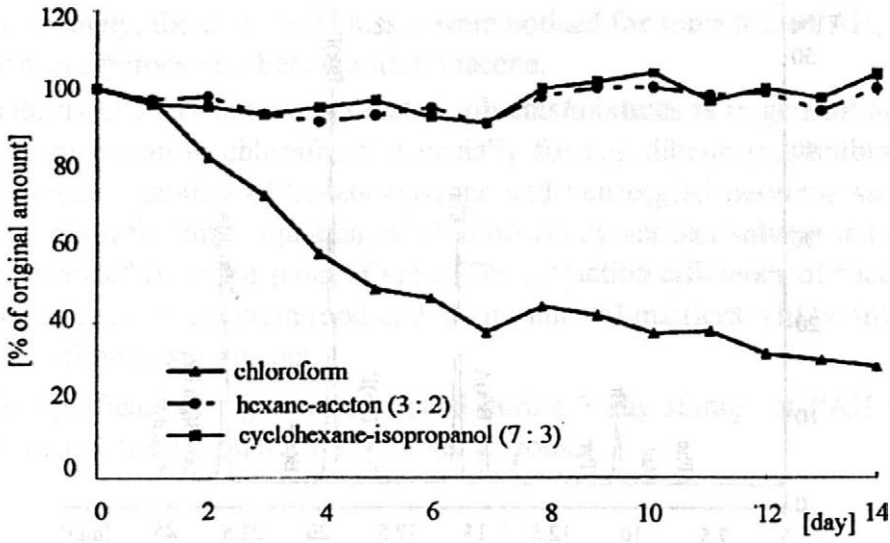


4. Stability of individiual PAH in hexane-acetone (3 : 2)

All PAH were much more stable – at least 2 days – either in hexane-acetone (3 : 2) or cyclohexane-isopropanol (7 : 3), with exception of benzo(a)pyrene and benzo(g,h,i)perylene, where losses were noticed after 1–2 days (compare Figs. 4, 5, and 7). Whereas losses of dibenzo(a,h)anthracene are significant just after 2–3 days in chloroform, 100% of its original amount was recovered after 2 weeks in the other solvents.

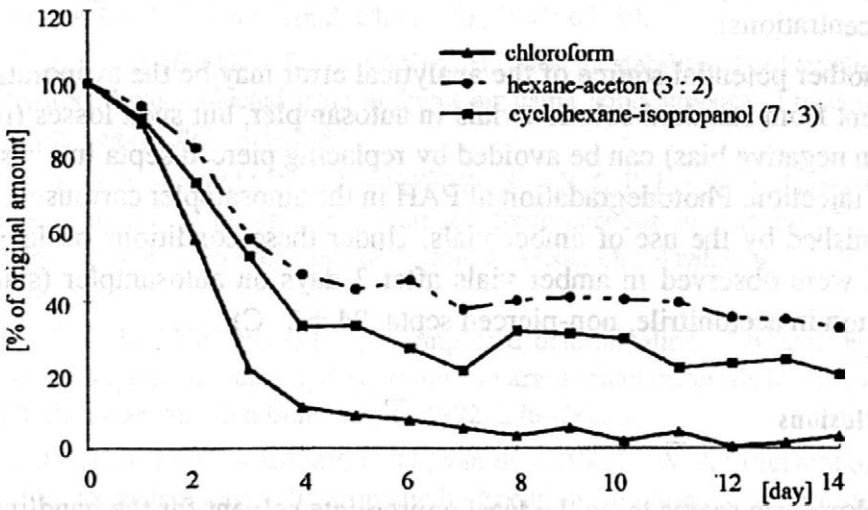


5. Stability of individual PAH in cyclohexane-isopropanol (7 : 3)

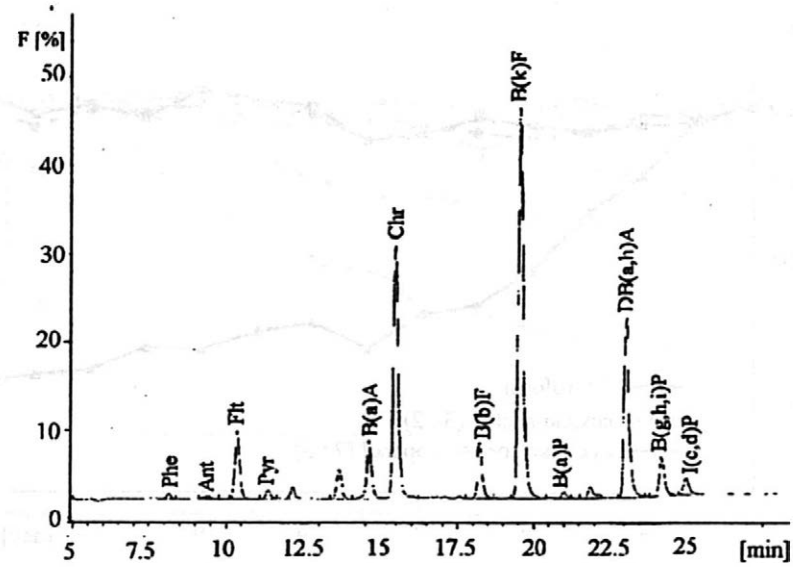


6. Stability of dibenzo(a,b)anthracene in solvents/mixtures

The repeatability of PAH determination expressed as the coefficient of variation (relative standard deviation) is about 4% for fluoranthene, 5–6% for benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, phenanthrene, benzo(b)fluoranthene benzo(k)fluoranthene, benzo(a)anthracene and



7. Stability of benzo(g,h,i)perylene in solvents/mixtures



8. Chromatogram of PAH mixture in chloroform after 3 days on daylight (original amount: 50 ng/ml each compound)

chrysene, and 7–8% for indeno(1,2,3-c,d)pyrene, pyrene, and anthracene. It could be objected that the relatively high uncertainty is connected with individual results, apparently due to very low concentrations of analytes. However, these experimental conditions reflect real food sample analysis (concentrations).

Another potential source of the analytical error may be the evaporation of solvent from standard solution vials in autosampler, but such losses (resulting in negative bias) can be avoided by replacing pierced septa in vials after each injection. Photodegradation of PAH in the autosampler carrousel can be diminished by the use of amber vials. Under these conditions no losses of PAH were observed in amber vials after 3 days on autosampler (standard solution in acetonitrile, non-pierced septa, 24 ± 3 °C).

Conclusions

Chloroform seems to be the least appropriate solvent for the handling and storage of PAH extracts, due to the susceptibility of some PAH to photodegradation. The rate of degradation differs significantly for individual PAH.

Unfortunately, the most rapid losses were noticed for some toxic PAH, such as benzo(a)pyrene or dibenzo(a,h)anthracene.

Stability of PAH in all other tested solvents/mixtures is in general apparently better than in chloroform, especially for e.g. dibenzo(a,h)anthracene and pyrene. Stability of benzo(a)pyrene and benzo(g,h,i)perylene still remains relatively low. Replacing of chloroform by another solvent is highly recommended from this point of view. The extraction efficiency of such solvent mixtures for common food and environmental matrices will be investigated in future experiments.

No significant changes were noticed during 3 day storage of PAH solutions in amber vials on the autosampler carrousel.

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Stabilita polycyklických aromatických uhlovodíků (PAH) v některých extrakčních rozpouštědlech

Byla porovnávána stabilita prioritních PAH v rozpouštědlech používaných při jejich stanovení v potravinách. Nejvýznamnější změny byly pozorovány v případě, kdy chloroformové roztoky PAH byly ponechány v odměrné baňce při laboratorní teplotě na nepřímém denním světle. Nejrychlejší pokles koncentrace byl zaznamenán pro benzo(a)pyren, benzo(g,h,i)perylen a pyren, zatímco poměrně stabilní jsou fluoranthen, chrysen a benzo(b)fluoranthen. V prostředí chloroformu byly metodou HPLC/FLD již po třech dnech pozorovány navíc další chromatografické píky, které se v původním roztoku nevyskytovaly. Směsi hexan – aceton (3 : 2) a cyklohexan – isopropanol (7 : 3), které byly v analytickém postupu navrženy jako náhrada chloroformu, se pro přechovávání extraktů PAH jevily jako podstatně vhodnější s tím, že nejnižší stabilitu jeví opět benzo(a)pyren a benzo(g,h,i)perylen; neidentifikované píky detekovány nebyly. Významným zdrojem chyb při stanovení PAH mohou být také změny koncentrace standardních roztoků vložených do autosampleru, u kterých byla porušena těsnost septa (při opakovaném nástřiku).

polycyklické aromatické uhlovodíky; stanovení; stabilita; extrakce; HPLC; PAH; degradace

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COLOUR CHANGES DURING THE PROCESSING OF POTATO TUBERS*

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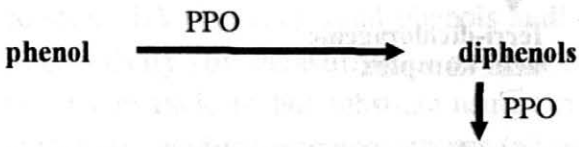
Abstract: During the technological or culinary processing potato tubers undergo colour changes (enzymatic browning reactions, non-enzymatic browning reactions and after-cooking blackening) which significantly affect the processing quality of potatoes. About 36 potato cultivars released in the Czech Republic were studied during three seasons, the principal chemical composition and intensity of colour changes were evaluated during all storage periods. The obtained data were used to divide the table potato cultivars according to various uses. The found correlations between the chemical composition and colour changes were compared with literature data.

potato processing; potato products; colour changes; enzymatic browning; non-enzymatic browning; after-cooking blackening; potato varieties

The processing quality of potatoes associated with structure, composition and culinary properties includes pigmentation – the tendency to colour changes during the technological and/or culinary processing of tubers. The following ways of colour changes occur during potato processing: enzymatic browning reactions, non-enzymatic browning reactions and after-cooking blackening. Enzymatic browning (Fig. 1) is based on the polyphenoloxidase



1. Enzymatic browning

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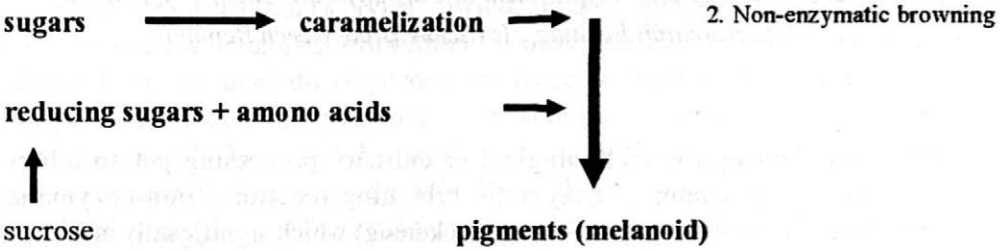
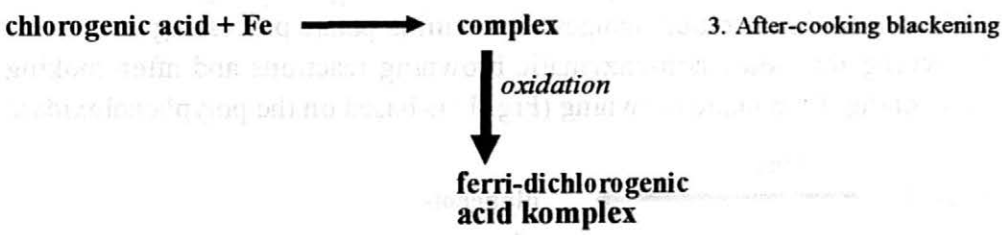
(PPO) oxidation of phenols in native tissues (peeled, cut potatoes, peeled chilled potatoes, etc.). The intensity of colour changes depends mainly on tyrosine and o-diphenols (chlorogenic acid) concentration, PPO activity in tubers (Mapson et al., 1973; Kadam et al., 1991a). Non-enzymatic browning (Fig. 2) is important for fries and chips. The limiting factor



affecting the colour intensity is the content of reducing sugars (and ascorbic acid, sometimes sucrose) in processed potatoes. The course of colour changes during the frying also depends on the temperature of oil bath and duration of processing. (Kadam et al., 1991a, b) The after-cooking blackening (Fig. 3) takes place when tubers are cooked and cooled. During cooking complexes of chlorogenic acid and iron ions are produced, during subsequent oxidation grey-black products are formed. The formation of coloured products is affected by several factors, the most important seems to be the ratio of chlorogenic and citric acid in tubers (Muneta et al., 1985; Mondy et al., 1991).



The aim of the presented work was to divide 36 potato cultivars released in the Czech Republic according to their proper use and to evaluate the effect of other factors such as e.g. season, storage conditions, etc. on the colour changes during technological or culinary processing of tubers.

MATERIAL AND METHODS

Potato tubers of 36 varieties (Agria, Ausonia, Bintje, Desirée, Dita, Eba, Folva, Gloria, Granola, Impala, Karin, Karla, Klera, Kobra, Korela, Koruna, Krasa, Krista, Krystala, Lada, Lipta, Lomnica, Lukava, Nela, Nicola, Ostara, Premiere, Radka, Resy, Rosella, Sante, Svatava, Tara, Vilma and Zlata) were grown under the same conditions in the seasons 1993–1995 in the fields of Research Station Valečov of the Potato Research Institute in Havlíčkův Brod. Potatoes were cultivated according to usual conditions, with nitrogen fertilisation at 60 kg/ha, other standard treatments were applied. Potatoes were harvested in full maturity. After harvest the tubers were stored three or four weeks at 20 °C and 80% rel. humidity, then they were treated with anti-sprouting inhibitor (CIPC) and were stored at dark at 6 °C.

