ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH
INFORMACÍ

POTRAVINÁŘSKÉ VĚDY
FOOD SCIENCES

1

ČNÍK 15
HA 1997
SSN 0862-8653

ČESKÁ AKADEMIE ZEMĚDĚLSKÝCH VĚD

POTRAVINÁŘSKÉ VĚDY
FOOD SCIENCES

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

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

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Subscription information: Subscription orders can be entered only by calendar year and should be sent to the contact address.
Subscription price for 1997 is 306 Kč, 76 USD (Europe) and 80 USD (overseas)

Periodicity: The journal is published six times a year.

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Potrav. Vědy, *15*, 1997 (1) : 1–12

GC/MS PROCEDURE FOR ANALYSIS OF SYNTHETIC PYRETHROID RESIDUES IN TREATED CEREALS*

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Abstract: Optimisation of procedure for determination of 6 widely used pyrethroid residues in cereals was investigated. Grains were extracted with ethyl acetate, gel permeation chromatography (GPC) was employed for clean-up of crude extracts. High resolution gas chromatography coupled with mass selective detector (HRGC/MSD) performed in selected ion monitoring mode (SIM) was used for isomeric separation of these insecticides and their subsequent quantitation. The recoveries at 0.1 mg/kg spiking level were 90–99%. The detection limits ranged from 0.0002 to 0.05 mg/kg. Compared to existing methods, the procedure presented in this study is less laborious and highly selective.

pyrethroids; insecticides; residues in grains; gas chromatography-mass spectrometry

Synthetic pyrethroids represent a group of "modern" insecticides being nowadays widely used for the control of insects during the growth of fruits and vegetables as well as for the protection of stored grains. Residue limits for some pyrethroids in food commodities have been prescribed by FAO/WHO (Anonym, 1985), the demand for their control also follows from EU legislation.

The concern in control of pyrethroid occurrence in food supply is reflected by several recent papers reviewing the state-of-art in the field of multiresidue methods applicable for analysis of these contaminants and/or proposing new improved/optimised procedures. Extraction with acetonitrile, partition into n-hexane, Florisil clean-up and capillary GC/ECD, respectively, are the prin-

* Supported by COST Action 66.30 and by Ministry of Education, Youth and Culture of Czech Republic, project of FRVS: IS 0193/7.

cipal steps of a method developed for determination of natural pyrethrins and 12 synthetic pyrethroids in plant matrices (Nakamura et al., 1993). In another study (Pang et al., 1994a), acetone-petroleum ether mixture was used to extract 10 pyrethroid insecticides from various crops. Mixed Florisil-charcoal-alumina column was used to reme of co-isolated compounds from grains and/or oily sample extracts; GC/ECD employing packed column was then used for quantitation. A small-scale partition of ethanol extract into toluene followed by Florisil mini-column clean-up was used (Wan et al., 1994) prior to GC/ECD determination of 13 organochlorine and pyrethroid pesticides in spiked vegetables. Similar method (methanol used for extraction) was described (Pang et al., 1994b) for determination of 11 pyrethroids in six different fruits and vegetables. The same authors (Pang et al., 1995a) suggested modification of AOAC Method 970.52.: residues are extracted with acetonitrile-water (for fruits and vegetables) or with acetonitrile (for grains). After a transfer of residues to hexane, removing of co-extracts by acetonitrile partition and Florisil clean-up were carried out. Altogether 20 crops spiked with 8 pyrethroids were involved in respective experiments. In the second part of this extensive study (Pang et al., 1995b), an acetone extraction system was compared with the previous one utilising acetonitrile. Both procedures were evaluated with regard to the achieved recoveries and occurrence of interfering materials.

The purpose of our study was to examine the applicability of GC/MS(SIM) procedure for unbiased and simple multi-residue determination of six most common synthetic pyrethroids in grains. Although the persistence of these insecticides under the field conditions is relatively low and, consequently, the residues in harvested crops are often below the detection limits, the reduction of residues in stored grains in case of post-harvest treatment is a relatively slower process, moreover, bran/grain concentration factors greater than 1 were recorded in many studies concerned with the fate of residues during cereal processing. The control of pyrethroid occurrence in this commodity representing an important component of human diet by a reliable method is therefore urgently needed.

MATERIALS AND METHODS

Wheat grains were purchased in retail store (the history of chemical treatment was unknown).

Standards, Chemicals: Standards of alphametrin (91%), bifenthrin (98%), cyhalothrin (97%), deltamethrin (98.5%), fluvalinate (96%) and permethrin (97%) were supplied by Dr. Ehrenstorfer (Germany).

Stock solutions of individual standards (1 mg/ml) were prepared in isooctane and kept refrigerated. The concentration of analytes in standard mixture *S1* used in *Experiment A* and *B* (see below) was 0.5 mg/ml. Test mixture *S2* used in *Experiments C–G* was 0.5 μg/ml. Concentrations of solutions used for calibration ranged from 0.01–5 μg/ml (isooctane). Solution *S3* used for spiking of "blank" samples in recovery study was prepared in ethyl acetate, its concentration was 0.1 μg/ml.

All solvents were pesticide grade, supplied by Merck (Germany).

Apparatus: ASPEC XL (Gilson) liquid chromatograph equipped with steel column (500 x 8 mm) containing styrene-divinyl benzene copolymer Bio Beads SX-3 was used for automated gel permeation chromatography (GPC) of sample extracts.

Hewlett Packard GC/MS system consisted of HP 5890 gas chromatograph (equipped with split/splitless injector, electronic pressure control and autosampler HP 7673) and HP 5972 mass selective detector (MSD); DB-5 (30 m x 0.25 mm, film 0.25 μm) capillary was employed as an analytical column.

Optimisation of GC conditions – test mixture *S1* was analysed under the following conditions:

Experiment A

- injection port: 250 °C
- carrier gas: helium, constant flow, 0.87 ml/min., linear velocity 42.1 cm/s
- injection mode: splitless
- injection volume: 1 μl
- oven temperature program: 90 °C (2 min), 30 °C/min. to 280 °C, held 15 min.
- interface temperature: 280 °C
- setting of MSD: scan mode – 50–510 amu; dwell time – 100 ms; autotune (default m/z – 69, 219, 502 used for adjusting of detector parameters).

Experiment B

Injection port temperature was increased up to 300 °C. All other conditions were the same as those specified in *Experiment A*.

Optimisation of MS detection – test mixture *S2* was analysed under the following conditions:

Experiment C

GC conditions were the same as specified in *Experiment B*. MSD was performed in SIM acquisition mode. The ions selected for this purpose are summarised in Table I together with respective time groups.

Experiment D

Instead of "Maximum sensitivity" tune autotune option, "User tune" was executed. Following ions were used for this purpose: m/z 131, 219, 264. All other conditions were the same as specified in *Experiment C*.

Experiment E

Pressure pulse was applied during injection period. The pressure program was as follows:

250 kPa 2 min., 99 kPa/min to 120 kPa, then constant flow. All other conditions were the same as in *Experiment D*. Volumes of standard solution injected ranged from 1 to 6 μl. Dwell time was 100 ms.

Experiment F

Six repeated injections were carried out with dwell time settings 50, 100 and 200 ms, respectively. All other conditions were the same as specified for *Experiment E*, 3 μl of sample were injected.

Analytical procedure consisted of the following steps:

Experiment G

a) *Isolation of residues* – 50 g of representative wheat sample were extracted with 200 ml ethyl acetate. Sonication was applied to enhance extraction efficiency. After separation of organic solvent, the grains were rinsed with additional 100 ml of ethyl acetate. The combined extracts were dried over anhydrous sodium sulphate and then evaporated to dryness. The residue was dissolved in chloroform.

b) *Clean-up of raw extract* – 2 ml of chloroform solution were loaded on GPC column. Chloroform was used as a mobile phase (flow rate 0.8 ml/min.). Fraction corresponding to elution volume 12–22 ml was collected. The residue left after removing of solvent was prior to GC/MSD examination dissolved in 0.5 ml isooctane.

c) *GC/MS analysis of wheat samples* – 3 μl of purified sample extract were analysed under conditions specified for *Experiment E.* For recovery study the sample was spiked with 50 μl of solution *S3.*

RESULTS AND DISCUSSION

In the initial phase of our experiments, thorough optimisation of GC/MSD conditions was carried out. The first parameter considered was the selection of appropriate GC resolution. Contrary to the most of other pesticides, certain pyrethroid insecticides exist as a mixture of several stereoisomers, which is due to the presence of a cyclopropane ring in their molecule. Individual isomers may be under appropriately adjusted conditions resolved by gas chromatography. However, when packed columns (Pang et al., 1994a) and/or wide-bore capillaries are employed, limited or no isomeric resolution is obtained (Pang et al., 1994b; Wan et al., 1994). Composite peaks have to be used for quantitation which is thus made on a summation basis regardless of actual isomeric contents. Therefore, for getting more detailed information on the individual isomeric contents, narrow-bore capillary was employed in our study. Rather surprisingly, none of the aforementioned studies utilised mass selective detector (MSD) for quantitation of pyrethroid residues, although, as it was emphasised by several authors (Pang et al., 1995a; Fernandez-Alba et al., 1994), organohalogen and/or other co-extracted compounds may interfere with low-resolution GC/ECD determination. In such case, either high performance GC or GC/MS was recommended for confirmation purpose. However, the lather method was used only in the case of samples with incurred residues (Nakamura et al., 1993; Pang et al., 1995b).

Prior to the optimisation of MSD setting aimed at a highly sensitive detection, attention was paid, as it was mentioned, to GC procedure (MSD operated in full scan mode in this phase). Under conditions specified in *Experiment A* which are commonly used for most classes of pesticides, a fairly small detector response was obtained in this run esp. for the late eluting analytes. This phenomenon was attributed to a relatively very low volatility of pyrethroids (see also high molecular weights of these compounds shown in Table I) and therefore the temperature of injection port was elevated (*Ex-*

periment B) up to 300 °C. As can be seen from Table II, significant increase of the amount of analytes transferred onto the GC column was recorded. Cyhalothrin, permethrin and fluvalinate were eluted in two isomeric peaks, the ratio of which was found to be stable throughout our experiments, more abundant component of this pair was utilised for all further quantitations.

Based on the analysis of electron impact (EI) mass spectra measured in *Experiment C*, appropriate ions were selected for operation of MSD in "selected ion monitoring mode" (Table I). It should be noted that relative abun-

I. "Selected ion monitoring" (SIM) – basic characteristics utilized for data acquisition (*Experiment C*)

Analytes (mol. weight)	m/z set in SIM cycle	Relative abundance [%]	Analytes retention time [min.]	Time setting for SIM group [min.]
Bifenthrin (422.9)	165	33	10.55	10.0
	166	29		
	181	100		
Cyhalothrin (449.9)	181	100	11.28	11.1
	197	76	11.48	
	208	44		
Permethrin (391.3)	163	27	12.59	12.1
	165	23	12.77	
	183	100		
Alphamethrin (416.3)	163	100	14.34	14.0
	165	65		
	181	88		
Fluvalinate (502.9)	208	10	16.42	16.0
	250	100	16.60	
	252	34		
Deltamethrin (505.2)	181	100	18.33	18.0
	251	46		
	253	90		

II. Comparison of MSD response in relation to the injection port temperature (*Experiment A* and *B*)

Analyte	Peak area – *A* (*Experiment A* inj. *t* = 250 °C)	RSD [%]	Peak area – *A* (*Experiment B* inj. *t* = 300 °C)	RSD [%]	$A_{300\,°C}$ $A_{250\,°C}$
Bifenthrin	11 549.3	3.7	39 402.3	3.7	3.4
Cyhalothrin	1 099.8	2.5	5 270.6	5.6	4.7
Permethrin	1 107.0	3.8	7 470.8	5.3	6.7
Alphamethrin	414.1	3.3	2 802.0	6.4	6.7
Fluvalinate	135.6	16.8	738.1	8.5	5.4
Deltamethrin	88.3	6.8	684.9	8.1	7.7

dances of particular m/z were distinctly different from those reported in study employing double focusing mass spectrometer (Nakamura et al., 1993); in general, more extensive fragmentation leaving smaller molecular ion and other ions from higher mass region was observed in our records obtained by quadrupole analyser.