The samples of 5 kg of tubers were taken after healing and then three times during the storage period. Colour changes of fresh homogenate, fried chips and peeled boiled tubers were evaluated using the standard procedures comparing with the scales (Heilinger, 1975; European Association for Potato Research). In the seasons 93/94 and 94/95 tristimulus colorimeter Minolta was used. For the chemical analyses a homogenate of 1 kg sample of whole tubers was prepared. The following chemical parameters were evaluated: starch content (polarimetry), reducing sugars (glucose and fructose) and sucrose (HPLC on OSTION LGKA in Ca^{2+} cycle, with RI detection), ascorbic acid (HPLC Si-HH_2 column, mobile phase: 50 : 50 (v/v) methanol – 0.25% KH_2PO_4 buffer solution pH 3.5, detection UV 270 nm, total ascorbic acid was determined after reduction using cysteine), chlorogenic acid (HPLC Si-C_{18}, 80% methanol, 2% acetic acid, linear gradient 5–50% of methanol solution, UV 234 nm), total phenols and/or tyrosine (spectrophotometry), PPO activity (by measuring of oxygen consumption of protein extracts using 4-methylcatechol substrate using the oxygen Clark electrode), malic acid, citric acid, phosphates, nitrates (all using capillary isotachophoresis), dry matter content (drying at 105 °C), total nitrogen content (Kjeldahl method). Data were statistically processed using correlation analysis, LDA (linear discriminant analysis) and PCA (principal components analysis).

I. Ranges of selected parameters during the storage of 36 potato varieties in the seasons 93/94, 94/95, 95/96

Parameter	Mean	Minimum	Maximum
CA	129.1–148	44.0–68.0	255.2–312
AA	99.5–214	13.4–155	236.8–338
TA	171.3–246	49.8–165	412–436.5
PPO	106.4–600	17.1–161	124.4–226.1
DM	20.1–22.3	16.6–19.3	23.4–26.35
ST	14.6–15.7	11.9–12.1	16.9–19.2
TN	0.31–0.38	0.26–0.29	0.37–0.44
GF	0.12–0.42	0.04–0.1	0.36–0.96
TS	0.18–0.24	0.05–0.11	0.45–0.55
NI	406–647.8	145–452.0	644–808
MA	0.82–1.37	0.41–0.47	1.74–5.4
AC	4.95–6.17	3.1–3.58	6.92–11.02
PH	0.32–0.81	0.17–0.46	0.57–1.43

CA – chlorogenic acid [mg/kg]; AA – ascorbic acid [mg/kg]; TA – total ascorbic acid [mg/kg]; PPO – activity of polyphenoloxidase [μmol O_2/kg.s]; DM – dry matter [%]; ST – starch content [%]; TN – total nitrogen [%]; GF – glucose + fructose [%]; TS – saccharoe [%]; NI – nitrates [mg/kg as $NaNO_3$]; MA – malic acid [g/kg]; AC – citric acid [g/kg]; PH – phosphate [g/kg]

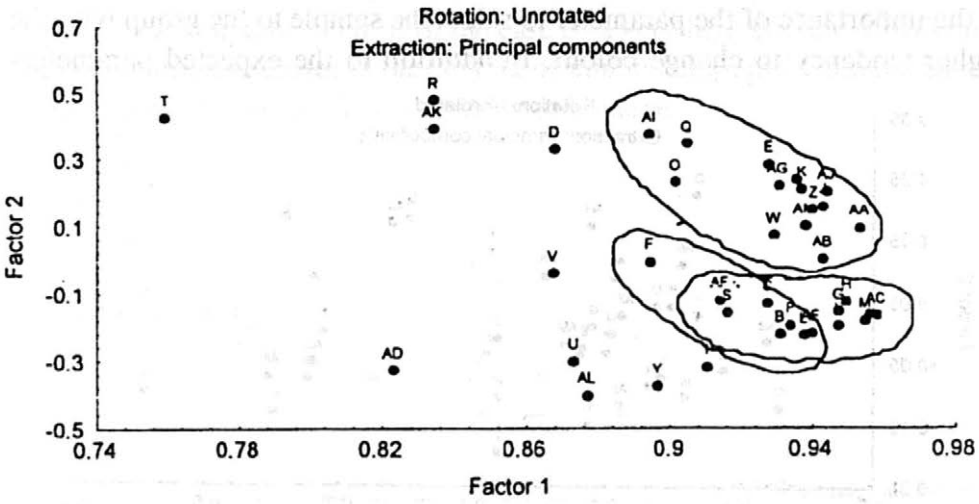
RESULTS AND DISCUSSION

Results of selected parameters of 36 potato cultivars within the seasons 93/94; 94/95; 95/96 are given in Table I. In spite of very similar cultivation conditions, agrotechnical treatments, etc. the variation between the results was very high. The tubers changed during the storage, dry matter content slightly increased, similar tendency was observed in the case of phenols and PPO activity. Ascorbic acid content and starch content decreased, other parameters remained almost stable.

The climatic conditions affected our results in the season 94/95, when wet July and subsequent higher temperatures caused the new formation of tubers and the crop contained tubers of various physiological stage.

The expected correlations were confirmed in the 36 varieties during three years (the critical value of correlation coefficient for $\alpha = 0.05$ is $r = 0.32$). The nonenzymatic browning reaction (browning of fried potatoes) correlated with reducing sugars content (0.28–0.89), at the beginning of storage ascorbic acid content also affected the colour of chips ($r = 0.33$). Enzymatic browning of potatoes correlated with PPO activity ($r = 0.34$–0.47), dry matter content ($r = 0.30$–0.53) and no effect of phenols (total phenols, tyrosine, chlorogenic acid) was observed. After-cooking blackening was dependent on the phosphate content ($r = 0.35$–0.53) and iron content ($r = = 0.28$–0.44). In the case of after-cooking blackening and enzymatic browning the highest correlation coefficient values were obtained after healing, during storage the correlations were less significant. These changes are probably more "complex" being affected by more parameters compared with reducing sugars in nonenzymatic browning of fried chips.

The "complexity" of factors is obvious also from the results of discriminant analysis of data in Table II. In the table the most important parameters

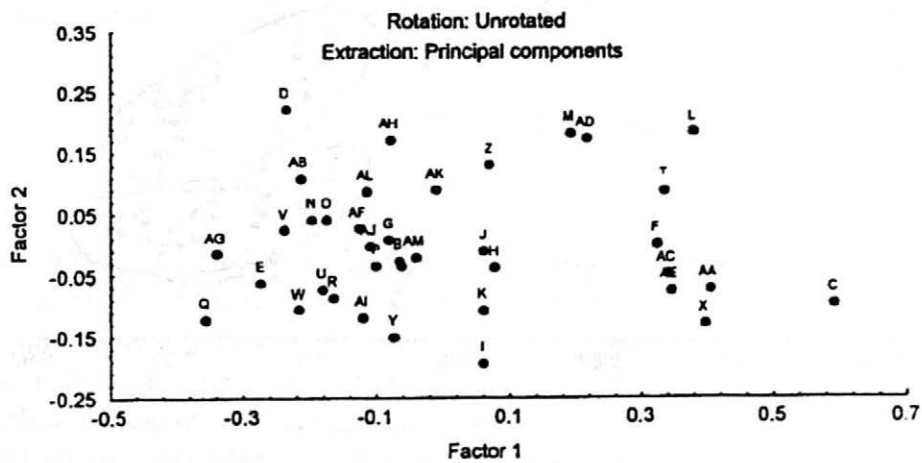


4. Principle components analysis, colour data only, upper group – varieties convenient for frying, down-left group – for peeling, down-right group – varieties for cooking

II. Discriminant analysis – parameters and values

	Nonenzymatic browning – IBVL	Enzymatic browning – lightness after 2 hours	After-cooking blackening – surface of potato immediately after cooking
Glucose, fructose	0.721	0.784	0.804
Ascorbic acid	0.642	0.689	
Citric acid			0.852
Sucrose		0.918	0.906
Potassium		0.854	
Nitrates			0.733
Phosphates			0.671
Malic acid			0.594
Iron			0.570
Total ascorbic acid	0.599	0.738	
Total nitrogen content	0.489		
PPO		0.599	

affecting the selected colour change are given. The value λ gives a measure of the importance of the parameter to select the sample to the group with the higher tendency to change colour. In addition to the expected parameters

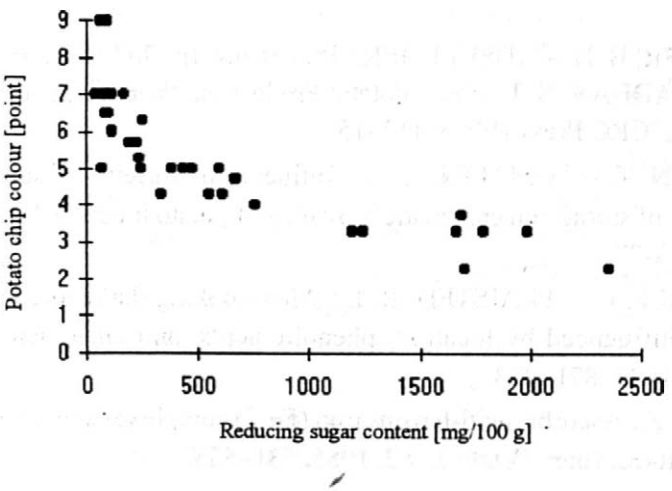


5. Principle components analysis, all data (chemical parameters and colour data)

(underlined λ values) high λ values were also found for other factors without any expected participation in the mechanisms of these colour changes.

Similar conclusion could be drawn from the results of a preliminary processing of obtained data using the PCA method. The calculated factors covered majority of the followed parameters. When the colour data only (without results of chemical analyses) are processed (Fig. 4) in the plot there are the clusters of varieties convenient for different ways of processing (with lowest tendency to change colour). But taking all results of colour and chemical parameters (Fig. 5), the plot is without visible groups of varieties of similar properties.

The results of PCA confirmed the complexity of factors affecting colour changes during the processing of potatoes. When tubers with higher content of one of the limiting parameters are processed, only a possible higher tendency to change colour could be expected. This high level of the parameter need not be the reason of colour changes during processing of the tubers. The difficulties and indefiniteness of any predictions are not true (with some exceptions) in the case of the relationship of reducing sugars content and nonenzymatic browning reaction in fried chips. It is possible in the range of the usual content of reducing sugars to predict well the intensity of colour changes during frying, this conclusion is also obvious from our results given in Fig. 6, where a very close correlation between reducing sugars and colour of chips was found ($r = 0.89$). In the case of a very high content of reducing sugars the limiting factor is the content of amino compounds, but the colour is unacceptable and prediction need not to be done.



6. Correlations between colour quality (IBVL) and reducing sugar content of potato chips

According to the results of analyses of tubers in the seasons 93/94 and 95/96 the most convenient varieties for different ways of technological or culinary processing are:

- Fresh, peeled potatoes (regarding the colour changes only) Ausonia, Gloria, Krystala, Kobra, Lipta, Lomnica, Tara and Desirée.
- Cooking, products sensitive to the "after-cooking blackening" – Agria, Dita, Folva, Granola, Lada, Lomnica, Radka and Nicola.
- Frying, drying, etc: Ausonia, Gloria, Impala, Koruna, Krasa, Krystala, Albína, Desirée and Lukava.

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Barevné změny během zpracování brambor

Během technologického zpracování nebo kulinární úpravy konzumních brambor dochází k barevným změnám, které mohou být do určité míry žádoucí, většinou však negativně ovlivňují kvalitu a prodejnost výrobku. Práce shrnuje výsledky několikaletého projektu zaměřeného na ověření možností predikce barevných změn podle chemického složení, posouzení v ČR povolených odrůd z hlediska jejich vhodnosti pro různé způsoby zpracování, zhodnocení dalších faktorů jako jsou povětrnostní podmínky, vliv fyziologického stáří hlíz, podmínky skladování atd. Výsledky jsou postupně zpracovávány; v následujícím textu jsou uvedeny předběžné závěry vyplývající z dosud provedených statistických zpracování souborů výsledků.

Brambory 36 odrůd povolených v České republice byly pěstovány za stejných podmínek během sezón 1993/94, 1994/95 a 1995/96 na polích VÚB Havlíčkův Brod ve stanici Valečov. Barevné změny brambor byly hodnoceny standardními postupy porovnáním barvy s etalony i instrumentálními metodami měření barvy. Paralelně s hodnocením barevných změn bylo analyzováno i velké množství chemických parametrů. Výsledky byly zpracovány korelační analýzou a analýzou hlavních komponent.

Přes velmi podobné podmínky kultivace a maximální snahu o snížení chyby měření v důsledku nehomogenity analyzovaných vzorků byla pozorována velká variabilita sledovaných hodnot.

Některé z očekávaných korelací chemického složení a barevných změn byly potvrzeny, u některých hodnot se korelace prokázat nepodařila.

Z jednoduché korelační analýzy výsledků je zřejmé, že barevné změny jsou komplexnější a zahrnují kromě vlastních reagujících složek také další parametry, které vytvářejí prostředí reakce, případně reagují v dalším kroku.

Z dosud provedených statistických výpočtů je zřejmé, že barevné změny jsou podmíněny komplexem vlastností. Při vysokém obsahu některého z parametrů je možné předpokládat větší sklony hlíz k určitým barevným změnám, ale vysoký (nebo jinak extrémní) obsah nemusí ještě znamenat významné barevné změny během zpracování. Obtížnost a neurčitost předpovědi s určitým omezením neplatí pro vztah redukujících cukrů a intenzity neenzymového hnědnutí.

V průběhu experimentů byl sledován také vliv skladování na barevné změny. Obecně se během skladování tendence k barevným změnám hlíz příliš neměnila, výše po-

psané změny složení hlíz během skladování nevedly ke statisticky významným změnám v intenzitě barevných změn. Výjimkou je v tomto směru případná akumulace cukrů, pokud však jsou hlízy sklizeny při sklizňové zralosti a jsou skladovány po retardaci klíčení za přiměřených teplot, udržují si také téměř konstantní tendenci k hnědnutí během smažení.

zpracování brambor; výrobky z brambor; barevné změny; enzymové hnědnutí; neenzymové hnědnutí; černání po uvaření; odrůdy brambor

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EFFECT OF NON-VOLATILE FLAVOUR SUBSTANCES ON THE PERCEIVED INTENSITIES OF VOLATILE AROMA SUBSTANCES

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Abstract: Sucrose suppresses the perceived bitterness of quinine sulphate when present in excess *et vice versa*. Sucrose enhances and quinine sulphate suppresses the perceived aroma intensity of cinnamic alcohol, 3-phenyl-1-propanol, eugenol, and benzaldehyde.

aroma; sweetness; bitterness; sucrose; quinine sulphate; benzaldehyde; eugenol; cinnamic alcohol; 3-phenyl-1-propanol

The aroma of foods and beverages is affected by up to several hundreds of flavour active compounds, both non-volatile and volatile substances. The antagonistic or synergistic effect of bitter, acid or sweet substances is well known (Barylko-Pikielna, 1975; Solms, 1971). They can influence the perceived intensities of other components by interactions of either type. The often observed antagonism may be explained by competition of two or more substances for the same sites on the receptor surface (Beidler, 1958).