To enhance the detectability of analytes, "User tune" procedure was carried out in *Experiment D*. Instead of ions covering a wide range of masses utilised in "Maximum sensitivity tune" default option, m/z from perfluorinated tributyl amine (PFTBA) spectrum (reference compound used for mass axis calibration) close to those selected for SIM acquisition (Table I) were chosen for tuning and then stored in "tune file". Remarkable increase (approx. by half order of magnitude) of detector response was obtained, never-

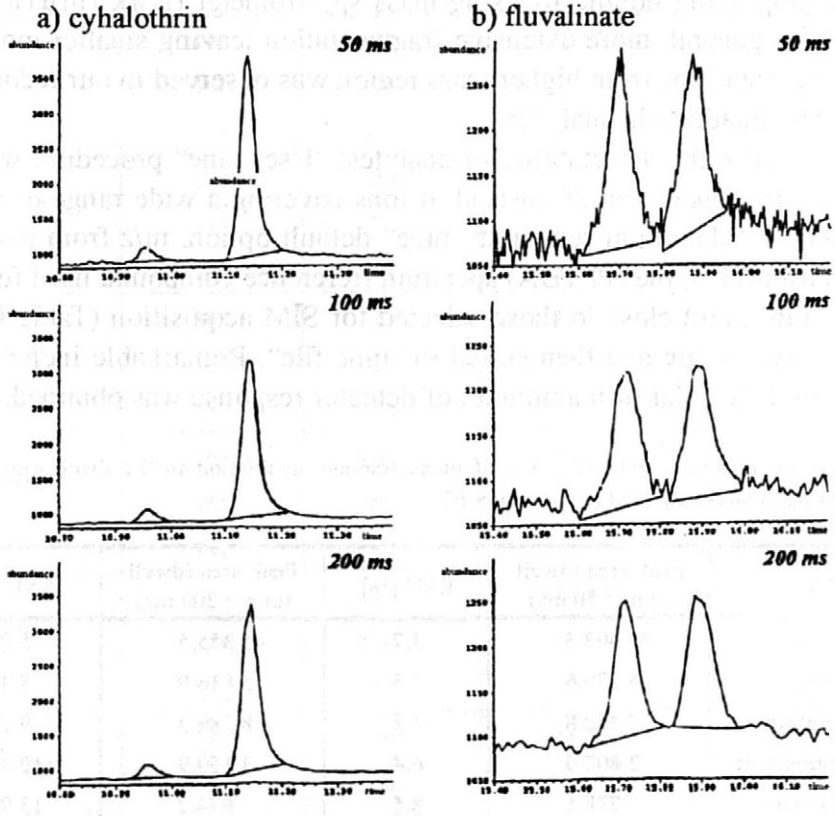
III. Detector response and precission of measurements in relation to the dwell time setting, 1500 pg per analyte injected (*Experiment F*)

Analyte	Peak area (dwell time = 50 ms)	RSD [%]	Peak area (dwell time = 200 ms)	RSD [%]
Bifenthrin	39 402.3	3.7	45 355.5	5.7
Cyhalothrin	5 270.6	5.5	5 816.9	8.4
Permethrin	7 470.8	5.3	8 289.3	9.2
Alphamethrin	2 802.0	6.4	3 150.9	12.3
Fluvalinate	738.1	8.5	674.2	13.9
Deltamethrin	684.9	8.1	769.1	13.8

theless, gradual decline of peak heights with increasing analyte retention time could be seen from these records.

To increase the amount of sample transferred onto chromatographic column, pressure pulse (*Experiment E*) was applied in the course of splitless injection period. The detector response was found to be linear up to 3 μl for all analytes tested and therefore this volume was injected in all following experiments.

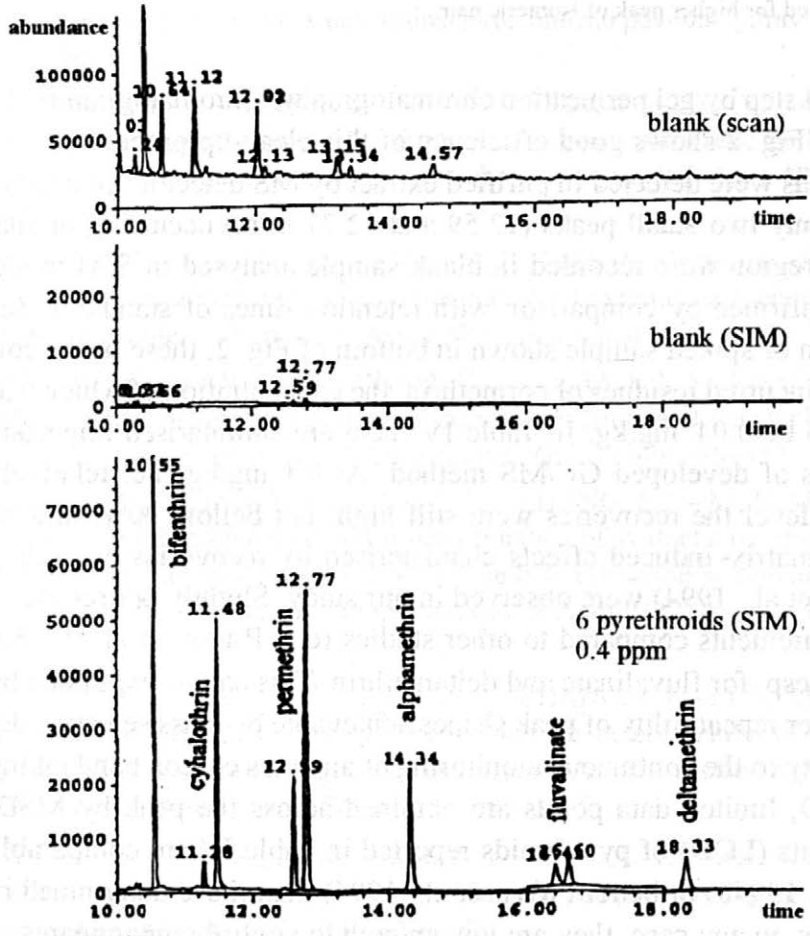
To document the influence of another adjustable MS acquisition parameter, default dwell time (the length of time an ion is monitored) 100 ms was replaced in *Experiment F* by half and double values, i.e. 50 and 200 ms, respectively. Nevertheless, as illustrated in Fig. 1, to get peak profile reflecting true chromatographic peak shape eluting from GC, 100 ms is a good compromise in this case. Faster monitoring (50 ms) results in a certain loss of sensitivity,



1. SIM acquisition of two pyrethroids, the influence of dwell time setting

while lower scan cycle rate leads to some peak distortion and, consequently, to the decrease of precision of peak area measurements (Table III).

As regards analysis of wheat grain samples (*Experiment G*), ethyl acetate was applied for extraction of residues. Although its use for isolation of pyrethroids from plant crops was mentioned some years ago (Kocourek et al., 1988), other solvents are mostly specified in a procedure concerned with this single class of pesticides (see Introduction). We have decided for this extraction agent because of its wide use in many general multiresidue methods (e.g. Andersson, Palsheden, 1992). The clean-up step consisting normally in partition followed by adsorption chromatography was distinctly simplified in our procedure: lipoid materials and other co-extracts were removed in sin-



2. GC/MS(SIM) analysis of six pyrethroids in wheat grains under optimised conditions

IV. Recoveries (at 0.1 mg/kg spiking level) and detection limits (LOD) of pyrethroids in wheat grains

Analyte	Recovery [%] (RSD – %)	LOD (pg per injection)	LOD (mg/kg in sample)
Bifenthrin	93.2 (4.1)	5	0.0002
Cyhalothrin*	97.5 (6.9)	50	0.002
Permethrin*	95.4 (10.3)	50	0.002
Alphamethrin	99.2 (10.2)	125	0.005
Fluvalinate*	90.2 (16.1)	1 250	0.05
Deltamethrin	91.0 (15.0)	500	0.02

* Values calculated for higher peak of isomeric pair

gle automated step by gel permeation chromatography. Chromatogram in the upper part of Fig. 2 shows good efficiency of this clean-up process – very few compounds were detected in purified extract by MS detector operated in scan mode; only two small peaks (12.59 and 12.77 min.) occurring in analytes elution region were recorded in blank sample analysed in SIM mode. As it was confirmed by comparison with retention times of standards, see chromatogram of spiked sample shown in bottom of Fig. 2, these peaks corresponded to incurred residues of permethrin, the concentration of which was determined to be 0.04 mg/kg. In Table IV there are summarised important characteristics of developed GC/MS method. At 0.1 mg/kg (i.e. relatively low) spiking level the recoveries were still high: not bellow 90%; and, in addition, no matrix-induced effects characterised by recoveries exceeding 100% (Wan et al., 1994) were observed in our study. Slightly poorer precision of measurements compared to other studies (e.g. Pang et al., 1995a) was obtained esp. for fluvalinate and deltamethrin. This can be explained by generally lower repeatibility of peak shapes achievable by mass selective detector. Contrary to the continuous monitoring of analytes elution band taking place by ECD, limited data points are obtained across the peak by MSD. Detection limits (LOD) of pyrethroids reported in Table IV are comparable (Pang et al., 1994b) or better (Wan et al., 1994) than those determined in similar studies, in any case, they are low enough to control contaminants at common legislation levels.

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Received December 6, 1996

Potrav. Vědy, *15*, 1997 (1) : 1–12

GC/MS metoda pro analýzu reziduí syntetických pyrentroidů používaných v obilninách

Byla optimalizována metoda pro stanovení šesti běžně používaných insekticidních pyrethroidů v obilninách. Pšeničná zrna byla extrahována etylacetátem, k přečistění surového extraktu byla využita gelová permeační chromatografie (GPC) na BioBeads SX-3. Izomerická separace analytů byla dosažena pomocí vysokoúčinné plynové chromatografie (HRGC); k jejich identifikaci a kvantifikaci byl užit hmotnostně selektivní detektor (MSD). Výtěžnosti analytů na hladině 0,1 mg/kg byly v rozmezí 90–99 %, detekční limity se pohybovaly mezi 0,0002 a 0,5 mg/kg. Ve srovnání s dosud dostupnými postupy je naše metoda méně pracná a vyznačuje se vysokou selektivitou detekce. Detekční limity umožňují kontrolu na úrovni hygienických limitů (pokud tyto ovšem existují).

pyrentroidy; insekticidy; rezidua v zrnu; plynová chromatografie – hmotnostní spektroskopie

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Potrav. Vědy, *15*, 1997 (1) : 13–28

REACTION OF DEHYDROASCORBIC ACID WITH ASPARTAME*

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Abstract: Solutions of dehydroascorbic acid and its mixture with Aspartame were heated to 80, 90 and 100 °C. The content of dehydroascorbic acid decreased in complicated manner, but in the early stage – after the first order kinetics; the reaction rate increased with increasing temperature and in presence of Aspartame. The browning proceeded more rapidly at early reaction stages. At later stages, brown pigments absorbing at longer wavelength were accumulated at greater rate; the browning was stimulated by Aspartame. Acidic and sweet tastes were not substantilly affected by heating. In the dehydroascorbic acid solution, caramel flavour note appeared from the begining, followed by burnt and bitter notes at later reaction stages. In presence of mixtures with Aspartame, caramel flavour was substantially weaker, and burnt flavour did not develop. Contrary to solutions of sole dehydroascorbic acid, musty, mouldy and rotten flavour notes were produced in heated mixtures of dehydroascorbic acid with Aspartame, most probably due to N-(2-pyron-3-yl)aspartame, which posssesses similar flavour, in addition to floral and fruity flavour notes. The pyronylaspartame was previously identified in heated mixtures of dehydroascorbic acid and Aspartame.

Aspartame; browning reactions; dehydroascorbic acid; pyronylaspartame

Nonalcoholic fruit beverages are subject to nonenzymic browning, and probably to enzymic browning as well, when stored in original packages. On storage of lime and orange juices, browning resulted from interactions between reducing sugars and free amino acids, and also between amino acids and dehydroascorbic acid formed by oxidation of ascorbic acid; dehydroas-

* Work was partially supported by Nihon University grant, partially by Czech Grant Agency (Project No. 509/93/0419).

corbic acid produced intensively coloured compounds with amino acids (Heikal et al., 1972). Moderate browning was observed, accompanying the oxidation of ascorbic acid into dehydroascorbic acid in orange juice and orange drinks (Kacem et al., 1987). Ascorbic acid was more active precursor of brown pigments than sugars (Kacem, 1987). Removal of oxygen and the subsequent anaerobic storage inhibited the browning because of reduced formation of dehydroascorbic acid (Kacem et al., 1987). The presence of amino acids increased the browning rate (Cornwell, Wrolstad, 1981) in pear juice concentrate containing both sugars and ascorbic acid and/or dehydroascorbic acid. Browning reactions involving ascorbic acid and dehydroascorbic acid were found more important than the effect of sugars in the discolouration of stored kiwifruit juice (Wong, Stanton, 1989). The content of dehydroascorbic acid and reducing sugars increased in stored *Myrciaria dubia* juice, which is likely to increase the browning rate (Zapata, Dufour, 1993). Reaction with cysteine may inhibit browning reactions with dehydroascorbic acid, as was tested in canned lansat (Suhaila, Leong, 1987).

The effect of dehydroascorbic acid on browning reactions was studied in model systems as well (Miková, Davídek, 1975). In the initial phase, intensive browning was observed, but its rate decreased at later stages. The browning rate of systems containing dehydroascorbic acid, glycine and cellulose increased with the increasing water activity (Ranganna, Srinivasan, 1979). Both yellow (Hayashi et al., 1983b) and purple pigments (Hayashi et al., 1984) were formed during the reaction of ascorbic acid with amino acids. The red pigment was identified as L-scorbamic acid (2-amino-2-deoxy-L-ascorbic acid), resulting from Strecker degradation (Kurata et al., 1973). The presence of N-[(2-nitrilo-2-deoxy-L-ascorbic acid)acetyl]-DL-leucine was identified among reaction products of dehydroascorbic acid and glycyl leucine (Sakurai, Ishii, 1988). The kinetics of red pigment formation was studied in systems containing dehydroascorbic acid and α-amino acids (Shin, Bae, 1984). Dehydro-ascorbic acid may react with the ε-amino group of lysine as well, even if it is bound in protein (Dunn et al., 1990).

Dehydroascorbic acid does not react only with amino acids and peptides, but also with proteins, such as casein or ovalbumin (Hayashi et al., 1985;

Nishimura et al., 1989). The reaction with ovalbumin already takes place during the oxidation of ascorbic acid into dehydroascorbic acid (Homma et al., 1973).

The reaction of dehydroascorbic acid probably proceeds via free radicals (Namiki et al., 1974; Yano et al., 1976). The free radical products were separated by TLC, and their ESR spectra were determined (Yano et al., 1976a). The free radical is produced from *tris*(2-deoxy-2-L-ascorbyl)amine (Hayashi, Namiki, 1986), and two conformational isomers spectra can be resolved by ESR. Free radical could be produced by reaction of the above substance with dehydroascorbic acd, and the subsequent reduction of the product with another molecule of ascorbic acid (Hayashi et al., 1983a). Analogous free radicals my be produced by reaction of dehydroascorbic acid with amines (Yago et al., 1976b). Amino acids are easily converted into amines by reaction with dehydroascorbic acid, which possesses strong Strecker activity (Co, Sanderson, 1970), e.g. in tea fermentation.

The formation of brown pigments is not the sole reaction of dehydroascorbic acid with amino acids. Dehydroascorbic acid reacted with glycylglycine forming N-(2-pyron-3-yl)glycylglycine (Sakurai et al., 1989). Aspartame is added often as a sugar substitute in low-energy ("light") fruit beverages. As it contains an amine group as well, it can contribute to the pyrone formation; we have identified N-(2-pyron-3-yl)aspartame among the reaction products (Sakurai et al., 1996, 1997), and detected the presence of off-flavours due to the pyrone derivative. In this paper, we present our results on the kinetics of the rection of dehydroascorbic acid with Aspartame, the course of the discolouration reactions and the deterioration of the sensory quality.

MATERIAL AND METHODS

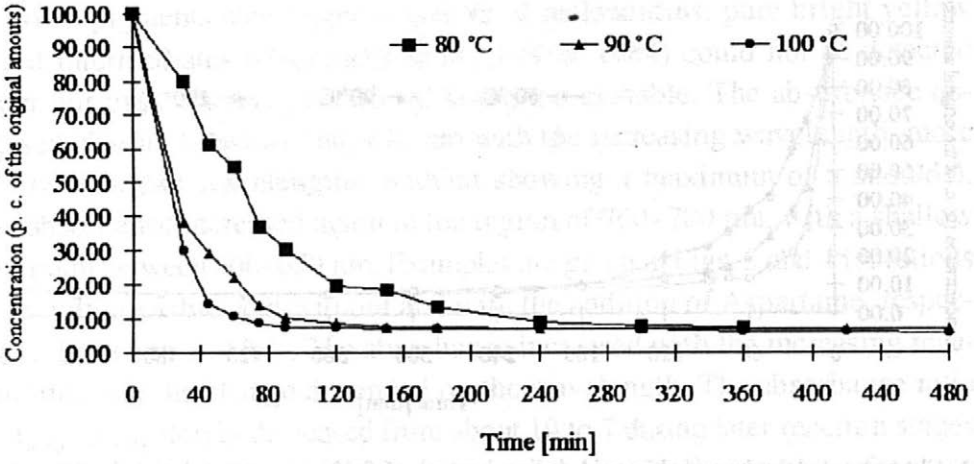
Dehydro-L-ascorbic acid was prepared by oxidation of L-ascorbic acid with silver oxide, and by crystallization of the product from acetone; the substance was found pure by HPLC. Aspartame (L-aspartyl-L-phenylalanine methyl ester) was produced by Wamo Pure Chemical Industries, Japan; the purity was tested by HPLC.

The browning was determined by measuring the solutions in 10 mm quartz cells (with distilled water in the reference cell), using the spectophotometer Spectromom 195D (MDR, Budapest, Hungary). The contents of dehydroascorbic acid and Aspartame were determined by HPLC (sampler: Spectroflow 480; stainless steel column Tessek, 4 mm x 250 mm, packed with Separon SGX C 18.5 μm; injected volume: 100 μl; isocratic pump Knauer; integrator CI-100; Line Recorder TZ 4200; refractometer RIDK 101; manufactured by Laboratorní přístroje, Prague). The mobile phase for the determination of dehydroascorbic acid was distilled water; the mobile phase for the separation of Aspartame consisted of methanol and 0.2M sodium dihydrogen phosphate (1 : 1 v/v); flow rate 0.23 ml/min. For the determination of reaction products of dehydroascorbic acid with Aspartame, the mobile phase of methanol and 0.2M aqueous monosodium dihydrogen phosphate was used, but in the ratio of 1 : 3 (v/v); the flow rate was 0.20 ml/min. The apparatus was calibrated using pure standards.

The sensory analysis consisted of a sensory intensity profile. Samples were served after instruction of the international standard (ISO 6564), and tested in a standard test room (ISO 8589). The panel consisted of selected trained assessors (ISO 8586) with the experience of at least 6 months. Intensities were rated using an unstructureed graphical scale (160 mm straight lines) with anchor points only at the two ends (0% = imperceptible; 100% = very strong). Descriptors (ISO 6658) were selected on the basis of free choice profiling, and their names are given in the sensory diagrams. The descriptors not significantly changing during heating have been omitted.

RESULTS AND DISCUSSION

Decrease in the concentration of dehydroascorbic acid in 0.2% aqueous solution was determined during the heating to 80, 90 and 100 °C, respectively, for up 540 min, using HPLC (Fig. 1). The reaction kinetics is, naturally, very complicated, but in the first reaction stage, it can be approximated as a first order reaction with respect to dehydroascorbic acid. The reaction in 0.1% aqueous solution of dehydroascorbic acid containing 0.1% Aspartame (Fig. 2) had very similar course. Aspartame did not change appreciably on heating, therefore, its changes are not given here. The stability of Aspartame



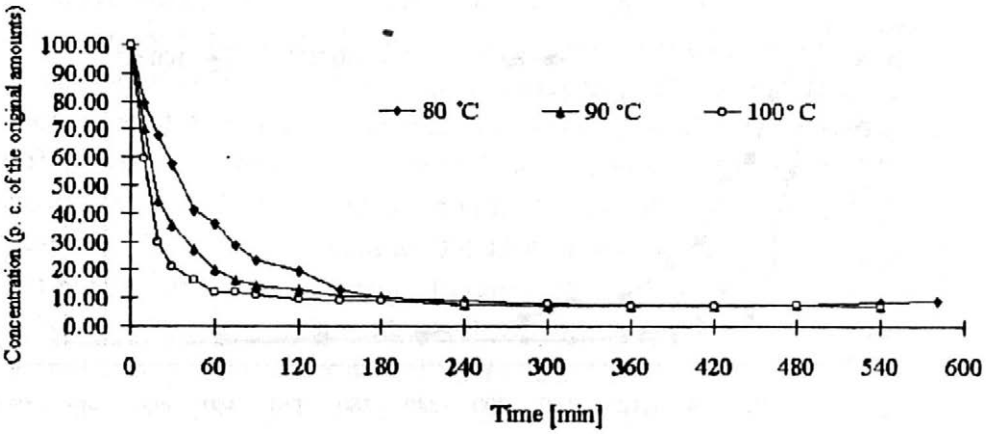
1. Changes of dehydroascorbic acid during heating of 0.2% aqueous solution

was reported (Ripper et al., 1986) relatively good in the medium, corresponding to the acidity of the 0.1% solution of dehydroascorbic acid. The reaction rate rapidly increased with increasing temperature (Table I); the reaction was accelerated by the presence of Aspartame.

I. Rate of degradation of dehydroascorbic acid on heating (expressed in $-\log C/\text{min} \times 10^3$, where C is expressed as p. c. of the original concentration)

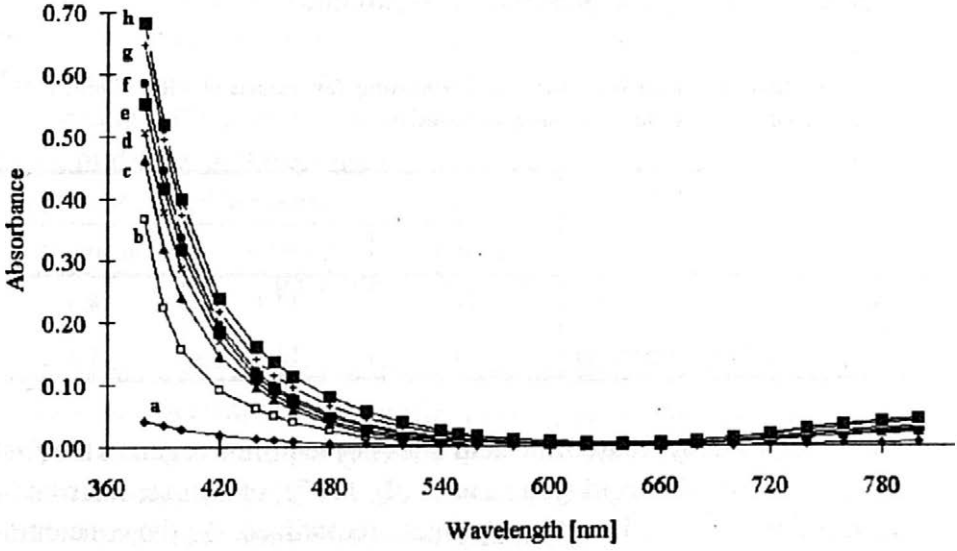
Heated system	Decrease		
	at 80 °C	at 90 °C	at 100 °C
Dehydroascorbic acid	6.8	10.4	18.4
Dehydroascorbic acid with Aspartame	6.1	12.4	19.4

The browning of dehydroascorbic acid does not require oxygen in the first stage, contrary to ascorbic acid (Kacem et al., 1987); of course, intermediary condensation products do not react, unless reoxidized. As the concentration of oxygen is very low in hot water, it may easily become the rate determining factor. The reaction rate then degenerates to that of a first or zeroth order reaction.