The flavour intensity may be mechanically suppressed by coating the receptor surface with fat or oil layer (Lynch et al., 1993; Ohta et al., 1979) if fatty food has been consumed.

The synergism between sucrose and low concentrations of sodium chloride is well known (Pangborn, 1962; Pangborn, Trabue, 1967). The salty taste of sodium chloride solutions is enhanced by various amino acids and esters (Tamura et al., 1989), and monosodium glutamate shows synergism with 5'-ribonucleotide (Wada et al., 1985).

The mutual interaction between acids and sugars is well known (Barylko-Pikielna, 1975). Citric acid interacts not only with sucrose, but also with synthetic sweeteners (Hoppe, 1981). Sucrose and tartaric acid mutually interact in white wines (Pokorný, Kalinová, 1994) and in fruit juices (Stampanoni, 1993, 1994).

Interactions between non-volatile taste and volatile aroma substances were discussed as well (Solms, 1971; Pokorný, Kalinová, 1994). Examples were reported (Lawless, 1986) from the inhibition of taste and odour sensations till relative independence of taste from odour stimuli. Trimethylamine affects the concentration/odour intensity of hexanal (Miller et al., 1983). Flavour-odour interactions were observed in solutions of acetic acid and coffee (Garcia-Medina, 1981). In a mixture of odour and taste substances, the effect is not exactly additive, but is always slightly antagonistic (Ennis, Hornung, 1985). In combinations of ethyl butyrate and sucrose, the overall sensations, estimated by the magnitude method, could be expressed as the summation of the contributions of both the smell and taste stimuli (Hornung, Ennis, 1986).

The effect of non-volatile flavour substances on the perceived intensities of volatile aroma-active components needs, however, still additional detailed study. Therefore, we have studied mutual interactions of sucrose and quinine sulphate, and their effect on various volatile food flavourings.

MATERIAL AND METHODS

Material

Pure sucrose (p. a., Lachema Brno), quinine sulphate (Pharmaceutical Reagents), *Cortex chinae*; cinnamic alcohol, 3-phenyl-1-propanol, eugenol, benzaldehyde (Aldrich) were found pure by gas chromatography. Extract from *Cortex chinae* was prepared by extraction with 50% aqueous ethanol.

Analytical Methods

The sensory analysis was performed conform to the requirements of the international standard (ISO 6658). Aqueous solutions of sucrose and quinine sulphate were prepared by weighing the appropriate amount of the chemicals with redistilled water purified from sensory active substances by evacuation. The samples were served in coded 25ml glass beakers and 10 ml were tasted. The next sample was ingested after mouth washing with tap water and expectorating.

The sensory analysis was carried out in the standard test room (ISO 4121) provided with standard test booths. The assessor panel consisted of selected trained persons (ISO 8586) with the experience of at least half a year.

The intensity was rated with use of unstructured graphical scales (ISO 8589), represented by straight lines 100 mm long, oriented by verbal description (0 mm = absent, imperceptible; 100 mm = very strong). The sweet and bitter tastes were determined in separate sessions.

RESULTS

The results given in the tables are average values of 10 responses. The standard deviations of the means were between 1–5% in testing solutions Nos. 1–2 and Nos. 5–6, but moderately higher in Nos. 3–4 of experimental series.

Results of sucrose and quinine sulphate solutions are given in Table I for bitter and sweet tastes, respectively, and the sweet and bitter tastes of the mixtures of sucrose and quinine sulphate (the two tastes were rated in different sessions) are given in Table II. Sucrose suppressed the bitterness of quinine sulphate, while quinine sulphate suppressed the sweetness of sucrose. Extract of *Cortex chinae* had similar effect on the sweetness of sucrose as quinine sulphate (the results are not shown). Differences between the perceived intensities in sucrose (100 g/l) and quinine sulphate (100 mg/l) solutions are also shown in Table II.

The effect of sucrose and of quinine sulphate on the perceived intensities of aroma substances is shown in Table III. The concentration of cinnamic

I. Intensities of sweetness of sucrose solutions and intensities of bitterness of quinine sulphate solutions

Concentration of sucrose [g/l]	Intensity of sweetness [p. c. of the scale]	Concentration quinine sulphate [mg/l]	Intensity of bitterness [p. c. of the scale]
0	0	0	0
20	11	20	16
40	30	40	36
60	66	60	54
80	74	80	73
100	82	100	81

II. Taste interactions of sucrose and quinine sulphate in aqueous solutions

Sucrose [g/l]	Quinine sulphate [mg/l]	Sweetness perceived [p. c.]	Sweetness expected [p. c.]	Bitterness perceived [p. c.]	Bitterness expected [p. c.]
0	100	0	0	88	–7
20	80	4	+6	66	+7
40	60	19	+11	40	+14
60	40	48	+18	21	+15
80	20	66	+8	1	+16
100	0	85	–2	0	0

alcohol, eugenol and benzaldehyde in the tested solutions was 0.005% (g in 100 ml), in the case of 3-phenyl-1-propanol, the concentration was 0.05% (g in 100 ml). In all experiments, quinine sulphate suppressed the perceived intensities of aroma compounds tested. On the contrary, the presence of sucrose increased the perceived intensities of aroma substances tested; the perceived aroma intensities in the sucrose solutions compared with those in the quinine sulphate solutions were as follows: 136% for benzaldehyde, 191% for cinnamic alcohol, 231% for 3-phenyl-1-propanol, and 305% for eugenol.

III. Effects of sucrose and quinine sulphate on the perceived aroma intensity of volatiles

Quinine [g/l]	Quinine sulphate [mg/l]	Benzaldehyd [p. c.]	Cinnamic alcohol [p. c.]	3-Phenyl-1--propanol [p. c.]	Eugenol [p. c.]
0	100	33	35	16	19
20	80	38	47	10	24
40	60	36	46	25	37
60	40	47	48	28	47
80	20	49	66	40	57
100	0	48	67	37	58

DISCUSSION

The masking of bitter flavour by sucrose has been generally known for many centuries, and the effect was utilized in the pharmaceutical practice for serving bitter medicines. Not only alkaloids, but also naringin or sucrose octaacetate have similar effect on the sweetness of neohesperidin dihydrochalcone (Naim et al., 1986). The addition of sweeteners diminished the bitterness, but also the astringency (Lyman, Green, 1990).

The antagonism of bitter substances and volatile aroma substances cannot be explained by competition for the same receptor sites as the two receptors are different. Similar effect was observed in systems of ethyl benzoate or anisole and caffeine, and the suppression took place in the gas chromatographic analysis (Tunaley et al., 1985). Therefore, some interactions are possible, e.g. formation of physical bonds. Analogous interactions were reported (King, Solms, 1979) in systems containing benzyl alcohol and serum albumin or soybean protein. In our experiments, the flavour intensities of volatile aroma substances were determined separately from the determination of taste substances, which could influence the results as well (Ennis, Hornung, 1985).

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Vliv netěkavých chuťově aktivních látek na intenzitu vjemu těkavých aromových složek

Degustací souborem školených hodnotitelů byly zkoumány intenzity aroma vybraných těkavých potravinářsky významných látek ve vodném roztoku sacharosy (100 g/l) a síranu chininu (100 mg/l) a jejich směsí v různých poměrech. Intenzita byla vyhodnocována s použitím nestrukturovaných grafických stupnic. Sacharosa potlačovala hořkost síranu chininu a naopak přítomnost síranu chininu potlačovala sladkost sacharosy. Sacharosa zvyšovala intenzitu vjemu vyvolaného přítomností skořicového alkoholu, 3-fenyl-1-propanolu, eugenolu a benzaldehydu. Přítomnost síranu chininu naopak působení aromových složek mírně potlačovala.

aroma; sladkost; hořkost; sacharosa; síran chininu; skořicový alkohol; 3-fenyl-1-propanol; eugenol; benzaldehyd

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STANOVENIE BIOGÉNNYCH AMÍNOV V POTRAVINÁCH ŽIVOČÍŠNEHO PÔVODU METÓDOU HPLC

Determination of Biogenic Amines in Foods from Animal Sources by HPLC Method

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Abstract: A method for determination of hygienically important components (cadaverine, putrescine, histamine and tyramine) present in foods was developed and tested. HPLC method on NUCLEOSIL C18 column was used for this determination, with the mobile phase methanol: a cetonitril : water at a ratio 2 : 1 : 1 (v/v/v) and detection in UV zone at a wavelength of 254 nm. The limit of determination was 10 ng for cadaverine, putrescine, histamine and 20 ng for tyramine in feed, which corresponds to 1 or 2 mg per 1 kg sample at a sample weight of 10 g. The method yield was 85–102% with maximum standard deviation 2.3%. In the next part, the presence of the above amines in foods from animal sources is discussed.

biogenic amines; cadaverine; putrescine; histamine; tyramine

Abstrakt: Bola vypracovaná a overená metóda pre stanovenie hygienicky významných zložiek (kadaverínu, putrescínu, histamínu a tyramínu) vyskytujúcich sa v potravinách. Stanovenie bolo realizované HPLC metódou na kolóne NUCLEOSIL C18 použitím mobilnej fázy metanol : acetonitril : voda v pomere 2 : 1 : 1 (v/v/v) s detekciou v UV oblasti pri vlnovej dĺžke 254 nm. Medza stanoviteľnosti pre kadaverín, putrescín a histamín bola 10 ng a pre tyramín 20 ng v nástreku, čo pri návažke 10 g zodpovedá 1, resp. 2 mg na 1 kg vzorky. Výťažnosť metódy bola 85–102 % s maximálnou smerodajnou odchýlkou 2,3 %. V ďalšej časti poukazujeme na výskyt sledovaných amínov v potravinách živočíšneho pôvodu.

biogénne amíny; kadaverín; putrescín; histamín; tyramín

Biogénne amíny (BA) patria do skupiny endogénnych cudzorodých látok hygienicky významných vo výžive. Ide o nízkomolekulové organické zásady rozličnej štruktúry s charakterom alifatických, aromatických a heterocyklických zlúčenín biologicky aktívnych, s pomerne dobre preštudovaným fyziologicko-chemickým a farmakologickým účinkom. BA sú vytvárané, riadené, metabolizované a za určitých podmienok môžu byť tiež hromadené pri látkovej premene u človeka, zvierat, rastlín a mikroorganizmov. Tým môžu ovplyvňovať rad procesov prebiehajúcich v organizme (napr. reguláciu telesnej teploty, príjem potravy, znižovanie, resp. zvyšovanie krvného tlaku, alergie).

Vyššie koncentrácie BA vyskytujúce sa v rade potravín, nápojov a potravinárskych surovín vznikajú prevažne mikrobiálnou dekarboxyláciou príslušných aminokyselín, predovšetkým ich L-formy (Askar, Treptow, 1986). Preto je nevyhnutné venovať zvýšenú pozornosť výberu surovín, ich mikrobiologickej kontaminácii, ale aj následným technologickým operáciám a skladovacím podmienkam.

Aj napriek skutočnosti, že BA sú nevyhnutné z hľadiska normálneho priebehu fyziologických funkcií, ako aj skutočnosti, že v tráviacom trakte človeka je vybudovaný ochranný systém na báze enzýmov (monoamínooxidázy MAO a diamínooxidázy DAO), ktoré detoxifikujú prebytočné amíny, je potrebné venovať zvýšenú pozornosť koncentráciám BA v potravinách.

Analýze BA v potravinách sa v poslednom období venuje náležitá pozornosť. Hľadajú sa vhodné extrakčné činidlá a spôsoby izolácie, pri ich delení sa využívajú rôzne separačné techniky a dôraz sa kladie na ich detekciu. Je popísaný celý rad metód stanovenia BA v potravinách, predovšetkým sú využívané metódy tenkovrstvovej chromatografie (TLC), plynovej chromatografie (GC), ionexovej chromatografie (IEC) a vysokoúčinnej kvapalinovej chromatografie (HPLC).

Napriek problémom pri vyhodnocovaní chromatogramov sa TLC stále využíva pre svoju rýchlosť a jednoduchosť (Wortberg et al.,1981; Ramantanis et al., 1985; Nahodilová, Lát, 1988). Prevažne je táto metóda zameraná na identifikáciu amínov (Pavelka, Šubrtová, 1987). Plynová chromatografia nie je často využívaná pre stanovenie amínov. Separácia BA na náplňových kolónach nedáva uspokojivé výsledky z dôvodov nepravidelných píkov (Křížek, 1991). GLC metódy vyžadujú derivatizačný krok, ktorý je technicky náročný a je ťažké dosahovať reprodukovateľnosť v prí-

tomnosti interferujúcich látok, nachádzajúcich sa v potravinách, ako sú syry (Joosten, Olieman, 1986). Separácia amínov na kapilárnych kolónach v spojení s hmotnostným detektorom (GC-MS) (Slemr, Beyerman, 1984) dosiahla vysoký stupeň citlivosti a selektivity, avšak zariadenia sú značne nákladné.

Najviac využívanou metódou pri stanovení BA je HPLC. Separácia nederivatizovaných amínov chromatografickou technikou iónových párov (Ion-Pair-RP-HPLC) je známy postup (Reuves et al., 1986; Pavelka, Šubrtová, 1988; Izquierdo-Pulido et al., 1993). Pri použití HPLC sa často využíva pokolónová derivatizácia amínov s ninhydrínom za vzniku farebného produktu (Joosten, Olieman, 1986) alebo o-ftalaldehydu v prítomnosti 2-merkaptoetanolu (Seiler, Knodgen, 1985; Suzuki et al., 1990; Hernandez-Jover, et al. 1995). Pre zvýšenie citlivosti detekcie sa využíva aj predkolónová derivatizácia danzyl chloridom (Hui, Taylor, 1983; Antila et al., 1984; Ibe et al., 1991), benzoyl chloridom (Křížek, 1991) a 9-fluorenylmetyl chloromravčan (FMOC-Cl) (Kirschbaum et al., 1994).