2. Changes of dehydroascorbic acid during heating of 0.1% aqueous solution in presence of 0.1% Aspartame

The browning of dehydroascorbic acid was rather rapid in the beginning, but it became much slower in later stages [when the concentration of dehydroascorbic acid, determined by HPLC (Figs. 1 and 2), was also very low], similarly as observed in earlier studies (Mikoyá, Davídek, 1975).



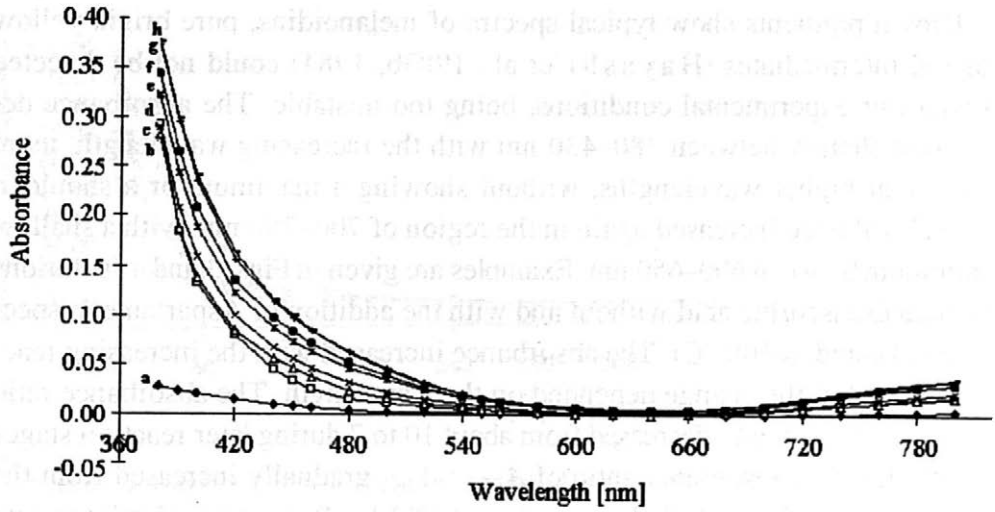
Reaction time: a = 0 min; b = 30 min; c = 60 min; d = 120 min; e = 180 min; f = 240 min; g = 360 min; h = 480 min

3. Changes of the visible spectrum during the heating of 0.2% dehydroascorbic acid to 100 °C

Brown pigments show typical spectra of melanoidins; pure bright yellow or red intermediates (Hayashi et al., 1983b, 1984) could not be detected under our experimental conditions, being too unstable. The absorbance decreased sharply between 380–430 nm with the increasing wavelength, more slowly at higher wavelengths, without showing a maximum or a shoulder. The absorbance increased again in the region of 700–780 nm, with a shallow minimum between 600–650 nm. Examples are given in Figs. 3 and 4 (solutions of dehydroascorbic acid without and with the addition of Aspartame, respectively, heated to 100 °C). The absorbance increased with the increasing reaction time, but the change depended on the wavelength. The absorbance ratio of $A_{400} : A_{500}$ slowly decreased from about 10 to 7 during later reaction stages (Table II). The absorbance ratio of $A_{500} : A_{760}$ gradually increased from the beginning till the end of the experiment (Table II). In case of mixtures of dehydroascorbic acid with Aspartame, the absorbance ratios of $A_{400} : A_{500}$

II. Changes of the absorbance ratios at 400 nm, 500 nm and 760 nm during heating to 100 °C (DAA = dehydroascorbic acid, ASP = Aspartame)

Heated system	Time [min]	$A_{400} : A_{500}$	$A_{500} : A_{760}$	$A_{400} : A_{760}$
DAA	0	9.7	0.75	7.2
	30	9.8	1.00	9.8
	60	11.4	1.17	13.3
	120	11.0	1.24	13.7
	180	9.9	1.33	13.2
	240	9.3	1.50	14.0
	360	8.2	1.48	12.1
	480	7.2	1.68	10.8
DAA + ASP	0	6.7	1.5	10.0
	30	12.1	0.9	11.1
	60	8.6	1.2	10.6
	120	6.9	1.4	9.9
	180	5.4	1.5	7.8
	240	5.3	1.6	8.3
	360	5.1	1.7	8.7
	480	5.4	1.9	8.6



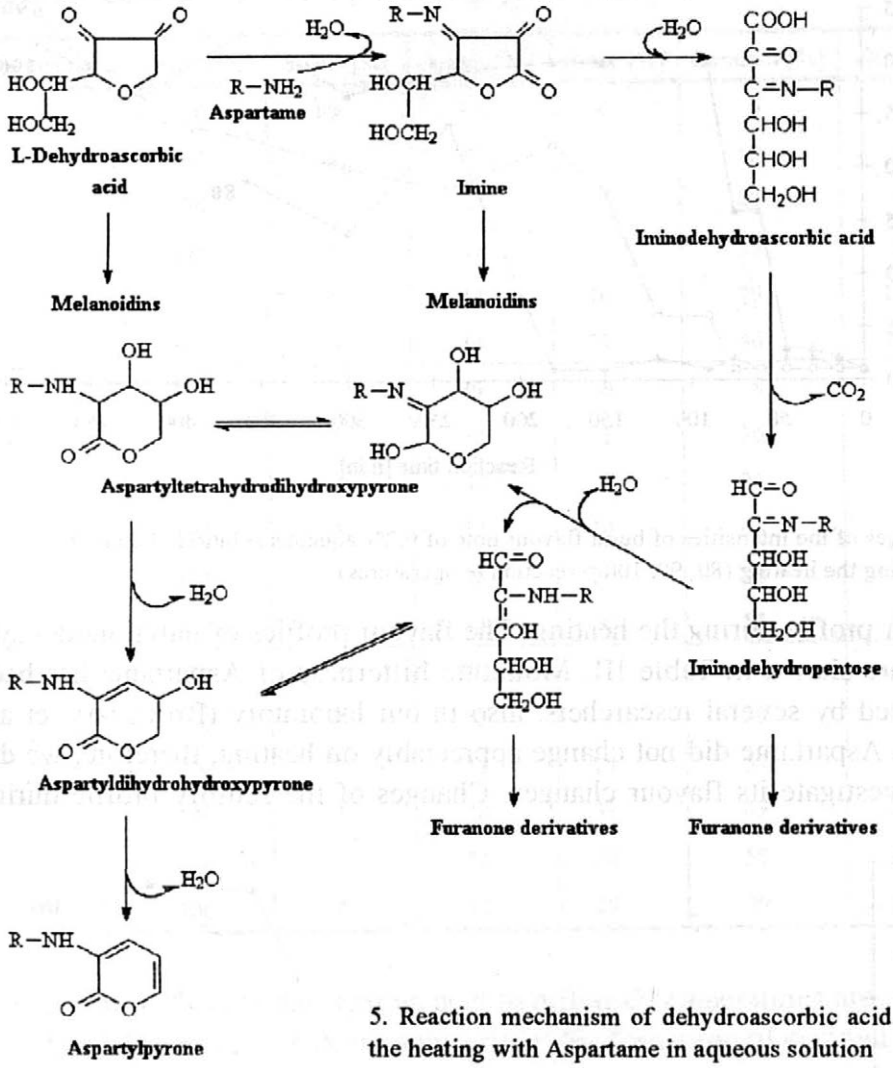
Reaction time: a = 0 min; b = 30 min; c = 60 min; d = 120 min; e = 180 min; f = 240 min; g = 360 min; h = 480 min

4. Changes of the visible spectrum during the heating of 0.1% dehydroascorbic acid in presence of 0.1% Aspartame to 100 °C

were, generally, lower, but the values of the absorbance ratios $A_{500} : A_{760}$ nearly the same.These changes show gradual lengthening of the conjugated double bond system during heating, and the chromophore effect of nitrogen groups from the bound Aspartame or its fragments.

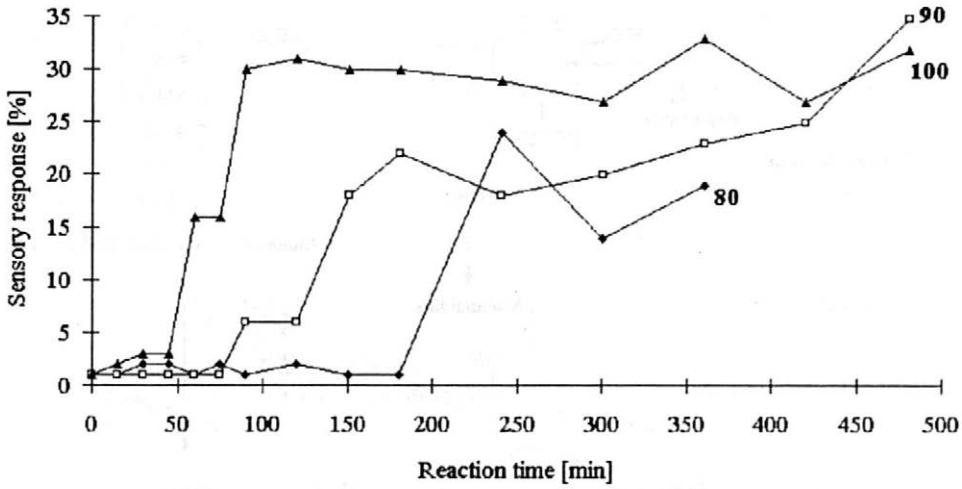
III. Sensory profiles of solutions of reacting substances (results are expressed in p. c. of the graphical scale)

Flavour descriptor	Dehydroascorbic acid (0.2%)	Aspartame (0.2%)	Mixture of both (0.1% + 0.1%)
Sweet	4	54	37
Acidic	55	1	41
Bitter	1	13	4
Total off-flavour	1	7	2
Other flavours	1	3	5



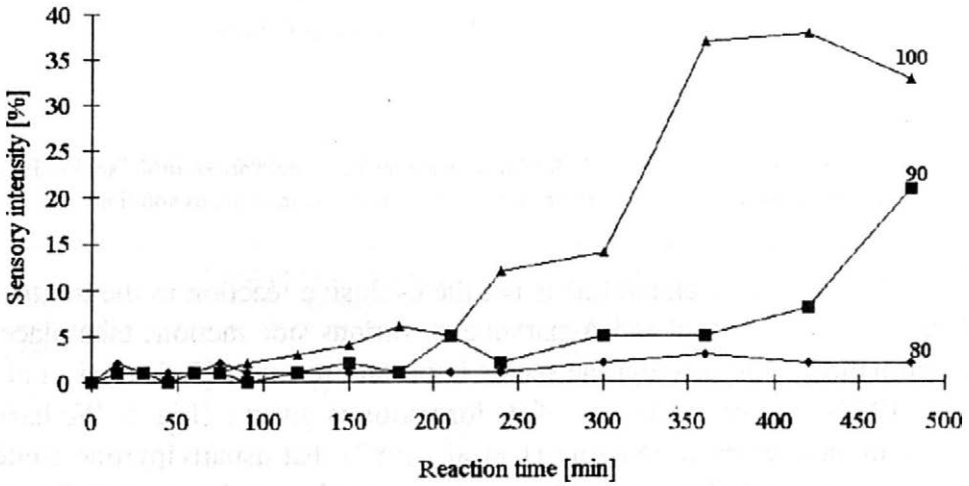
5. Reaction mechanism of dehydroascorbic acid during the heating with Aspartame in aqueous solution