Rozšírenou je aj IEC metóda, pri ktorej sa nevyžaduje náročná príprava vzorky a je možné stanoviť väčší počet amínov s dostatočnou presnosťou a citlivosťou (Zee et al., 1985; Ingles et al., 1986; Ababouch et al., 1991; Baráth et al., 1995; Simon-Sarkadi, Hodosi, 1995). Nevýhodou tejto metódy je dlhý čas potrebný na získanie amínového profilu.

Cieľom práce bolo vyvinúť metódu rýchlu, dostatočne citlivú a opakovateľnú, ktorá by mohla byť aplikovaná v bežných laboratórnych podmienkach, ale aj pri sledovaní produkcie amínov rôznymi kmeňmi mikroorganizmov.

MATERIÁL A METÓDY

Materiál

Na analýzy boli použité fermentované výrobky z mlieka (jogurty, syry, bryndza), mäsové výrobky tepelne opracované (diétne párky, safalátky), mäsové výrobky tepelne neopracované (Lovecká saláma, suchá saláma, domáca klobása) a rybie konzervy (treska – šalát, rybací šalát, tuniak). Tieto výrobky boli zakúpené v obchodnej sieti. Sledované výrobky boli v respiračnej dobe.

Chemikálie

Štandardy: kadaverín, putrescín, histamín boli vo forme danzylderivátov (Sigma, USA); tyramín chlorid a 1,7-diamínoheptán dichlorid (Sigma, USA), danzyl chlorid (Sigma, USA), acetonitril, metanol (Merck, SRN). Ostatné použité chemikálie boli čistoty p.a. (Lachema Brno, ČR).

Prístroje

Na analýzy bol použitý kvapalinový chromatograf fy Laboratorní přístroje Praha, vybavený UV detektorom LCD 2040 a zapisovačom TZ 4620.

Metódy stanovenia

Izolácia BA zo vzoriek: 10 g zhomogenizovanej vzorky potraviny bolo extrahované 30 cm^3 10% kyseliny trichlóroctovej (80 °C) 30 minút v sonifikátore. Získaný homogenát bol ochladený v mrazničke a odstredený (5 000 min^{-1}, 10 minút).

Derivatizácia BA: 400 µl supernatantu bolo zmiešaných v zatemnenej zábrusovej skúmavke (obalenej alobalom) so 400 µl deionizovanej vody a 200 µl nasýteného roztoku Na_2CO_3. Takto pripravená zmes bola temperovaná vo vodnom kúpeli pri teplote 40 °C 20 minút. Potom bolo pridané 100 µl roztoku danzyl chloridu (50 mg/ml) a nechalo sa temperovať pri teplote 40 °C 1 hodinu. Po uplynutí 1 hodiny bol pridaný roztok glutamátu sodného (50 mg/ml) v objeme 100 µl a zmes bola temperovaná ďalšiu hodinu. Glutamát sodný slúži na zreagovanie prebytočného danzyl chloridu, ktorý ruší stanovenie. Po ochladení skúmaviek bol do každej pridaný octan etylový (1 ml), skúmavky boli dôkladne pretrepané a po rozdelení vrstiev bolo z hornej vrstvy odobraté 400, resp. 800 µl a doplnené na 1 ml metanolom. Pripravená vzorka bola aplikovaná na kolónu HPLC. Pri väčšom počte vzoriek bola vrstva octanu etylového oddelená, vysušená na vákuovej odparke (40 °C) a vyfúkaná dusíkom. Vysušenú vzorku je potom možné skladovať pri teplote 0 až 5 °C aj niekoľko týždňov. Derivatizácia štandardov (tyramín a 1,7-diamínoheptán) bola robená obdobne. Derivatizované štandardy (kadaverín, putrescín a histamín) boli rozpustené v metanole (1–2 mg) a po príslušnom nariedení aplikované na kolónu.

Podmienky HPLC

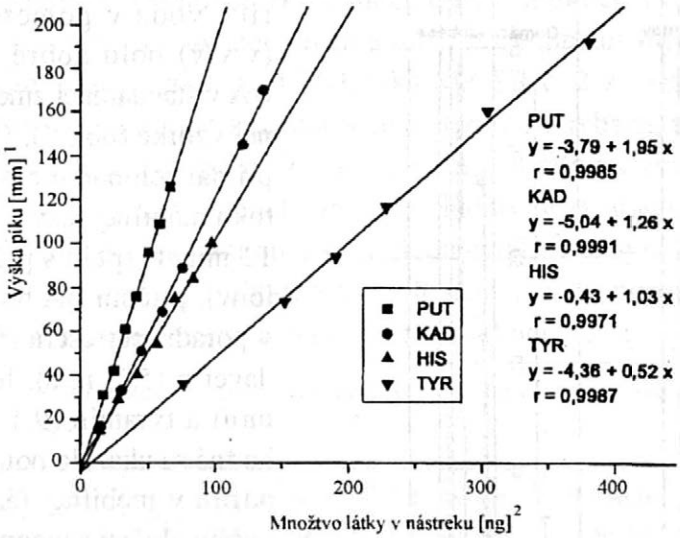
Stanovenie obsahu BA bolo realizované za nasledujúcich podmienok:

Kolóna: kovová kolóna 250 x 8 x 4 mm so stacionárnou fázou NUCLEOSIL 100 C18, 5 mm (Macherey Nagel, Germany), temperovaná na 40 °C, mobilná fáza – metanol : acetonitril : voda v objemovom pomere 2 : 1 : 1 s prietokom 1 cm^3/min

– slučkový dávkovač LCI 30 s 20ml slučkou

– detektor UV-VIS LCD 2040, vlnová dĺžka 254 nm

Obsah BA bol vyhodnocovaný metódou vnútorného štandardu (1,7-diaminoheptán) a kalibračnej krivky (obr. 1).



PUT = putrescín – putrescine; KAD = kadaverín – cadaverine; HIS = histamín – histamine; TYR = tyramín – tyramine;

[1]peak height; [2]substance quantity in feed

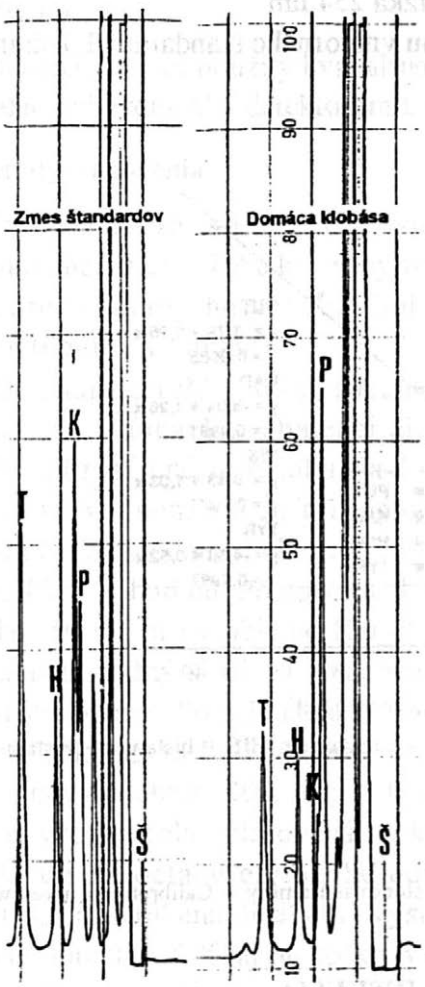
1. Kalibračné krivky s regresnými rovnicami pre sledované amíny – Calibration curves with regression equations for determined amines

VÝSLEDKY A DISKUSIA

Optimalizácia chromatografických podmienok

V úvodnej časti práce boli hľadané podmienky vhodné pre stanovenie BA vo forme danzylderivátov metódou HPLC. Pozornosť bola venovená výberu stacionárnej fázy a hľadaniu vhodnej mobilnej fázy. Najskôr bola testovaná

kolóna plnená reverznou fázou C_{18} fy Tessek (Ltd. Praha ČR). Pri použití mobilnej fázy metanol : voda v rôznych pomeroch sa podarilo dosiahnuť uspokojivé rozdelenie štandardnej zmesi nami sledovaných BA v poradí: tyramín, putrescín, kadaverín, histamín pri mobilnej fáze metanol : voda 75 : 25 (v/v). Avšak pri reálnej vzorke dochádzalo k prekrývaniu tyramínu inými zložkami, ktoré boli eluované v prvej časti analýzy.



P = putrescín – putrescine; K = kadaverín – cadaverine; H = histamín – histamine; T = tyramín – tyramine; Š = štart – start
[1]blend of standards; [2]home-made sausage

2. Chromatogram biogénnych amínov – Chromatograms of biogenic amines

Pri použití kolóny s náplňou NUCLEOSIL 100 C18 a mobilnej fázy metanol : acetonitril : voda v pomere 2 : 1 : 1 (v/v/v) bolo dobré rozdelenie BA v štandardnej zmesi aj v reálnej vzorke (obr. 2). Čas analýzy pri daných podmienkach a prietoku mobilnej fázy 1 m^3/min bol 12 minút (spolu s premytím kolóny), pričom BA boli eluované v poradí: putrescín (5. min), kadaverín (5,7. min), histamín (7. min) a tyramín (9,1. min). Výhodné sa ukázalo použitie acetonitrilu v mobilnej fáze pre jeho vyššiu elučnú schopnosť.

Výťažnosť metódy bola študovaná na vzorke domácej klobásy použitím 1,7-diamínoheptánu ako vnútorného štandardu a tyramínu v množstve 100 mg/kg. Tento diamín bol použitý preto, lebo sa nevyskytuje v potravinách (Etter et al., 1990). Výťažnosť sa pohybovala v rozmedzí 85–102 % s maximálnou smerodajnou odchýlkou 2,3 %.

Citlivosť, resp. medza stanoviteľnosti BA v roztokoch štandardov bola pre putrescín, kadaverín, histamín 10 ng a tyramín 20 ng v nástreku (20 ml), čo pri návažke 10 g zodpovedá 1, resp. 2 mg amínu na 1 kg vzorky. Tieto hodnoty sú dostatočne nízke pre toxikologické štúdie aj pre sledovanie produkcie amínov rôznymi kmeňmi mikroorganizmov (Greif et al., 1995a, b). Výsledky sú v súlade s literárnymi údajmi pre stanovenie BA metódou HPLC (Pavelka, Šubrtová, 1987, 1988).

V ďalšej časti práce sme sa zamerali na hodnotenie potravinárskych výrobkov živočíšneho pôvodu z hľadiska výskytu BA. V tab. I sú uvedené nami namerané hladiny koncentrácií BA v mliečnych výrobkoch a v tab. II mäsových výrobkov tepelne opracovaných a tepelne neopracovaných a konzerv z rýb. Na Slovensku zatiaľ platí Vyhláška MZ SR č. 2 z roku 1994, ktorá uvádza prípustné limity pre koncentráciu histamínu v rybách, rybacích konzervách (200 mg/kg), v pive (20 mg/dm^3) a tyramínu v tvrdých syroch (200 mg/kg). Ako vidieť z oboch tabuliek, hladiny histamínu sú prekročené len u vzoriek tuniaka a tyramínu u vzoriek ementálskeho syra a jarnej bryndze. Aj napriek tomu, že Vyhláška MZ SR neuvádza prípustné maximálne koncentrácie tyramínu v mäse a mäsových výrobkoch, ale aj iných výrobkov

I. Hladiny biogénnych amínov v mliečnych výrobkoch – The concentrations of biogenic amines in milk products

Vzorka[1]	Počet[9]	Kadaverín[10] [mg/kg]	Putrescín[11] [mg/kg]	Histamín[12] [mg/kg]	Tyramín[13] [mg/kg]
Syr Niva[2]	6	25–705	7–61	1–28	3–82
Syr Primátor[3]	6	1–42	5–30	1–20	2–120
Syr Ementál[4]	8	5–30	4–20	5–50	130–420
Bryndza – jarná[5]	4	930–1208	520–610	180–202	408–435
Syr Balkán – slaný[6]	3	1–20	1–35	58–102	3–29
Syr Hrudka[7]	3	1–24	5–40	5–83	0–10
Jogurt[8] I	5	ND	ND	1–5	10–45
Jogurt II	5	ND	ND	1–16	8–54

[1]sample; [2]Niva blue cheese; [3]Emmenthaler-type cheese Primátor; [4]Emmenthal cheese; [5]Brynza sheep cheese – spring; [6]Balkán pickled cheese; [7]Hrudka lumpy cheese; [8]yoghurt; [9]number; [10]cadaverine; [11]putrescine; [12]histamine; [13]tyramine

II. Hladiny biogénnych amínov v mäsových výrobkoch – The concentrations of biogenic amines in meat products

Vzorka[1]	Počet[10]	Kadaverín[11] [mg/kg]	Putrescín[12] [mg/kg]	Histamín[13] [mg/kg]	Tyramín[14] [mg/kg]
Dietne párky[2]	6	80–167	72–220	0–20	ND
Safalátky[3]	6	30–85	15–98	0–13	ND
Lovecká saláma[4]	3	105–190	80–186	2–49	10–58
Domáca klobása[5]	8	24–140	35–93	8–35	20445
Suchá saláma[6]	3	14–255	7–101	1–30	1–31
Treska – šalát[7]	4	0–15	0–24	11–75	30–182
Rybací šalát[8] I	4	1–20	0–35	1–5	109–136
Tuniak – konzerva[9]	3	20–180	124–171	35–1820	0–35

[1]sample; [2]dietary frankfurters; [3]wurst-type sausages; [4]hunter sausage; [5]home-made sausage; [6]salami; [7]cod – salad; [8]fish salad; [9]tuna – canned; [10]number; [11]cadaverine; [12]putrescine; [13]histamine; [14]tyramine

predovšetkým studenej kuchyne, je potrebné poukázať na šaláty z rýb a na výrobok domáca klobása, ktorý patrí do skupiny výrobkov tepelne neopracovaných. Vysoké koncentrácie tyramínu poukazujú na pomnoženie grampozitívnych kokov, a to predovšetkým rodov *Enterococcus faecalis* a *Enterococcus faecium*, ktoré sú zodpovedné za produkciu tyramínu a sú indikátorom úrovne hygieny a sanitácie v danej výrobni. Hoci vyhláška neuvádza prípustné hladiny koncentrácií pre putrescín a kadaverín, je potrebné ich sledovať vzhľadom na skutočnosť, že sú indikátormi pomnoženia baktérií z čeľade Enterobacteriaceae, a to predovšetkým *Escherichia coli, Enterobacter, Proteus, Klebsiella* a pseudomonád (*Pseudomonas fluorescens, Pseudomonas aeruginosa*), ako aj skutočnosti, že v tráviacom trakte oslabujú činnosť MAO a DAO, čo má za následok zvýšenie koncentrácií histamínu a tyramínu v krvi.

Prísun BA u nás sledovaných potravín, z ktorých najvyššie hodnoty boli namerané vedľa histamínu v rybách (tuniak) a tyramínu v jarnej bryndze a ementálskom syre, by za predpokladu normálneho konzumu nemal u zdravých ľudí vyvolať zdravotné problémy. Iná je však otázka u chorých osôb, ktoré užívajú lieky s inhibičnými účinkami na MAO a DAO, ako sú antihis-

taminiká (Alfadryl, Bromadryl, Methiaden), tuberkulostatiká (Ninrazid), ale predovšetkým psychofarmaká s antidepresívnym účinkom (Narval, Nardin, Stinerval), kde je potrebné zobrať do úvahy zmenený metabolizmus BA, ich možné nahromadenie, čo môže viesť k vážnym zdravotným poruchám. U takýchto osôb je preto potrebné brať v diéte ohľad na celkovú bilanciu prísunu BA potravou do organizmu.

Záver

Bola vypracovaná a overená metóda pre stanovenie biogénnych amínov (putrescínu, kadaverínu, histamínu a tyramínu) v potravinách živočíšneho pôvodu. Okrem toho je možné ju použiť aj pri stanovení amínov produkovaných mikroorganizmami a iných druhov potravín. Osvedčilo sa použitie HPLC metódy s využitím kolóny plnenej stacionárnou fázou NUCLEOSIL 100 C18. Ako mobilná fáza bola používaná zmes metanol : acetonitril : voda v pomere 2 : 1 : 1 (v/v/v). Detekcia bola robená v UV oblasti pri vlnovej dĺžke 254 nm. Medza stanoviteľnosti bola pre putrescín, kadaverín, histamín 10 ng a pre tyramín 20 ng v nástreku, čo pri návažke 10 g zodpovedá 1, resp. 2 mg amínu na 1 kg vzorky. Výťažnosť metódy bola 95–102 %, s maximálnou smerodajnou odchýlkou 2,3 %.

Zistené koncentrácie biogénnych amínov v nami sledovaných vzorkách nepredstavujú s ohľadom na literárne údaje zdravotné riziko u zdravých jedincov, okrem koncentrácií BA u vzoriek tuniaka, jarnej bryndze, ementálskeho syra a domácej klobásy. Aj napriek tomu považujeme za žiadúce pravidelne sledovať výskyt týchto endogénnych cudzorodých látok, pretože práve mäsové a mliečne výrobky sú významnou položkou stravy v našej krajine.

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Vyhláška MZ SR č. 2/94 Z. z., ktorou sa ustanovujú hygienické požiadavky na cudzorodé látky v poživatínách.

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Potrav. Vědy, *15*, 1997 (2): 131–136

DISTRIBÚCIA CHRÓMU A NIKLU V MLIEKU

Distribution of Chromium and Nickel in Milk

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Abstract: The distribution of chromium and nickel was studied in different fractions of raw cow's milk, gained after fractionation by centrifugation and acid coagulation. 40% of chromium and 27% of nickel went to the casein. 6% chromium and 7% nickel went to the cream and whey contained 53% Cr and 63% Ni. The element content in milk was determined in 1995 and 1996. Results were the basis for the change levels of these elements for milk products from 0.1 mg/kg to 0.5 mg/kg.

chromium; nickel; distribution; fractionation; milk

Abstrakt: V práci je sledovaná distribúcia chrómu a niklu v jednotlivých frakciách surového mlieka, získaných frakcionáciou pomocou odstreďovania a kyslého zrážania. Do kazeínu prešlo 40 % chrómu a 27 % niklu. V mliečnom tuku bolo 6 % chrómu a 7 % niklu. Srvátka obsahovala 53 % chrómu a 63 % niklu. Bola zistená hladina chrómu a niklu v mlieku v rokoch 1995 a 1996. Práca bola jedným z podkladov pre zmenu platného limitu množstva chrómu a niklu v mliečnych výrobkoch z 0,10 mg/kg na 0,50 mg/kg.

chróm; nikel; distribúcia; frakcionácia; mlieko

Obsah toxických kovov v životnom prostredí stále vzrastá. Prispievajú k tomu odpady a exhaláty oceliarskych podnikov, kožiarenského i chemického priemyslu, ale aj produkty uvoľňované spaľovaním fosílnych palív. Kovy chróm a nikel sa môžu dostať do pitnej vody z koróznych inhibítorov používaných pre vodné potrubie. Chróm na oxidačnej hladine 3 je esenciálny stopový prvok (pre udržiavanie glukózového, lipidového a proteínového metabolizmu v ľudskom organizme), Cr^{VI} má toxický účinok na biologický systém, je karcinogénny. Zlúčeniny niklu sú toxické pre vyvolávajúcu karci-

nogenitu dýchacích ústrojov, ale v malých dávkach je nikel pravdepodobne základným prvkom pre živé organizmy.

Chróm a nikel sú prvky kategorizované ako kontaminujúce cudzorodé látky, pretože prechádzajú zo životného prostredia do potravinového reťazca. Najvyššie prípustné množstvá definuje vyhláška Ministerstva zdravotníctva (Zb. yákonov č. 2/1994, čiastka 1, ktorá od 15. 7. 1996 bola nahradená čiastkou 9–13 Vestníku MZ SR 1996).

Výskyt Cr a Ni v potravinovom reťazci je od roku 1987 predmetom kontroly v rezorte pôdohospodárstva SR. Súhrnné zhodnotenie výsledkov ukázalo, že v niektorých komoditách, najmä však v mliečnych výrobkoch a syroch, bol obsah chrómu a niklu často vyšší ako najvyššie prípustné množstvo, definované pre danú komoditu obsahom 0,1 mg/kg pre Cr a Ni. Išlo niekedy o 100 až 250% prekročenie hygienicky limitovaného množstva. Zvýšený obsah Cr a Ni bol priebežne identifikovaný v tuzemských i dovážaných výrobkoch. Pre posúdenie reálnosti platného limitu pre mlieko a mliečne výrobky sme sledovali distribúciu Cr a Ni v mlieku, nakoľko sme nenašli v publikáciách prácu, ktorá by sa zaoberala týmto problémom. Sledovaním distribúcie kovov v mlieku a jeho frakciách sa zaoberalo už v minulosti viacero prác. Už v roku 1959 sa King et al. (1959) zaoberali sledovaním distribúcie medi a železa v mlieku. Roh et al. (1975) sledovali distribúciu kadmia a ortuti. U nás sa distribúciou ortuti zaoberali v roku 1995 Koreňovská a Poláčeková (1995).

MATERIÁL A METÓDY

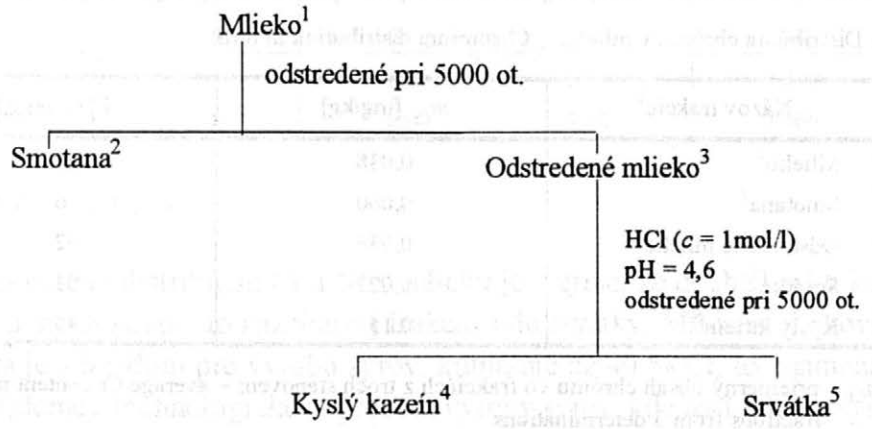
Metóda frakcionácie mlieka vychádza z fyzikálno-chemických vlastností mlieka, zo schopností vystúpenia tuku na povrch mlieka a jeho následného oddelenia. Spodnú vrstvu tvorí odtučnené mlieko, ktoré obsahuje len 0,5 % tuku. Jeho ďalšie delenie je založené na typickej vlastnosti mlieka, ktorá súvisí s jeho prechodom zo stavu koloidného roztoku do stavu zrazeniny, t. j. gélu. Druhotným javom je postupné sťahovanie sa zrazeniny a vylučovanie čírej srvátky z nej. Zrazeninu predstavuje kyslý kazeín. Srvátka obsahuje vysokomolekulárne bielkoviny – globulíny – a nízkomolekulové bielkoviny – albumíny.

Na distribúciu Cr a Ni v mlieku sme používali surové mlieko odobraté z roľníckych družstiev Stredoslovenského kraja v období september až december 1995. Priemerné fyzikálno-chemické hodnoty mlieka: tuk 3,85 %, kyslosť 6,4 °SH, BTS 8,73 %. Obsah chrómu a niklu v mlieku bol vyšší ako medza stanovenia použitej metódy (detekčný limit pre Ni a Cr 0,002 mg/kg, medza stanovenia 0,006 mg/kg), a preto sme nepoužili umelú kontamináciu a sledovali sme len prirodzenú distribúciu týchto kovov v mlieku. Na meranie množstva Cr a Ni sa použil atómový absorpčný spektrometer Perkin Elmer 4100 s grafitovou kyvetou HGA 700 po predchádzajúcej mineralizácii mokrou cestou v mikrovlnnom systéme Milestone 1200 MEGA. Chróm sa meral pri vlnovej dĺžke 357,9 nm a nikel pri 232,0 nm. Všetky použité chemikálie boli čistoty p.a. a Suprapur, voda bola dvakrát destilovaná.

Metóda stanovenia chrómu a niklu bola kontrolovaná na referenčnom materiáli „Green algae" SMÚ P-ACHK s certifikovanou hodnotou Cr = 2,4 mg/kg a referenčnom materiáli „Lucerna" SMÚ P-ALFALFA s certifikovanou hodnotou Ni = 2,54 mg/kg, nakoľko príbuzny referenčný materiál – mlieko s deklarovaným obsahom chrómu a niklu – sme nemali k dispozícii.

Postup frakcionácie

Frakcionácia mlieka sa robila v laboratórnych podmienkach. Surové mlieko sa premiešalo, zvážilo 500 g a odobralo na stanovenie obsahu Cr a Ni.



[1]milk; [2]cream; [3]skim milk; [4]acid casein; [5]whey

1. Schéma frakcionácie surového mlieka – Scheme of fraction of raw milk

Potom sa odstredilo na odstredivke pri 5 000 ot. po dobu 10 minút. Smotana sa oddelila. Z odstredeného mlieka sa oddelila kazeínová frakcia po prevedení do izoelektrického bodu kazeínu pri pH = 4,6 opatrným pridávaním HCl (1 mol/l). Po vyvločkovaní v chladničke sa odstredili príslušné frakcie srvátky a kazeínu pri 5 000 otáčkach za 10 minút. Kazeín a smotana sa nechali pri laboratórnej teplote mierne presušiť a všetky získané frakcie sa zvážili a zmineralizovali mokrou cestou v mikrovlnnom systéme. Mineralizačná zmes obsahovala 3 ml konc. HNO_3 a 0,5 ml H_2O_2.

V smotane, odstredenom mlieku, srvátke a kazeíne sa stanovilo množstvo chrómu, niklu a vyjadrilo sa v hmotnostných percentách. Publikované obsahy Cr a Ni sú priemernou hodnotou z troch frakcionácií.

VÝSLEDKY A DISKUSIA

Z celkového obsahu chrómu v mlieku sme vo frakciách namerali 98 % Cr. Do smotany prešlo približne 6 % a do odstredeného mlieka 92 % chrómu. V kazeínovej frakcii sa zachytilo 40 % a v srvátke zostalo 53 % Cr.

Z celkového množstva Ni v mlieku sme vo frakciách namerali 97 % niklu. Do smotany prešlo 7 % a do odstredeného mlieka 90 %. V kazeínovej frakcii sa zachytilo 27 % niklu a v srvátke zostalo 63 % (tab. I a II).

V rokoch 1995 a 1996 sme monitorovali obsah chrómu a niklu v surovom mlieku ako vstupnej surovine pre výrobu mliečnych výrobkov. Zisti-

I. Distribúcia chrómu v mlieku – Chromium distribution in milk

Názov frakcie[1]	m_{Cr} [mg/kg]	$\bar{x}$ [% hmot.]
Mlieko[2]	0,038	100
Smotana[3]	0,060	6
Odstredené mlieko[4]	0,036	92
Srvátka[5]	0,027	53
Kyslý kazeín[6]	0,15	40

m_{Cr} = priemerný obsah chrómu vo frakciách z troch stanovení – average Cr content measured in fractions from 3 determinations

$\bar{x}$ = priemerná hodnota percentuálneho obsahu Cr v jednotlivých frakciách mlieka – average value of Cr percentage content in different milk fractions

[1]fraction name; [2]milk; [3]cream; [4]skim milk; [5]whey; [6]acid casein

II. Distribúcia niklu v mlieku – Nickel distribution in milk

Frakcia[1]	m_{Ni} [mg/kg]	$\bar{x}$ [% hmot.]
Mlieko[2]	0,045	100
Smotana[3]	0,10	7
Odstredené mlieko[4]	0,040	90
Srvátka[5]	0,035	63
Kyslý kazeín[6]	0,12	27

m_{Ni} = priemerný obsah niklu vo frakciách – average nickel content measured in fractions from 3 determinations

$\bar{x}$ = priemerná hodnota percentuálneho obsahu Ni v jednotlivých frakciách mlieka – average value of Ni percentage content in different milk fractions

[1]fraction name; [2]milk; [3]cream; [4]skim milk; [5]whey; [6]acid casein

li sme, že priemerný obsah týchto kovov v roku 1995 bol 0,045 mg Cr/kg a 0,047 mg Ni/kg. V roku 1996 bol 0,023 mg Cr/kg a 0,037 mg Ni/kg (tab. III). V roku 1995 bola jedna vzorka mlieka nadlimitná na obsah niklu, ale pre chróm platný limit nebol prekročený. Hygienický limit pre množstvo chrómu a niklu v mlieku zostal nezmenený (0,10 mg/kg).