The formation of melanoidins is not the exclusive reaction in the mixture of dehydroascorbic acid and Aspartame as various side ractions take place; the formation of aspartylpyrone seems important reaction (Sakurai et al., 1996, 1997). The probable way of its formation is given in Fig. 5. We have shown in another place (Sakurai et al., 1997) that aspartylpyrone could impart foreign off-flavours to the solution, which may become distinctly negative in higher concentrations. Therefore, we investigated changes of the



6. Changes of the intensities of burnt flavour note of 0.2% aqueous solution of dehydroascorbic acid during the heating (80, 90, 100 = reaction temperatures)

flavour profile during the heating. The flavour profiles of initial model systems are shown in Table III. Moderate bitterness of Aspartame has been observed by several researchers, also in our laboratory (Pokorný et al., 1984). Aspartame did not change appreciably on heating, therefore, we did not investigate its flavour changes. Changes of the sensory profile during



7. Changes of the intensities musty flavour note of 0.1% aqueous solution of dehydroascorbic acid heated to in presence of 0.1% Aspartame (80, 90, 100 = reaction temperatures)

IV. Changes of the sensory profile of aqueous 0.2% dehydro-L-ascorbic acid solution in course of heating (results are expressed in p. c. of the graphical scale)

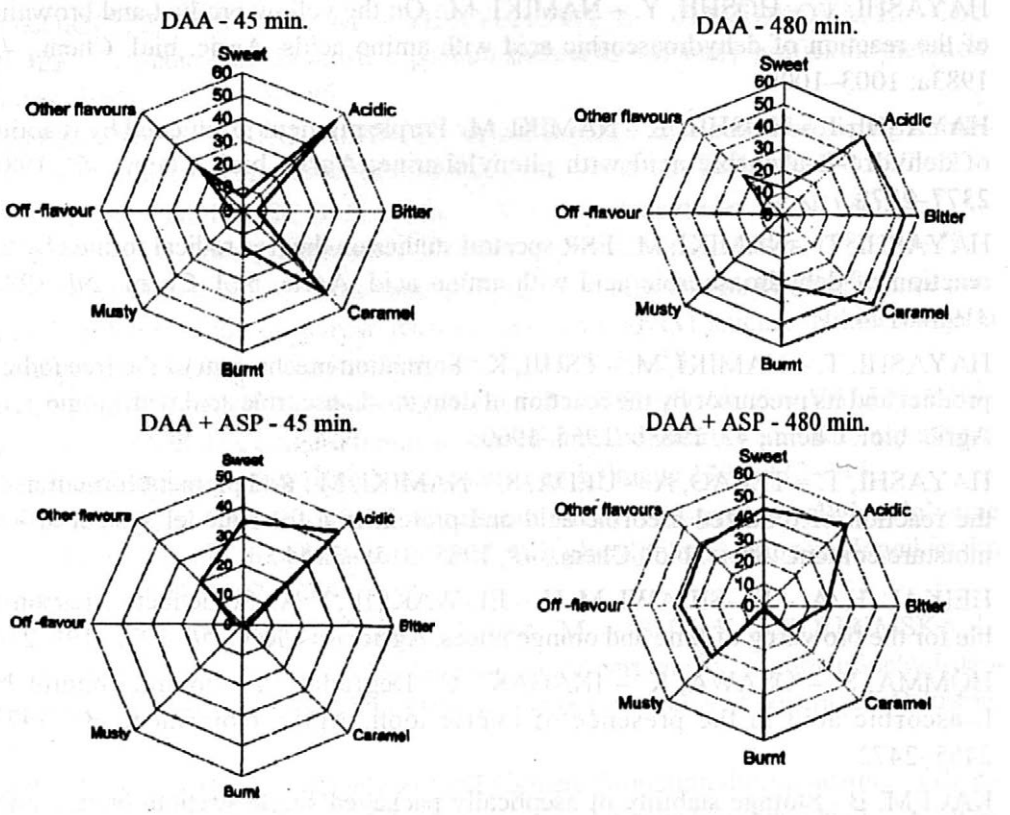
Temperature	Time [min]	Sweet [%]	Acidic [%]	Bitter [%]	Caramel [%]	Burnt [%]
80 °C	30	10	61	4	4	1
	60	8	60	7	12	1
	120	7	48	12	47	7
	180	10	44	7	25	3
	240	8	44	16	29	12
	300	10	34	21	46	16
90 °C	30	4	49	0	8	2
	60	3	50	1	26	2
	120	6	47	7	26	6
	180	5	52	14	48	7
	240	3	48	20	48	9
	300	6	43	30	55	22
100 °C	30	2	50	2	36	4
	60	3	55	6	50	16
	120	7	41	24	57	31
	180	6	48	33	63	28
	240	6	54	34	59	29
	300	4	52	29	49	17

heating of 0.2% dehydroascorbic acid to different temperatures are given in Table IV. The acidity changed only a little; the formation of caramel flavour is interesting, and of burnt and bitter off-flavours at later reaction stages. Therefore, we give changes of the burnt flavour in more detail on Fig. 6. The caramel note was produced from the beginning of heating, but bitter and burnt flavour notes were produced only after a certain lag period (Fig. 6 for the burnt flavour). In presence of Aspartame, the sweetness and the acidity did not appreciably change on heating, but the caramel flavour note was much weaker, and appeared at later reaction stages (Table V). Burnt off-flavour was only extremely weak or not perceived at all. In addition to these

V. Development of the sensory profile during heating of aqueous solutions containing 0.1% dehydroascorbic acid and 0.1% Aspartame (results expressed in p. c. of the graphical scale)

Temperature	Time [min]	Sweet [%]	Acidic [%]	Bitter [%]	Caramel [%]	Musty [%]
80 °C	30	43	39	10	13	1
	60	40	48	13	10	1
	120	42	40	9	16	1
	180	44	37	10	6	1
	240	47	30	12	8	2
	300	46	44	26	18	4
90 °C	30	52	35	4	2	0
	60	48	43	4	2	1
	120	48	42	10	3	1
	180	45	36	11	4	2
	240	40	41	22	6	4
	300	38	48	25	16	6
100 °C	30	42	42	1	1	1
	60	41	46	2	6	1
	120	43	40	4	4	4
	180	44	42	10	5	6
	240	40	40	22	16	12
	300	44	42	12	16	14

flavour notes, musty, mouldy and rotten off-flavours were produced, again after lag period; we give the development of musty off-flavour in more detail in Fig. 7). These off-flavours are typical for aspartylpyrone; in a solution of pure aspartylpyrone, these flavour notes, together with floral and fruity notes were detected (Sakurai et al., 1997); the solution was very bitter as well, and bitterness was pronounced in our experimental solutions as well. The sensory profiles of the two heated systems, heated for very short and very long times, are compared in sensory profile diagrams in Fig. 8.



DAA = dehydroascorbic acid (0.2% solution); DAA + ASP = mixture of dehydroascorbic acid and Aspartame (concentrations of 0.1% each)

8. Comparison of sensory profiles of 0.2% dehydroascorbic solution and of 0.1% dehydroascorbic acid with 0.1% Aspartame heated to 100 °C for extreme time intervals of 45 min. and 480 min.

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Received December 5, 1996

Reakce dehydroaskorbové kyseliny s aspartamem

Vodné roztoky kyseliny dehydroaskorbové (0,2 %) a její směsi s aspartamem (0,1 % + 0,1 %) byly zahřívány na 80, 90 a 100 °C po dobu 540 min a ve vhodných intervalech byly odebírány vzorky k spektrální, chromatografické a senzorické analýze. Změny reagujících složek byly stanoveny metodou HPLC, která byla pro tento účel modifikována. Úbytek kyseliny dehydroaskorbové probíhal podle složitého mechanismu; v první fázi se blížil kinetice reakce 1. řádu. Reakční rychlost rostla s rostoucí teplotou záhřevu. Destrukce kyseliny dehydroaskorbové byla větší v přítomnosti aspartamu, i když se jeho obsah při reakci podstatně nesnižoval. Hnědnutí probíhalo od počátku reakce, nejprve rychleji, v pozdějších stadiích reakce pomaleji. Postupně se zvyšoval podíl látek absorbujících při delších vlnových délkách, zvláště v přítomnosti aspartamu. Byly stanoveny změny senzorického profilu chuti. Během záhřevu roztoku samotné kyseliny dehydroaskorbové se od počátku tvořily produkty s karamelovou příchutí, později také s připálenou a hořkou příchutí. V přítomnosti aspartamu se karamelová příchuť vyvinula až později a s menší intenzitou, zatímco připálená příchuť nevznikala vůbec. Naproti tomu se objevily zřetelné plesnivé, zatuchlé a zkažené pachutě, které jsou vedle ovocné a květové příchuti typické pro N-(2-pyron-3-yl)aspartam, jehož vznik z dehydroaskorbové kyseliny a aspartamu jsme prokázali.

aspartam; dehydroaskorbová kyselina; neenzymové hnědnutí; pyronylaspartam

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Potrav. Vědy, *15*, 1997 (1) : 29–38

INFLUENCE OF KH_2PO_4 ON THE CONCENTRATION OF NISIN PRODUCED BY *Lactococcus lactis* subsp. *lactis* NIZO R5*

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Abstract: Stimulatory effects of inorganic phosphorus source in form of KH_2PO_4 in the batch fermentations in the CM-medium and in skim milk supplemented with yeast extract and Tween 80 were found for the growth rate and extent and for the concentration of nisin produced by *Lactococcus lactis* subsp. *lactis* NIZO R5. The maximum nisin concentration (1400 IU/ml) in CM-medium was achieved with initial KH_2PO_4 concentration 3% (w/v), while in milk based medium with 1% (w/v) KH_2PO_4. The KH_2PO_4 concentration higher than 5% (w/v) in the CM-medium and 3% (w/v) in the milk based medium had already inhibiting effect on the growth and the concentration of nisin produced by *Lactococcus lactis* subsp. *lactis* NIZO R5 in the media.

nisin; *Lactococcus lactis* subsp. *lactis;* KH_2PO_4; higher nisin yield

The antimicrobially active peptide nisin is synthetized by certain strains *Lactococcus lactis* subsp. *lactis*. It is bactericidal against a broad range of gram-positive organisms including *Lactococcus,Lactobacillus* (Hurst, 1972), *Staphylococcus* and *Listeria* sp. (Harris et al., 1991) and prevents outgrowth of spores of *Bacillus* sp. (Thorpe, 1960) and *Clostridium* sp. (Hirsch et al., 1951).

The antimicrobial effect of nisin on vegetative cells is caused by disruption of the cytoplasmic membrane resulting in leakage of essential celullar material from the cells. Nisin inactivates sulphydryl groups in the cytoplasmic membrane acting as an inhibitor of both spore outgrowth and vegetative cells growth (Morris et al., 1984). Nisin is produced from a ribosomally synthetized precursor peptide, which is subsequently post-translationally enzymatically modified (Dodd et al.,1990; Kaletta, Entian, 1989).

* This work was supported by the Grant Agency of Czech Republic (grant 510/95/0990).

Nisin production shows primary metabolite kinetics (Vuyst, Vandamme, 1992). To increase the nisin yield the methods of genetic manipulation were tried but were not entirely succesful (Gasson, 1984; Tsai, Sandine, 1987). Another way for improving nisin yields is systematic examination of nutritional parameters in relation to growth kinetics and nisin metabolism in producing strains (Vuyst, Vandamme, 1992). In the past no very detailed data about nisin batch fermentation were published (Hust, 1966; Egorov et al., 1971). Nisin production influenced by phosphorus sources was studied by De Vuyst (Vuyst, Vandamme, 1993) with the aim to increase understanding of the physiology and metabolic control of nisin biosynthesis.