III. Obsah chrómu a niklu v mlieku – Chromium and nickel content in milk

	Rok[1]	Počet vzoriek[2]	$\bar{x}_{min}$ [mg/kg]	$\bar{x}_{max}$ [mg/kg]
Cr	1995	15	0,015	0,090
	1996	10	0,007	0,063
Ni	1995	15	0,005	0,230
	1996	10	0,025	0,066

[1]year, [2]number of samples

Zo sledovania distribúcie Cr a Ni v mlieku je zrejmé, že dochádza ku kumulácii daných kovov do kazeínovej frakcie a do srvátky. Mliečna bielkovina, ktorá je základom pre výrobu syrov, kumuluje až 40 % Cr, to znamená, že ak vyjdeme z technologického procesu výroby syrov, kde na 1 000 kg syra (45 % tuku v sušine) je spotreba asi 10 750 l mlieka, pri priemernom obsahu 0,038 mg Cr/kg mlieka sa v 10 750 l nachádza 408,5 mg Cr a pri zachytení

40 % do kazeínu zostáva v 1000 kg syra 163,4 mg Cr, čo je 0,164 mg Cr/kg syra , čo je hodnota nadlimitná. U niklu je situácia podobná. Z mlieka o obsahu 0,045 mg Ni/kg sa v 10 750 l mlieka nachádza 483,8 mg Ni a pri zachytení 27% do kazeínu zostáva v syre 130,6 mg Ni, čo je 0,131 mg Ni/kg syra a teda je tiež obsah niklu vo výrobku nadlimitný. No okrem mliečnej bielkoviny vo výrobe sa používa aj srvátka, ktorá ešte viac kumuluje chróm a nikel. V technologickom procese výroby syrov sa na zrážanie síce používa sýridlo, ale pri sledovaní výrobného procesu tavených a tvrdých syrov v mliekárni vo Zvolene sa nám kumulácia Cr a Ni vo výrobkoch potvrdila. Výsledky tejto práce budú publikované v nasledujúcom období.

Záver

Komisia Codex Alimentarius FAO/WHO odporučila jednotlivým štátom definovať také maximálne limity cudzorodých látok, ktoré sú reálne dosiahnuteľné. Prezentované výsledky boli jednou z prác, ktoré boli podkladom pre vytvorenie samostatnej kategórie „mliečne výrobky" s novým limitom 0,50 mg/kg pre nikel aj chróm.

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SHORT COMMUNICATIONS

THE USE OF ORGANIC ACIDS FOR SURFACE DECONTAMINATION OF POULTRY

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It is advantageous to decontaminate the surface of chilled chickens to increase their shelf-life and to enable the safe distribution. A number of preparations were suggested for the surface decontamination. Frequently, a suitable combination of organic acids can be used. In this case their properties are combined and they act together with pH value drop (synergistic effect), etc. The mostly used lactic acid acts by means of decreasing pH value and by its bactericidal properties stops growth of bacteria.

Chilled poultry meat is of greater preference from the view of consumers than in frozen state because the former shows better organoleptic properties. On the other hand, the shelf-life of chilled poultry is limited and it is necessary to ensure that the surface is decontaminated (Palumbo, Williams, 1994).

In the moment of death the meat is almost sterile so that the primary contamination is concerning especially the meat surface. Later the micro-organisms penetrate into deeper layers of meat. When this primary contamination and further meat infesting are reduced the shelf-life of meat can be extended significantly.

Lactic acid buffers increased shelf-life of poultry meat, without adverse effects on sensory quality. Strong inhibition of H_2S-forming bacteria, especially *Pseudomonas* spp., was observed. Inhibition of *Listeria monocytogenes* increased with increasing lactic acid concentration in the buffer (Zeitoun, 1992).

Surface application of lactic acid reduces the initial bacterial count and causes a delay of the start of logarithmic phase of their growth. At the same time, the count of enterobacteria is reduced (PURAC, 1995).

Treatment with 1 and 2% lactic acid was used in order to improve the bacteriological quality of broiler carcasses. Immediately after treatment, colonisation on the skin was generally reduced by about 1 log. 2% lactic acid prevents post-decontamination colonisation with *Enterobacteriaceae* more effectively than 1% lactic acid (Marel et al., 1988).

For stored poultry, *Escherichia coli* was the recommended index organism as it could indicate post-process temperature abuse. Lactic acid decontamination (10%

w/v in buffer pH 3) significantly eliminated *Enterobacteriaceae* immediately and extended its effect by inhibiting psychrotrophic bacterial growth on chicken stored at 6 °C (Zeitoun et al., 1994).

Quail carcasses (dark and light meat) were dipped in 2% lactic acid and packaged and stored at 4 °C for 12 days. Dipped carcasses showed a small initial reduction in total plate count and staphylococcal count. Acid treatment destroyed coliforms completely, moreover it extended the lag phase of bacterial growth. No adverse effect of 2% lactic acid treatment on tenderness, colour, flavour and overall acceptability attributes of quail meat was observed (Singh et al., 1988).

Processing trials indicated that lactic acid can be used in a variety of ways to either decrease or eliminate *Salmonellae* from the carcass (Izat, 1989). The dip applications evaluated included 1 and 2% lactic acid. All carcasses treated with lactic acid exhibited mild skin discoloration, particularly in the highly pigmented areas (Izat et al., 1990). The results show that the concentration greater than or equal 1.0% lactic acid completely inhibited growth of *S. typhimurium* strain isolated from poultry (Mulder et al., 1987).

The lactic acid treatment may be done at different places of the slaughter line. However it should be done as soon as possible, i.e. when the most of micro-organisms are present on the meat surface and have not yet penetrated into the deeper layers.

MATERIAL AND METHODS

Materials

Lactic acid PURAC – 80% L(+)lactic acid (Purac Gorinchem, Netherlands).

Tarisol Fresh – mixture of organic acids (lactic, citric, acetic and tartaric acids), pH 1.7 (Giulini, Ltd, Ludwigshafen, Germany).

Methods

The pH value was measured using pH meter Gryf 209 S (Elektronické přístroje, Havlíčkův Brod, Czech Republic) with combined glass-calomel electrode. The measurement was carried out inside the muscle tissue as well as on the surface of the skin. The surface pH-value was determined as follows: a part of the skin was dehided and the electrode was covered by it. Each value represents the average value of five measurements.

Water activity (a_W) was determined using Thermoconstanter TH-200 (Defensor AG, Swiss) at 25° C.

The microbiological methods included the estimation of total counts, the counts of lactobacilli, coli, salmonellae and *Listeria monocytogenes*.

The total counts were determined according to the ČSN ISO 4833 standard. Each 10 g of sample was homogenised with 100 g of physiological solution and diluted following the expected microbial counts. One ml of diluted sample was placed on the Petri dish and covered by 15 ml of the GTK agar (temperature of 40–45 °C). After mixing, the Petri dishes were stored at the temperature of 37 °C. After 24 or 48 h of cultivation the total counts were related to 1 g of the sample.

The lactobacilli determination followed the standard ČSN 56 0094. The sample treatment was identical as at total counts determination. The cultivation was carried out 48 h at 28 °C on the MRS medium under anaerobic conditions in aerostat.

Coli counts were determined after ČSN ISO 4832 standard. The sample treatment was identical as at total counts determination. The Petri dishes were cultivated at 37 °C. The VČŽL medium (crystal violet, neutral red, gall and lactose) was used.

Salmonellae were observed using the standard ČSN ISO 6579. Each 25 g of sample is inoculated in the liquid medium after Rappaport and Vassiliadis (with magnesium chloride and malachite green) 24 h at the temperature of 42 °C. Such culture is inoculated using a loop on the surface of selective medium (agar with phenol red and brilliant green) and incubated 24 h at 37 °C. The presence of *Salmonella* colonies is identified (they cause the medium colour change from pink to red). Other colonies were identified using the suitable biochemical and serological tests.

Determination of *Listeria monocytogenes* was carried out by the following procedure: 25 g of homogenised sample was added to 225 ml of primary multiplying medium LEB I. This mixture was incubated 21–24 h under the temperature of 23 °C. After incubation, 0.1 ml of incubate was placed to 10 ml of medium LEB II and incubated again under the 23 °C for 21–24 h. 2 ml of such incubate are inactivated by heating for 15 minutes at 80 °C in order to inactivate the contaminant micro-organisms. After cooling, 135 ml was transferred to Rapid test, which in the presence of *Listeria monocytogenes* became blue.

Application of additives

The L(+)lactic acid (Purac) was sprayed in solution to the chicken's surface at an industrial scale. The concentration of lactic acid in original additive was 80%. It was added in concentrations of 1 or 2% lactic acid. The chickens were sprayed before chilling using the solution of lactic acid of temperature 40–45 °C in hot state. The whole surface of carcasses (outside and inside the soma) was sprayed using the corresponding solution.

The organic acid mixture Tarisol Fresh was applied in a similar way, i.e. the chickens were sprayed using a 1% solution of Tarisol Fresh of temperature 40–45 °C.

Warm water (40 °C) was always used as the control. All additives were applied immediately after washing the chicken at the end of the slaughter line. Then the

chickens were chilled in a cool air tunnel, packed into plastic film and stored under cooling temperature between 1 °C and 0 °C.

RESULTS AND DISCUSSION

The results of experiments (presented in the figures) show that the application of lactic acid and the mixture of acids is effective also at an industrial scale. In all cases the application of organic acids inhibited the growth of micro-organisms on the surface of poultry carcasses and in this way the shelf-life of chilled (not frozen) chicken was extended. The efficiency of treatment was influenced by the concentration of solution used. The water activity (a_W) was not influenced by the organic acids as seen in Table I.

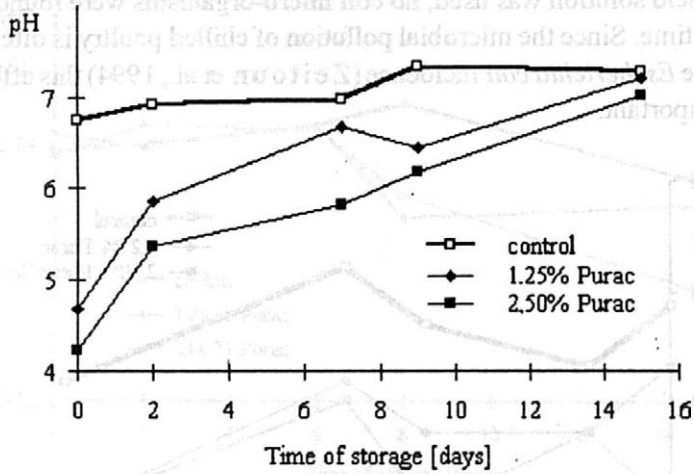
I. Water activity during chilling storage of chickens treated with lactic acid or Tarisol

Time of storage [days]	Control	1% lactic acid	2% lactic acid	1% Tarisol
0	0.976	0.975	0.972	0.971
2	0.976	0.973	0.973	0.973
7	0.976	0.976	0.975	0.976
9	0.977	0.978	0.976	0.976
15	0.976	0.976	0.974	0.975

The use of lactic acid

The pH value of surface layer fell (Fig. 1) significantly after the lactic acid (Purac) application. Naturally, the higher the concentration of lactic acid solution applied, the deeper the decrease of pH value was observed. The pH value in deeper layers of meat was not influenced significantly by the surface acid treatment. In a few days the pH value increased again due to the buffer capacity of meat, the diffusion of lactic acid into the deeper layers of the muscle tissue and/or due to its decomposition. Thus in another several days the pH values approached the initial ones of the non-treated samples. At the same time the pH value of the non-treated samples increased (evidently as a result of micro-organism metabolism). The pH value of the samples treated with 2% lactic acid did not however reach the values found in the control sample. Initial drop of the pH value and the presence of lactic acid however were important for inactivation of micro-organisms on the chicken's surface.

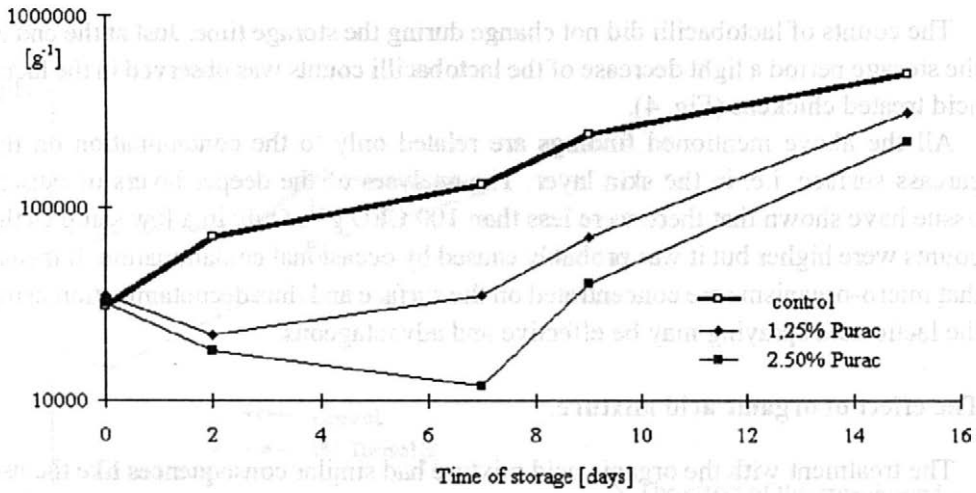
The treatment using lactic acid retarded the increase of total micro-organism counts at treated poultry (Fig. 2). Initially, after the lactic acid treatment a mild decrease of



1. The effect of lactic acid (Purac) on the pH values on the surface of chicken carcasses during chilling storage

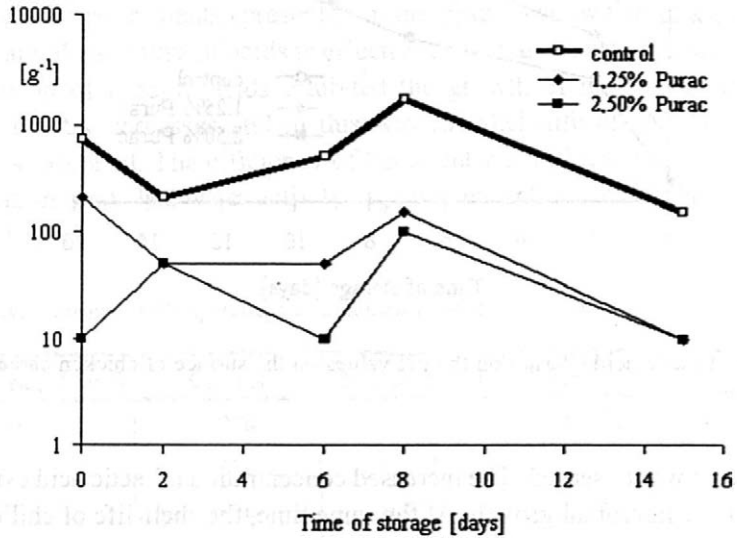
the total counts was observed. The increased concentration of lactic acid extended the lag phase of the microbial growth. At the same time, the shelf-life of chilled poultry was prolonged.

The counts of coli (Fig. 3) lightly decreased during the storage. These values for the lactic acid treated chickens were lower in comparison to the unreated ones. When



2. The effect of lactic acid (Purac) on the total counts of microorganisms on the surface of chicken carcasses during chilling storage

the 2% lactic acid solution was used, no coli micro-organisms were found during the whole storage time. Since the microbial pollution of chilled poultry is often estimated by means of the *Escherichia coli* incidence (Zeitoun et al., 1994) this effect of lactic acid is very important.



3. The effect of lactic acid (Purac) on the coli counts on the surface of chicken carcasses during chilling storage

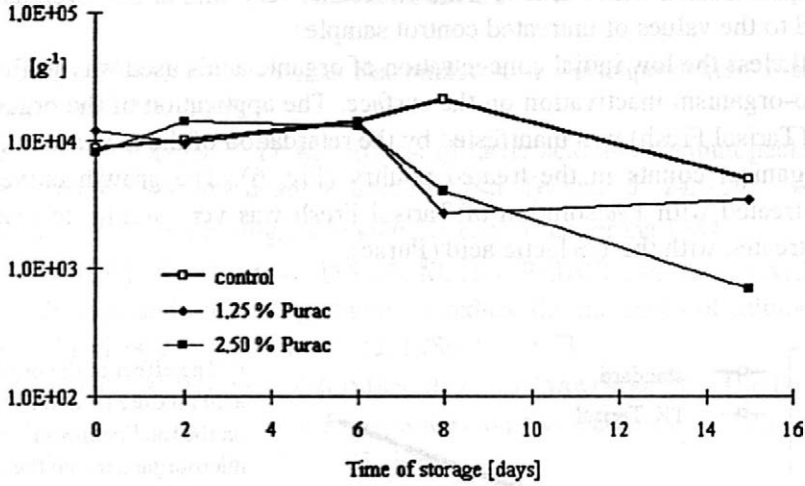
The counts of lactobacilli did not change during the storage time. Just at the end of the storage period a light decrease of the lactobacilli counts was observed in the lactic acid treated chickens (Fig. 4).

All the above mentioned findings are related only to the contamination on the carcass surface, i.e. in the skin layer. The analyses of the deeper layers of muscle tissue have shown that there were less than 100 CFU g^{-1}. Only in a few samples the counts were higher but it was probably caused by occasional contamination. It means that micro-organisms are concentrated on the surface and thus decontamination using the lactic acid spraying may be effective and advantageous.

The effect of organic acid mixture

The treatment with the organic acid mixture had similar consequences like the use of lactic acid alone.

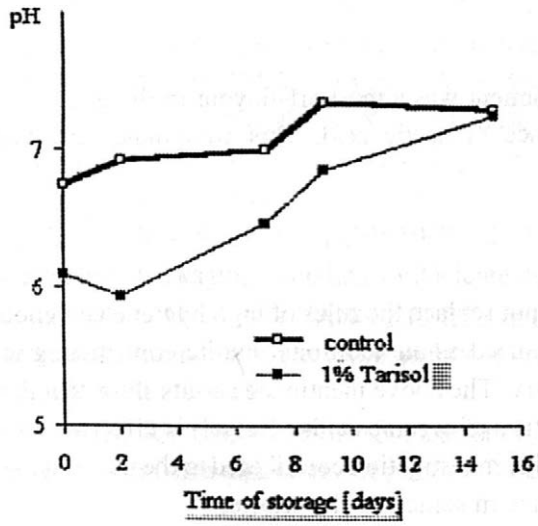
The pH value of the surface layer fell (Fig. 5) after the application of the organic acid mixture (Tarisol). The pH value increased consequently due to the buffer



4. The effect of lactic acid (Purac) on the lactobacilli counts on the surface of chicken carcasses during chilling storage

capacity of the meat, to the diffusion of organic acids in the deeper layer of the muscle tissue and/or due to their decomposition. In a few days the pH value of treated samples reached that of untreated samples.

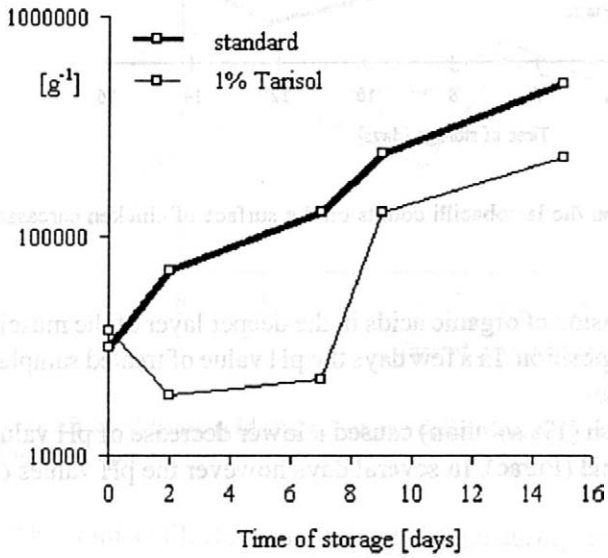
The acid mixture Tarisol Fresh (1% solution) caused a lower decrease of pH value in comparison with the lactic acid (Purac). In several days however the pH values of



5. The effect of the organic acid mixture (Tarisol Fresh) on the pH values on the surface of chicken carcasses during chilling storage

both samples treated with Purac or Tarisol became very similar and eventually they increased to the values of untreated control samples.

Nevertheless the low initial concentration of organic acids used was sufficient for the micro-organism inactivation on the surface. The application of the organic acid mixture (Tarisol Fresh) was manifested by the retardation of the increase in the total micro-organism counts in the treated poultry (Fig. 6). The growth curve of the samples treated with 1% solution of Tarisol Fresh was very similar to that of the samples treated with the 1% lactic acid (Purac).



6. The effect of the organic acid mixture (Tarisol Fresh) on the total counts of microorganisms on the surface of chicken carcasses during chilling storage

The disadvantage of the Tarisol treatment was a mild off-flavour in the bags with treated chicken caused by the presence of acetic acid. This compound however evaporates during heat treatment.

Conclusions

The application of organic acids cannot replace the rules of high hygiene and good manufacturing practice, but it may be used as an additional hurdle contributing to extending the shelf-life of chilled poultry. The above mentioned results show that the application of lactic acid or of the mixture of organic acids (Tarisol) is effective also at an industrial scale. So the results of this investigation contributed to the introducing of this method into the industrial practice in some Czech factories.

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Použití organických kyselin na dekontaminaci drůbeže

V průmyslových podmínkách byl sledován vliv postřiku povrchu drůbeže (kuřat) organickými kyselinami na údržnost během chladírenského skladování. Povrch kuřat byl ještě před vstupem do chladicího tunelu postříkán roztokem organických kyselin. Kyselina mléčná (Purac) byla aplikována ve formě 1% a 2% roztoku, směs organických kyselin (Tarisol Fresh) byla použita ve formě 1% roztoku. Ošetření kuřat organickými kyselinami vedlo ke snížení hodnoty pH na povrchu; tato hodnota se v průběhu skladování zvyšovala a dosáhla původní hodnoty. K hlubšímu poklesu pH došlo při aplikaci samotné kyseliny mléčné. Důsledkem snížení pH bylo omezení růstu mikroorganismů a tedy prodloužení údržnosti. Postřik 2% roztokem kyseliny mléčné vedl k úplnému vyloučení růstu koliformních bakterií.

organické kyseliny; kyselina mléčná; dekontaminace; drůbež; kuřata

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REVIEWS

PALM OIL

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Palm oil is one of vegetable oils the importance of which has increased enormously during recent decades. Palm oil is appreciated particularly due to its processing and application characteristics, and with respect to changed internal economic relations it has begun to be an interesting material also for the companies within the sector of fat industry.

The source of palm oil is the fruits of oil palm that reaches the height of 20–30 m. Oil palms form a typical tree top with bunches of fruits having 15–30 cm in diameter. The skeleton of the bunch bears several hundreds of fruits resembling plums as far as the size is concerned, the colour varying from orange to brown when ripe. The fruit consists of pulp (mesocarp) and nut. The pulp forms about 70% of the weight of the fruit and represents the source of oil. The nut is relatively hard and gives palm kernel oil whose composition closely resembles that of coconut oil.

I. World production of palm oil in thous. tons per year

	1960	1970	1980	1990	1995
Malaysia	92	431	2 573	6 095	7 811
Nigeria	638	449	433	580	630
Indonesia	141	216	691	2 413	4 040
Ivory Coast	14	50	182	270	290
Papua New Guinea	–	–	35	145	223
Colombia	–	27	74	226	388
Others	421	623	631	1 225	1 635
Total	1 306	1 796	4 619	10 954	15 017
Export from Malaysia	98	402	2 271	5 727	6 505

The cultivation of oil palm requires a typical tropical climate and that is why the main production areas are in Malaysia, Indonesia, Nigeria, etc. Favourable governmental policy, particularly in Malaysia, resulted in a remarkable increase of palm oil production (Oil World) (Table I).

Now the Malaysian export of palm oil forms 70–80% of the world export of this commodity. It should be stressed that the increase of palm oil production is accompanied not only by the export of refined palm oil but also by an increasing export of its fractions.

The biggest importers of palm oil are above all India, China, Pakistan, Middle East and also the countries of European Union and Russia in recent years. In the early 1980's smaller amounts of palm oil (particularly palm stearin) were also imported also into this country where it was used particularly in the manufacture of totally hardened whipped fat called Ceres Soft.

The price of palm oil was on the decline on the world market till the end of the 1980's but it has been increasing since 1991 (Table II) (Oil World). Nevertheless

II. Prices [USD/ton] of palm oil in world market in recent years

	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995
Crude palm oil [1)]	501	257	343	437	350	290	339	394	378	528	628
Palm oil [2)]	504	258	329	418	328	280	323	379	370	531	624

[1)] CIF Rotterdam, [2)] FOB Malaysia

III. Prices [USD/ton] of some oils in world market in recent years

		1987	1989	1991	1993	1995
Crude palm oil	*(CIF Rotterdam)*	343	350	339	378	628
Palm oil	*(FOB Malaysia)*	329	328	323	370	624
Coconut oil	*(CIF Rotterdam)*	442	517	433	450	670
Palm kernel oil	*(CIF Rotterdam)*	426	472	417	437	677
Rapeseed oil	*(FOB Dutch)*	305	413	414	466	614
Sunflower oil	*(ex-tank Rotterdam)*	361	481	474	540	693
Cottonseed oil	*(CIF Rotterdam)*	497	572	579	708	659
Peanut oil	*(CIF Rotterdam)*	500	775	895	739	991

the price is lower than that of rapeseed and sunflower oil (Table III) (Oil World). Favourable price and application characteristics together with the changes of internal economic system contribute to better chances of this raw material in Czech fat industry .

EXTRACTION OF PALM OIL

The extraction of palm oil requires to some extent different technology compared with that currently in use. Individual technological stages can be characterized as follows (Nielsen, 1972):

1. Sterilization – fruit bunches are sterilized in an autoclave at 0.2–0.3 MPa for a period of one hour.
   The purpose of this process is:
   A) Inactivation of plentiful enzyme; this process prevents the oil from lipolytic decomposition.
   B) Softening of pulp, which facilitates the release of oil.
   C) Facilitate loosing of kernel from the fruit.
2. Stripping – during this operation the fruit is stripped from the bunch in rotating drum threshers.
3. Mashing – this process is carried out in special tanks with rotating arms mashing the pulp at 80–90 °C and loosening the nuts.
4. Pressing – now carried out in screw presses (formerly in hydraulic ones). This operation yields a mixture containing 54% of oil, 40% of water and 6% of cellular mass.

The mixture obtained is then purified by settling with subsequent centrifuging and then drying. The crude palm oil obtained exhibits the following physicochemical characteristics: melting point 30.8–37.6 °C, iodine value 51.0–55.3%, saponification number 190.0–201.0 mg KOH/g, acid value 4–12 (and more) mg KOH/g, unsaponifiables 0.15–0.99% (Tan, Oh, 1981).

COMPOSITION OF PALM OIL

Palm oil is a mushy stuff at normal temperature, and possesses the characteristic composition of fatty acids with a dominant position of palmitic acid (about 45%) and oleic acid (about 40%). A low content of polyunsaturated fatty acids together with a high content of natural antioxidants results in a high oxidation resistance of this oil (Tan, Oh, 1981) (Table IV).

The presence of triacylglycerols (TAG) (in mole%) in palm oil expressed by carbon number is given in Table V and the solid fat content of palm oil is given in Table VI (Tan, Oh, 1981).