Nisin is commercially used mostly in the form of nisin concentrate Nisaplin (Aplin & Barrett, GB) in food production as an effective preservative in processed cheese and cheese spreads, milk, dairy desserts, canned foods, meat and alcoholic beverages (Delves-Broughton, 1990). In our work we tried to improve the nisin yield by *Lactococcus lactis* subsp. *lactis* NIZO R5 strain using supplementation of complex medium with KH_2PO_4.

MATERIAL AND METHODS

Bacterial strains

The nisin-producing strain used in this study *Lactococcus lactis* subsp. *lactis* NIZO R5 was maintained in sterile M17 broth (OXOID, GB) and subcultured once a week. Stock cultures were transferred at an inoculum size of 1% (w/v) and incubated at 30 °C for 15 h. For all tests only fresh culture was used. Its growth activity, absolute nisin production and morphological characteristics were checked before each trial. In all trials performed with *Lactococcus lactis* subsp. *lactis* the inoculum 1% (w/v) was used.

Media

The complex CM-medium used in this study was prepared by use of chemicals (Lachema, CR): sucrose (10.0 g/l), KH_2PO_4 (10.0 g/l), NaCl (2.0 g/l), $MgSO_4.7H_2O$ (0.2 g/l) and other components (OXOID): peptone (10.0 g/l), yeast extract (10.0 g/l) (Vuyst, Vandamme, 1992). Adjust-

ment of the reaction to pH 6.8 before sterilization by autoclaving at 121 °C for 15 minutes.

The skim milk based medium was prepared by dissolving of 10 g of dried skim milk in 90 ml of distilled water. Before steaming at 98 °C for 60 min. the yeast extract (0.5% w/v) (OXOID) and Tween 80 (0.3% w/v) (OXOID) were added.

Influence of KH_2PO_4 in CM-medium and Skim Milk based Medium on Absolute Nisin Production

The choice of the best phosphorus source was done according to previously published data (Vuyst, Vandamme, 1993). CM-medium was supplemented with KH_2PO_4 (Lachema, CR) in amounts (0, 0.5, 1, 3, 5% w/v) replacing KH_2PO_4 (10.0 g/l) present in the basal CM-medium. During fermentation in 100ml Erlenmeyer flask the growth as CFU (colony forming units) measurement and nisin activity was measured. The CFU were estimated by use of agar plate method on M17 agar (OXOID) after incubation at 30 °C for 72 h. The effect of KH_2PO_4 addition in the same amounts as in CM-medium on the increasing of nisin yields was tested also in sterile skim milk (10% w/v) supplemented with yeast extract (0.5% w/v) (OXOID) and Tween 80 (0.3% w/v) (OXOID).

Nisin Activity Determination

The nisin bioassay was performed by a slightly modified agar diffusion method (Tramer, Fowler, 1964). The modified agar diffusion assay was used to determine nisin concentration in CM-medium. Nutrient agar (OXOID) was inoculated by indicator strain *Bacillus stearothermophilus* 796 L to the final density of aprox. 10 cfu/ml. Volume 20 ml of nutrient agar per Petri dish (90 mm diameter) was used. The wells for the application of supernatant were cut by means of sterile cork borer (6.8 mm diameter) and 50 µl of supernatant from CM-medium were used.

Supernatant from CM-medium was prepared by mixing 1 ml culture with 9 ml sterile saline solution in 0.02 mol/l HCl. The suspension was heated at 98 °C for 5 min. to release nisin and centrifuged (6 000 g at 4 °C for 10 min). Supernatant from milk was prepared by mixing 1 ml culture with 9 ml sterile solution in 0.02 mol/l HCl. After pH adjustment to 2 with 0.1 mol/l HCl, the

suspension was heated at 98 °C for 5 min to release nisin and centrifuged (6000 g at 4 °C for 10 min).

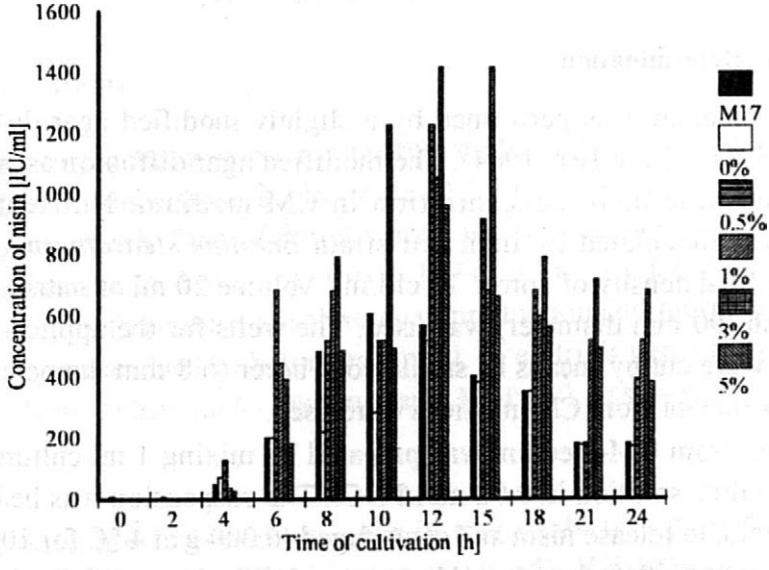
The plates were preincubated at 4 °C for 24 h and incubated at 55 °C for 24 h and the zones of growth inhibition were detected. For indicator strain the dependence of the zone of growth inhibition on the nisin concentration was measured (the size of inhibition zone was linear to the log of nisin concentration).

Nisin standards were prepared from Nisaplin (Aplin Barrett, GB). The working standard was prepared by dissolving 0.1 g of Nisaplin in 10 ml of sterile solution of saline in 0.02 mol/l HCl (i.e. 10 000 IU/ml). Only freshly prepared standards were used. The minimal detectable level of *Bacillus stearothermophilus* 796 L was 0.1 IU of nisin/ml.

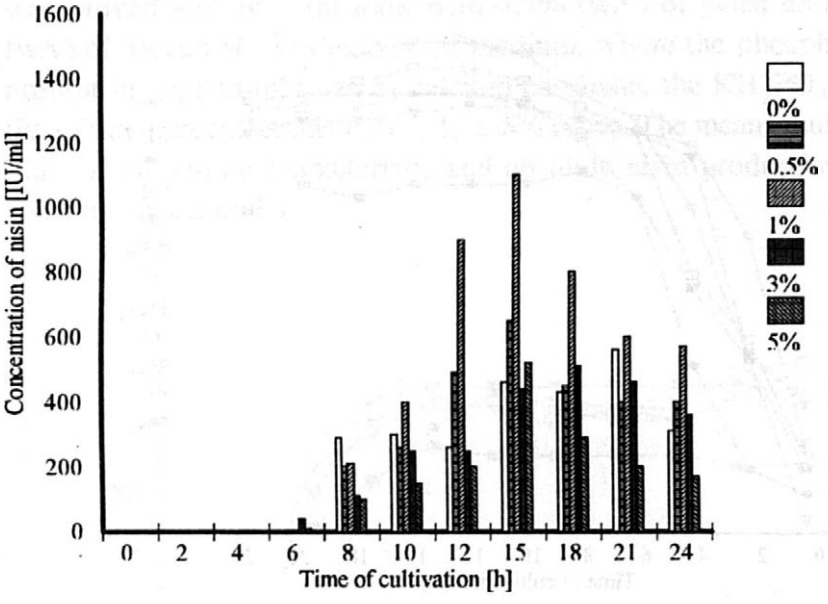
RESULTS AND DISCUSSION

Influence of Phosphorus Concentration

The influence of KH_2PO_4 on the concentration of nisin producted by *Lactococcus lactis* subsp. *lactis* NIZO R5 was studied in batch fermentations using the complex CM-medium, either skim milk supplemented with yeast



1. Influence of KH_2PO_4 concentration on the absolute nisin production by *Lactococcus lactis* subsp. *lactis* NIZO R5 in CM-medium compared with M17 medium



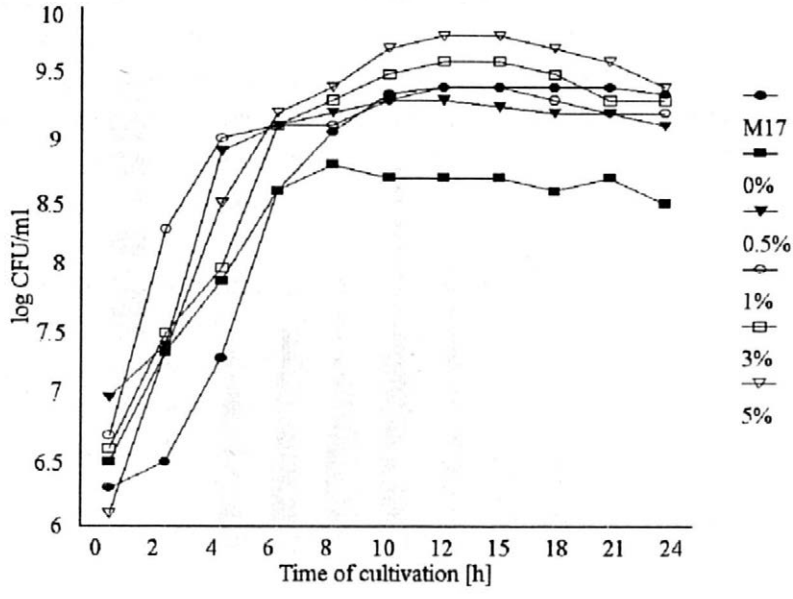
2. Influence of KH_2PO_4 concentration on the absolute nisin production by *Lactococcus lactis* subsp. *lactis* NIZO R5 in skim milk (10% w/v) with yeast extract (0.5% w/v) and Tween 80 (0.3% w/v)

extract (0.5% w/v) and Tween 80 (0.3% w/v) and compared with the parameters achieved in M17 broth.

The positive effect of KH_2PO_4 supplementation of the media for the growth and the concentration of nisin produced by *Lactococcus lactis* subsp. *lactis* NIZO R5 was proved in the CM-medium.

As a carbon source in CM-medium 2 % (w/v) of sucrose was used in our trial instead of 1% (w/v) of sucrose used usually in the basal medium. KH_2PO_4 was added to CM-medium in the initial concentrations 0; 0.5; 1; 3; 5% (w/v) replacing KH_2PO_4 present in the basal CM-medium. All measurements were done in duplicate and the mean values are present in Figs. 1 and 3.