IV. Composition of fatty acids of crude palm oil and palm kernel oil

Fatty acids [%]	Palm oil			Palm kernel oil
	min.	max.	average	
C 6:0				0.3
C 8:0				3.9
C 10:0				4.0
C 12:0	0.1	0.1	0.2	49.6
C 14:0	0.9	1.5	1.1	16.0
C 16:0	41.8	46.8	44.0	8.0
C 16:1	0.1	0.3	0.1	–
C 18:0	4.2	5.1	4.5	2.4
C 18:1	37.3	40.8	39.2	13.7
C 18:2	9.1	11.0	10.1	2.0
C 18:3	0.0	0.6	0.4	–
C 20:0	0.2	0.7	0.4	9.1

V. Carbon numbers of palm oil

Carbon number	Type of TAG	Min.	Max.	Average
C 46		0.4	1.2	0.8
C 48	PPP	4.7	10.8	7.4
C 50	PPO, PPS	40.0	45.2	42.6
C 52	POO, POL, POS	38.2	43.8	40.5
C 54	OOO, OOL, OOS	6.4	11.4	8.8

VI. Solid fat content of palm oil

Temperature [°C]	5	10	15	20	25	30	35	40
SFC [%]	50.7–68.0	40.0–55.2	27.2–39.7	14.7–27.9	6.5–18.5	4.5–14.1	1.8–11.7	0–7.5

VII. Content of minor components in crude palm oil and crude zero-erucic rapeseed oil

Minor components	Zero-erucic rapeseed oil	Palm oil
Carotenoids	95 [mg/kg]	500–700 [mg/kg]
Chlorophyll colourants	5–35 [mg/kg]	–
Tocopherols	500–1 000 [mg/kg]	300–500 [mg/kg]
alpha-	35–45%	30–35%
beta-	–	–
gamma-	55–60%	45–55%
delta-	0–5%	15–25%
Tocotrienols	–	400–700 [mg/kg]
alpha-	–	18–35%
beta-	–	0–7%
gamma-	–	30–45%
delta-	–	7–20%
Sterols	5 000–9 500 [mg/kg]	60–250 [mg/kg]
β-*sitosterol*	48–55%	60–75%
campesterol	28–35%	15–22%
stigmasterol	traces	8–15%
brassicasterol	9–12%	–
others	2–7%	3–7%
Phospholipids	0.7–1.5%	0.05%
PC	27%	36%
PE	30%	24%
PI	15%	22%
others	28%	18%

PC – phosphatidylcholine; PE – phosphatidylethanolamine; PI – phosphatidylinositol

Crude palm oil contains a considerable quantity of minor components comprising carotenoids, tocopherols, sterols, phospholipids, triterpenic and aliphatic alcohols, and hydrocarbons. Their content and relative abundance is given in Table VII.

When compared with zero-erucic rapeseed oil, crude palm oil contains significantly higher amount of carotenes and as one of few vegetable oils it contains a significant amount of tocotrienols. There is a lower content of sterols and phospholipids in this oil.

REFINING OF PALM OIL

Refining practices used in the refining of crude palm oil are to a great extent identical with those usually used in the industry.

They are:

- Hydration (0.1–0.2% of H_3PO_4 or citric acid)
- Neutralization
- Bleaching (1–2% of bleaching earth)
- Deodorization

As far as the export of oil is concerned, three basic commodities with a different degree of refining are at disposal, and their characteristics are given in Table VIII.

VIII. Characteristics of commercial palm oils from Malaysia

	N-PO	NB-PO	RBD-PO
Free fatty acids[%]	max. 0.25	max. 0.25	max. 0.1
Water content [%]	max. 0.1	max. 0.1	max. 0.1
Iodine value [%]	50–55	50–55	50–55
Melting point [°C]	33–39	33–39	33–39
Colour (Lovibond 5 1/4")	–	max. 20 R	max. 3 R
P [mg/kg]	–	–	max. 4
Fe [mg/kg]	–	–	max. 0.12
Cu [mg/kg]	–	–	max. 0.05
Peroxide value [mole/g]	–	–	0

N-PO – neutralized palm oil; NB-PO – neutralized and bleached palm oil; RBD–PO – fully refined palm oil

USE OF PALM OIL

Palm oil, due to its composition and physicochemical characteristics, is a much sought-for material for a number of products, including (Berger, 1981a, b; Teah, Ong, 1986):

- totally hardened fats for frying and deep fat frying
- emulsified fats of soft type
- industrial emulsified fats including ductile margarine

Table IX presents typical applications of palm oil in specified products (Přikryl, Novák, 1991).

IX. Examples of the use of palm oil in solid products

Component	Shortening [%]		Soft margarine [%]		Ductile margarine [%]		Table margarine [%]	
Palm oil	60	80	30	10	40	50	50	20
H_3PO_4 – 42 °C*	20	–	–	–	50	50	10	
Sunflower oil	20	20	50	60	10		15	10
Palm stearin	–	–	–	10				
Palm kernel oil							25	30
Hardened vegetable fat 34/36			20	20				40

*Palmolein hardened to the melting point of 42 °C

Palm oil and some fractions of palm oil exhibit polymorphic characteristics. Under rapid cooling and mechanical stress they yield fat of smooth texture with dominant β' crystalline modification, and on the contrary, under slow (spontaneous) cooling under isothermic conditions, β-type crystals are formed, which is utilized in the manufacture of Vanaspati-type fats popular in the Middle East region and in the Indian subcontinent.

The advantages of palm oil and some of its modified forms in the course of margarine and shortenings preparation can be seen from the following facts (Kheiri, 1982; Berger, 1984):

- palm oil helps to furnish product with suitable consistency characteristics,
- palm oil supports fat feed crystallization in the β'-form and contributes to the prevention of lumpy and gritty products and/or possible graining,
- using of palm oil decreases costs in connection with hydrogenation,
- palm oil increases oxidative stability of products.

MODIFICATION OF PALM OIL

A suitable modification of palm oil can further extend the range of its applications, which is fully utilized in practice. Usual modifications of palm oil comprise:

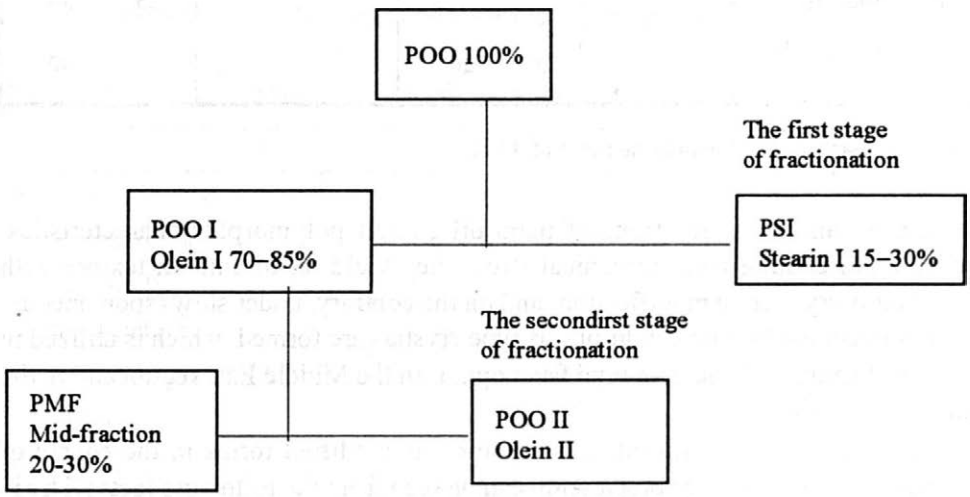
- fractionation,
- interesterification,
- hydrogenation.

These processes are mostly intended for the preparation of special fats or fats with improved application properties.

FRACTIONATION

In the course of fractionation the method of controlled crystallization enables the manufacturer to separate solid and liquid fractions of palm oil and obtain fat fractions with different physicochemical characteristics. During the fractionation of palm oil palmstearin as a solid and olein as a liquid fraction are produced.

As an illustration a classical two-stage fractionation of palm oil using detergent is presented in Fig. 1. Pursuant to the conditions of fractionation and the method chosen fractions of the following composition are obtained (Tables X and XI).



1. Example of a two-stage fractionation of palm oil with the use of detergent

The use of individual fractions:

POO – alone or in combination with vegetable oils as liquid fat for frying and deep fat frying
– modified (hydrogenated) for the manufacture of cocoa butter substitutes
– liquid fraction for the preparation of margarines

PS – solid fraction as a part of fat in emulsified fats and hardened fats

PMF – preparation of cocoa butter substitutes

INTERESTERIFICATION

During interesterification a statistical distribution of fatty acids in the molecules of triacylglycerols occurs, which becomes evident as a change of physical characteristics of the fat modified. As can be seen from the following table, within the framework of the interesterification of palm oil and its fractions, the solid fat

X. Characteristics of palm oil fractions

	POO I	PS I			PMF	
		soft	hard	average	50	34
I.V. [%]	56–60	24	50	40	49	35
Melting point [°C]	19–24	35	58	51	28	32
SFC [%] 10 °C	28–52	66	90	76	66	88
20 °C	3–9	35	84	57	32	42
30 °C	–	7	68	33	–	8
35 °C	–	3			–	–
40 °C	–	–	56	19	–	–
45 °C	–	–	40	12	–	–

content increases, and except for stearin, melting point increases too (Kheiri, 1985). Interesterified products are therefore harder (Table XII), interesterified palm oil does not spontaneously release liquid fraction, the products exhibit better crystallization characteristics, particularly in connection with the packaging of final products.

Other opportunities consist in the interesterification of the mixtures of palm stearin with vegetable oils or mixtures of palm oil with coconut oil or palm kernel oil

XI. Composition of fatty acids in palm oil fractions

Fatty acids [%]	POO I	PS		
		soft	hard	average
12 : 0	0.1–1.1	0.1	0.6	0.1
14 : 0	0.9–1.4	1.1	1.9	1.3
16 : 0	37.9–41.7	47.2	73.8	54.0
18 : 0	4.0–4.8	4.4	5.6	4.7
18 : 1	40.7–43.7	15.6	37.0	32.3
18 : 2	10.4–13.4	3.2	9.8	7.0
18 : 3	0.1–0.6	0.1	0.6	0.1
20 : 0	0.2–0.5	0.1	0.6	0.4

XII. Characteristics of interesterified palm oil and its fractions

Parameter	Palm oil	I.V. 55	Palm olein	I.V. 60	Palm stearin	I.V. 45
	before	after	before	after	before	after
Melting point [°C]	33.0	43.5	20.8	40.8	49.6	48.3
SFC [%] 20 °C	20.0	33.0	–	24.0	49.0	52.0
30 °C	7.0	17.0	–	11.0	28.0	31.0
35 °C	3.0	12.0	–	8.0	23.0	23.0
40 °C	2.0	9.0	–	5.0	19.0	18.0

which can be utilized in the manufacture of emulsified fats or hard fats with low *trans*-fatty acid content.

HYDROGENATION

As has already been said, palm oil as such has semi-solid consistency, and because its melting point ranges from 30 to 38 °C, hydrogenation is not necessary in most cases, and the oil is used in a number of fats directly in its natural form. The hydrogenation of palm oil or of its fractions is carried out when it further extends their application range (Kheiri, 1985; Grothues, 1985; Lefébvre, Baltes, 1975).

1. Hydrogenation of palm oil to melting point (M.P.) 40–42 °C

This material is used as solid fraction in margarine fats where it improves crystallization characteristics, and that is why it is preferred to palm stearin.

In the hydrogenation to M.P. 40–42 °C, fresh or partially exhausted Ni catalyst (0.06–0.20% Ni related to oil) is used, and the process runs at 180–200 °C. As the drop of iodine value is 4–5 units only, the cost of consumed hydrogen is very small as it will be shown in Table XIII in the final part of this chapter.

2. *Iso-* and *trans*-promoting hydrogenation

Fats with high dilation are necessary for some purposes as e.g. for applications in chocolate industry (fats for fillings and glazes, etc.). That is why, besides fractionated fats, palm oil hydrogenated in mixtures with vegetable oils with an intention to increase *trans*-fatty acids content is suitable. Special sulphur containing catalysts are used for this purpose.

Nevertheless, because *trans*-fatty acids are considered as not harmful but undesirable according to recent research works, this hydrogenation process loses its practical importance.

3. Hydrogenation of palm olein to M.P. 30–38 °C

Palm olein hydrogenated to M.P. 30–38 °C either alone or mixed with other oils is used as a special confectionery fat in chocolate glazes and fillings. As can be seen from Table XIII, these are fats melting relatively rapidly at the temperature around 35 °C.

XIII. Characteristics of palm oil (PO) and palmolein (POO) hydrogenated to various melting point

Parameter	Hydrogenated			
	PO	POO	POO	POO
Melting point [°C]	40.3	30.9	35.7	38.7
I.V. [%]	51	56	54	53
Trans-FA content [%]	13	9	18	25
SFC [%] 10 °C	75	57	72	84
20 °C	52	27	44	61
30 °C	25	7	18	30
35 °C	17	2	8	17
40 °C	7	0	0	3

4. Preparation of totally hardened palm oil

It is best to run hydrogenation for this purpose at 180 °C, pressure 3–5 bar and to use fresh Ni catalyst (0.2% Ni). The product having I.V. of 3 exhibits slip melting point 58 °C. Palm oil hydrogenated in such a way can be well used in the following cases:

- In 0.5–5.0% as crystallization promoting agent.
- As one of the components of mixtures with vegetable oils in interesterification, the purpose of which is to prepare fats free from cholesterol and *trans*-isomers.
- In the manufacture of distilled monoacylglycerols in mixtures with other vegetable oils (at 1 : 1 ratio).

The hydrogenation of palm oil and its fractions runs very easily, compared with other vegetable oils (zero-erucic rapeseed oil) when hardening to the same degree of hardening. Table XIV presents a comparison of the consumption of hydrogen per ton of oil in the hydrogenation of different oils to similar melting point, and the comparison of resulting energy consumption necessary for the electrolytic manufacture of hydrogen.

XIV. Consumption of hydrogen and power in the hydrogenation of various oils

Oil	Change of I.V.	M.P. [°C]	Consuption of H_2 in m^3	kWh/t
Soybean oil	130–70	36	60	348
Rapeseed oil	112–69	36	45	200
Sunflower oil	136–75	36	61	354
Peanut oil	92–66	36	26	151
Palm oil	55–51	40.5	4	23
Palmolein	58–54	36	4	23
Palmstearin	33–1	59	32	175

CONCLUSION

As this paper does not allow a more detailed review of the uses of palm oil and its modified forms, only the basic trends in use are indicated. If we consider the composition of palm oil and its modified forms, their resistance to oxidation, application and processing characteristics as well as prices, palm oil seems to be a suitable extension of the existing basis of domestic fats. Our own experience with palm oil so far can give evidence for this, and a larger use of palm oil in the fat industry can be recommended and should be supported.

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Palmový olej

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