It can be concluded from the results that in CM-medium without KH_2PO_4 the growth rate and nisin yields were lower compared with M17 broth where KH_2PO_4 was also used as a component of medium in amount 1%. The addition of initial concentration 0.5% (w/v) KH_2PO_4 to the CM-medium has increased the growth rate of *Lactococcus lactis* subsp. *lactis* NIZO R5 from 0.35 to 0.75 h^{-1}. And the absolute nisin production approx. by 100%. Further increase the initial concentration of phosphorus in CM-medium (1 and 3% w/v

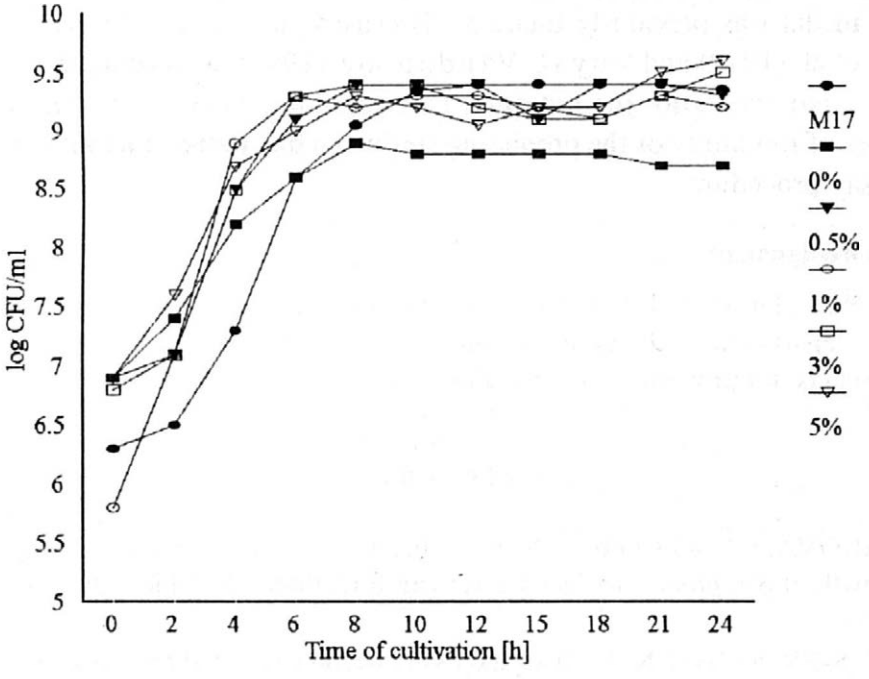


3. Influence of KH_2PO_4 concentration on the growth of *Lactococcus lactis* subsp. *lactis* NIZO R5 in CM-medium compared with M17 medium

KH_2PO_4) had further slightly stimulated effect on nisin yields, the same as on the number of CFU detected in CM-medium in the stationary phase of growth of *Lactococcus lactis* subsp. *lactis* NIZO R5. The maximum concentration of nisin (1400 IU/ml) in CM-medium was found using initial KH_2PO_4 concentration 3% (w/v) while the initial concentration of 5% (w/v) resulted in a decrease in the growth rate and nisin yields. As it has been found in the previous studies (Chumchalová et al., 1995) performed for the same strain the maximal nisin concentration was achieved in the M17 broth between 10.–12. hours of incubation, the maximum of nisin concentration found in CM-medium supplemented with KH_2PO_4 was achieved later. In the work of Vuyst, Vandamme (1993) the 5% (w/v) addition of KH_2PO_4 to CM-medium had still a stimulatory effect both on the growth rate and on the nisin yield by *Lactococcus lactis* subsp. *lactis* NIZO 22186 and the growth and absolute nisin production were suppressed by phosphate concentrations higher than 5% (w/v).

The positive effect of KH_2PO_4 supplementation of the media on the growth and absolute nisin production by *Lactococcus latis* subsp. *lactis* NIZO R5

was proved also in skim milk with 0.5% (w/v) of yeast extract and 0.3% (w/v) of Tween 80. In this type of medium, where the phosphorus was also present in the natural form as calcium caseinate, the KH_2PO_4 was added in the initial concentrations 0, 0.5, 1, 3, 5% (w/v). The mean results of duplicate trials of growth an characteristic and absolute nisin production are summarized in Figs. 2 and 4.



4. Influence of KH_2PO_4 concentration on the growth of *Lactococcus lactis* subsp. *lactis* NIZO R5 in skim milk (10% w/v) with yeast extract (0.5% w/v) and Tween 80 (0.3% w/v)

In this case the optimal initial KH_2PO_4 concentration added was 1% (w/v) which increased nisin yields by approx. 100–150% and positively influenced the growth rate. Improved nisin levels in the milk based medium with 1% (w/v) KH_2PO_4 achieved nearly the maximum of nisin concentration in CM-medium with the initial concentration of 3% (w/v) KH_2PO_4. The concentration of added KH_2PO_4 higher than 3% (w/v) into skim milk with yeast extract and Tween 80 had no stimulatory effect on the growth rate and nisin production by *Lactococcus lactis* subsp. *lactis* NIZO R5 strain.

In our work the positive effect of phosphorus in the form of KH_2PO_4 in CM-medium and skim milk supplemented with yeast extract and Tween 80 was proved for the growth rate and growth extent and for the concentration of nisin produced by the strain *Lactococcus lactis* subsp. *lactis* NIZO R5. The stimulatory effect was realized only till the concentration of initial KH_2PO_4 in the medium has reached a certain level.

The positive effect of KH_2PO_4 as a good phosphate source for the growth and absolute nisin production by another strains of *Lactococcus lactis* in the synthetic media was previously found by Baranova, Egorov (1967), Kozlova et al. (1979) and Vuyst, Vandamme (1993), who achieved slightly higher nisin yields for their strains. This could be explained by the various degrees of immunity of the producing strain and due to the non uniform nisin bioassay procedures.

Acknowledgement

We thank Dr. O. P. Kuipers, Department of Biophysical Chemistry and Genetics Department, Netherlands Institute for Dairy Research (NIZO), Ede, The Netherlands, for providing the strain *Lactococcus lactis* subsp. *lactis* NIZO R5.

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Received December 9, 1996

Potrav. Vědy, *15*, 1997 (1) : 29–38

Vliv přídavku KH_2PO_4 na absolutní produkci nisinu u kmene *Lactococcus lactis* subsp. *lactis* NIZO R5

Byl prokázán stimulační účinek přídavku anorganického fosforu ve formě KH_2PO_4 během jednorázové fermentace v CM-médiu a v odtučněném mléce obohaceném o kvasničný extrakt a Tween 80 v souvislosti se stanovením růstové rychlosti a absolutní produkce nisinu u kmene *Lactococcus lactis* subsp. *lactis* NIZO R5.

Maximální koncentrace nisinu – 1 400 IU/ml – bylo dosaženo v CM médiu za přídavku 3 % hmot. KH_2PO_4, v základním mléčném médiu za přídavku 1 % hmot. KH_2PO_4.

Koncentrace KH_2PO_4 vyšší než 5 % hmot. pro CM-médium a vyšší než 3 % hmot. pro základní mléčné médium vykazovaly inhibiční účinek na růst a absolutní produkci nisinu u kmene *Lactococcus lactis* subsp. *lactis* NIZO R5.

nisin; *Lactococcus lactis* subsp. *lactis*; KH_2PO_4; zvýšení absolutní nisinové produkce

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Potrav. Vědy, *15*, 1997 (1) : 39–48

ANTIFUNGAL ACTIVITY OF *Lactobacillus acidophilus*, CH5 METABOLITES*

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Abstract: In this preliminary study antifungal activity of supernatant containing acidocin CH5 (ACS) after cultivation of *Lactobacillus acidophilus*, CH5 in the MRS medium was found. ACS caused reduction in the growth of 3 (out of 4 tested) yeast strains belonging to the genera *Kluyveromyces* and *Candida* and suppression of the growth of mycelium and inhibition of spore formation of 5 (out of 13 tested) mold strains belonging to the genera *Penicillium* (3), *Cladosporium* (1) and *Alternaria* (1). *Penicillium* sp., DMF 0006 isolated from spoiled processed cheese as the most sensitive strain to ACS was suppresed in the growth on the GKCh agar and in a liquid complex medium with 2.5 and 5% (v/v) of ACS.

antifungal activity; *Lactobacillus* sp.; mold; *Penicillium* sp.; acidocin CH5; yeast

It is well known that the lactic acid bacteria (LAB) play an important role in the manufacture of fermented products including fermented milks and cheeses. LAB possess an effective enzyme system that enables them to degrade the milk components (lactose, proteins, lipids, citrate etc.) creating metabolites influencing the taste, odour and texture of products and synthetize the various secondary metabolites. Both the primary and secondary metabolites may be antimicrobial in character.

The antibacterial activity of metabolites of LAB (organic acids, diacetyl, hydrogen peroxide, bacteriocins) was studied in the past (Bruno, Montville, 1993; Daeschel, 1989; Ray, 1992; Lindgren, Dobrogosz, 1990; Holzapfel et al., 1995). Studies on the antifungal properties of LAB are relatively rare. Vandenbergh (1989) prepared yeast and mold inhibiting products from *Lactobacillus*, particularly *Lactobacillus casei* var. *rham-*

* This work was supported by the Grant Agency of Czech Republic (grant 510/95/0990).

nosus. Suzuki et al. (1993) tested many strains of LAB and discovered antifungal activity of chosen *Lactobacillus* and *Leuconostoc* strains.

Due to frequent incidence of yeasts and molds in dairy products and keeping in mind the danger of mycotoxin contamination of products the search for LAB strains with antifungal activity is very actual.

The purpose of this preliminary study was to evaluate the potential of *Lactobacillus acidophilus,* CH5 producing bacteriocin CH5, which was able to inhibit the growth of a broad spectrum of bacteria, to suppress the growth of chosen yeasts and molds.

MATERIAL AND METHODS

Microorganisms

- *Lactobacillus acidophilus,* CH5, isolated from dairy starter, Chr. Hansen's, Denmark,
- *Lactobacillus delbrüeckii* subsp. *lactis,* LTI30, isolated from the intestinal tract of a child, Czech Republic.

Both *Lactobacillus* strains were used in previous studies (Plocková et al., 1996; Chumchalová et al., 1995). They were maintained in sterile MRS broth (Oxoid) and subcultured once a week. Stock cultures were transferred at an inoculum size of 1% (v/v) and incubated at 30 °C for 16 hrs.

Strains of molds and yeasts were provided by the Culture Collection of Dairy Microorganisms Laktoflora, Prague, Czech Republic (LCC):

- *Kluyveromyces marxianus* var. *marxianus,* LCC 269
- *Kluyveromyces marxianus* var. *lactis,* LCC 255
- *Candida kefyr*, LCC 271
- *Candida famata,* LCC 262
- *Penicillium roqueforti,* LCC HAII
- *Penicillium camemberti,* LCC 797 + 799 (2:1)
- *Penicillium nalgiovensis*, LCC 3021

Strains of molds isolated from dairy products and dairy plant environment at the Department of Milk and Fat Technology, Prague Institute of Chemical Technology, Czech Republic and identified at the Department of Botany, Faculty of Natural Sciences, Charles University, Prague, Czech Republic:

- *Penicillium* sp., DMF 0006
- *Penicillium hirsutum*, DMF 0001
- *Penicillium rugulosum*, DMF 0003
- *Penicillium expansum*, DMF 0004
- *Penicillium chrysogenum*, DMF 0002
- *Penicillium glabrum*, DMF 0005
- *Fusarium* sp., DMF0101
- *Cladosporium herbarum*, DMF 0401
- *Alternaria* sp., DMF 0201
- *Geotrichum candidum*, DMF 0301

All strains of molds and yeasts were maintained on a GKCh agar (Milcom, Czech Republic) slants and transferred monthly.

The complex CM medium used for the cultivation of mold strain *Penicillium* sp. contained: sucrose (10.0 g/l), KH_2PO4 (10.0 g/l), NaCl (2.0 g/l), $MgSO_4.7H_2O$ (0.2 g/l) from Lachema (Czech Republic) and peptone (10.0 g/l), yeast extract (10.0 g/l) from OXOID. The pH of the medium to 6.8 before autoclaving (121 °C for 15 min) was adjusted.

The yeast strains were incubated at room temperature for 2 days, the mold strains under the same conditions for 5 days to provide the proper growth of mycelia and production of fungal spores.

For the assessment of antifungal activity the suspensions of yeast were obtained by washed out agar slant with the yeast strain (after 2 days incubation) with 5 ml of sterile saline. In the case of mold strains fungal spores and/or mycelia from the surface of agar slant (after 10 days of incubation) were washed out with 5ml of sterile saline containing 0.1% Tween 80 by brushing the surface of the slant with a sterile loop. Each suspension was used directly as an inoculum after adjusting to $A_{600} = 0.3$.

Preparation of Acidocin CH5 Containing Supernatant (ACS)

Supernatant after 16hr incubation of *Lactobacillus acidophilus*, CH5 at 37 °C (1% inoculum) of pH value 6.0 was centrifuged (4000 rpm/min at 4 °C for 10 min). After removing the cell biomass the supernatant was heated at water bath for 15 min to inactivate the potentially present labile antimicrobial substances e.g. hydrogen peroxide and inactivators (e.g. proteolytic en-

zymes). Obtained supernatant was stored at –20 °C, before each use was filter sterilized (Millipore 0,20 μm) and the activity of present bacteriocin was evaluated. All assessments in the preset work were realized by use of ACS containing 1280 IU/ml acidocin CH5.

Acidocin CH5 Titer Evaluation

The indicator strain *Lactobacillus delbrüeckii* subsp. *lactis*, LTI30 was subcultured before each use in MRS broth (16 hrs, at 37 °C, 1% inoculum). On the day of use the fresh culture of sensitive cells was prepared within 4 hrs, incubation at 37 °C (4% inoculum). 0.1ml of indicator organism suspension (A_{615} = 0.3) was mixed with 20 ml of molten sterile MRS agar (Oxoid) with 1.5% agar and used per Petri dish (90 mm). The wells for the application of sample were cut by means of sterile cork borer (6.8 mm diameter) after solidification of agar and 50 μl of the proper dilution of supernatant containing acidocin CH5 was applied. The plates were incubated at 37 °C for 24 h with preincubation at 4 °C for 20 hrs and after this time the clear zones of growth inhibition were detected. The bacteriocin titer (BT) was calculated using the formula:

$$BT\ (AU/ml) = 2^{x}.1000.V^{-1}$$

where: x – the number of the last dilution showing inhibition
V – the volume of used supernatant in μl

Assessment of Antifungal Activity

It was done using all strains of molds and yeast by agar diffusion assay with visual observation of clear zones of inhibition and morphological changes of mold and yeast growth on the surface of agar medium. Using chosen strains, for which the inhibition was proved by agar diffusion assay, slide cultures were prepared for the most precious evaluation of changes in morphological structures including the detection of spore forming. The most sensitive mold strain was tested for the growth inhibition in the liquid medium.

Agar Diffusion Assay

The spore suspension of each mold strain was prepared as described previously. 0.2 ml of each mold or yeast suspension was inoculated onto the

surface of solidified GKCh agar and after all saline has difunded into agar 10 and 20 μl of ACS on the surface of inoculated agar were stabbed. After 10 days (molds) and/or 2 days (yeasts) incubation at the light place at room temperature clear inhibition zones around the stabbing places were observed as well as changes in the colour and appearance of the mycelium.

Slide Cultures

On a glass slide placed on a glass curved stick in the petri dish with a drop of water on the bottom the GKCh agar was dripped on the two places keeping the aseptic conditions. Each of two agar drops was inoculated after solidification by loop with the same mold or yeast suspension (prepared as mentioned above). One inoculated agar drop was overlaid by 30 μl of ACS. After 10 days (molds) and/or 2 days (yeasts) cultivation at the light place at room temperature morphological changes were observed microscopically.

Evaluation of Growth Inhibition in the Liquid Medium

Into the 500ml Erlenmayer flasks with 250 ml of the complex medium inoculated with 2.5 ml of spore suspension of *Penicillium* sp. (containing 107 cfu/ml) were added 0, 1, 2.5 and 5% (v/v) of ACS. Each 3 hours the flasks were shaken by hand and the probes were taken aseptically for evaluating the cfu by standard agar plate method using GKCh agar and 10 days of incubation in the light place at room temperature.

RESULTS AND DISCUSSION

The first examination whether the yeasts and molds are inhibited by ACS was realized by agar diffusion assay. The strains inhibited by ACS on the GKCh agar were used for testing the changes in morphology of mycelia and conidiophores and the lack of conidiospore production in the form of slide cultures. The results are presented in Table I.

It can be concluded from the results that 3 out of 4 tested strains of yeasts and 5 out of 13 tested strains of molds were inhibited in the growth on the GKCh agar by the addition of ACS. All tested yeast strains were commercial strains used in the dairy industry. From the tested mold strains the following strains have been inhibited by ACS: 3 strains belonging to the genus *Penicil-*

I. Suppression of the growth mold and yeast strains on the GKCh agar and slide cultures (SC) by ACS

Strain	ACS	
	GKCh	SC
Yeast		
1. *Kluyveromyces marxianus* var. *marxianus*, LCC 269	+	+[1]
2. *Kluyveromyces marxianus* var. *lactis*, LCC 255	+	+[1]
3. *Candida kefyr*, LCC 271	+	nd
4. *Candida famata*, LCC 262	–	nd
Mold		
1. *Penicillium roqueforti*, LCC HAII	++	+[2]
2. *Penicillium camemberti*, LCC 797+799 (2 : 1)	–	nd
3. *Penicillium nalgiovensis*, LCC 3021	–	nd
4. *Penicillium* sp., DMF 0006	+++	+[2]
5. *Penicillium hirsutum*, DMF 0001	–	nd
6. *Penicillium rugulosum*, DMF 0003	++	+[2]
7. *Penicillium expansum*, DMF 0004	–	nd
8. *Penicillium chrysogenum*, DMF 0002	–	nd
9. *Penicillium glabrum*, DMF 0005	–	nd
10. *Fusarium* sp., DMF 0101	–	nd
11. *Cladosporium herbarum*, DMF 0401	++	+[2]
12. *Alternaria* sp., DMF 0201	++	+[2]
13. *Geotrichum candidum*, DMF 0301	–	nd

– no suppression

+ weak suppression, small detectable clear zone around the stabbing place 20 µl

++ strong suppression, detectable clear zone around all stabbing places, changes in colour and appearance around the stabbing place

+++ very strong suppression, detectable clear zone around the stabbing place, changes in colour and appearance of mycelia

nd = not detected

+[1] less amount of cells, changed morphology

+[2] changes in the size and morphology of conidiophores, no spore formation

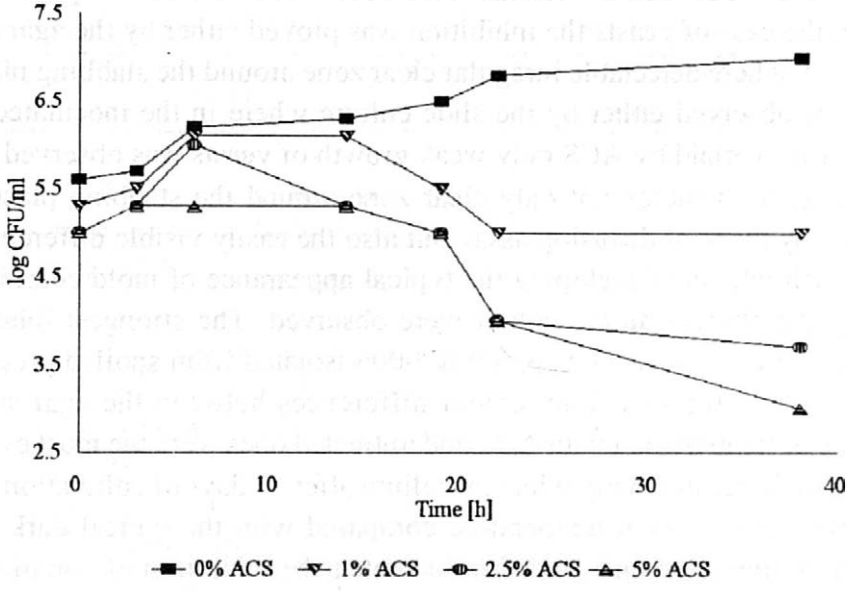
lium (out of 9 tested), 1 *Cladosporium* sp. and 1 *Alternaria* sp. One strain of *Penicillium* sp. was culture collection strain for application in the dairy industry, the other strains were isolated from spoilt dairy products and from the dairy plant environment.

Differences between the strains were observed in the intensity of inhibition. In the case of yeasts the inhibition was proved either by the agar diffusion assay where detectable inregular clear zone around the stabbing place of ACS was observed either by the slide culture where in the inoculated agar which was overlaid by ACS only weak growth of yeasts was observed.

In the case of molds not only clear zone around the stabbing place was detected by the agar diffusion assay but also the easily visible differences in the growth rate and developing the typical appearance of mold colonies including the changes in the colour were observed. The strongest inhibition was proved for *Penicillium* sp., DMF 0006 isolated from spoiled processed cheese, where the mycelium colour differences between the agar surface colonies of mold treated with ACS and untreated ones were the most evident. Treated mold created ivory white mycelium after 10 days of cultivation in the light place at the room temperature compared with the typical dark green created by untreated one. This fact indicated the inhibition of conidiospore forming at tested *Penicillium* sp., DMF 0006 strain and it was acknowledged by use of slide cultures, where the lack of conidiospores on the morphologically changed conidiophores was observed microscopically.

To be able to evaluate the behaviour of molds in the presence of ACS in the liquid medium the growth of *Penicillium* sp., DMF 0006 in complex medium, which was found in the past to be nutritionally suitable for the growth of tested mold strain was tested. The effect of various contents of ACS on the growth or survival of the mold strain in the complex medium was tested using the static incubation lasting for 36 hrs at the room temperature being interrupted by 1min shaking before each sampling.

The results are presented in Fig 1 and it can be read from them that the cfu of *Penicillium* sp., DMF 0006 in the complex medium were influenced by present ACS. After 36 hrs of cultivation of *Penicillium* sp., DMF 0006 without ACS the number of cfu increased by 1.5 log cycle while in the presence of 1% (v/v) ACS cfu remained approximately at the same level as at the beginning of cultivation and in the presence of 2.5 and/or 5% (v/v) of ACS cfu decreased by 1 and/or 1.5 log cycle. A similar degree of inhibition was found in the work of Vandenbergh (1989), who proved the effect of supernatant after cultivation of *Lactobacillus casei* subsp. *rhamnosus*, which suppressed *Penicillium oxalicum*.



1. The effect of ACS on the growth of *Penicillium* sp., DMF 0006

In our preliminary study the interesting fact of antifungal activity (inhibition of growth or reduction in the growth rate of yeasts, suppression of the growth of mycelium, inhibition of spore formation of molds especially in *Penicillium* sp.) of some metabolites of *Lactobacillus acidophilus,* CH5 was found by use of three methods. The same as in previously published studies, the substance responsible for the antifungal activity was not isolated (Suzuki et al., 1991). In contrary to the study of Suzuki et al. (1991), who found that for the antifungal action the presence of actively growing and metabolizing cells of lactobacilli and leuconostoci is necessary, in our case the antifungal action was due to the supernatant obtained after cultivation of *Lactobacillus acidophilus,* CH5 in MRS broth.

Vandenbergh (1989) found antifungal activity in supernatants after cultivation of several *Lactobacillus* sp. strains in the media similar to MRS (supplementing the strains with carbon and protein source including sulphur containing compounds, minerals such as manganese and magnesium salts and buffers). His most antifungally active compounds produced by *Lactobacillus casei* var. *rhamnosus* inhibiting *Penicillium oxalicum* were found to be

polar and had the molecular size of less than 1000 daltons but were not proteins or lipids.

Our product ACS did not contain any detectable viable cells and was neutralized to avoid the action of organic acids and treated by heat to avoid the action of hydrogen peroxide and other thermostable substances. It is clear from this fact that in our case was for the antifungal activity responsible any thermostable metabolite other different from the organic acids in the contrary to Batish (1989) according to whom the antifungal activity of lactic acid bacteria appeared to be related to lactic and acetic acids.

In future we shall continue our studies to prove if for the antifungal activity of *Lactobacillus acidophilus,* CH5 is responsible acidocin CH5, which was found to be bactericidal and fulfilled the characteristic features mentioned above, or if it is any other product of fermentation.

The strains of lactic acid bacteria possessing the antifungal activity are very promising from the point of view of application in the dairy industry as well as in the other branches of the food industry.

Acknowledgement

We thank RNDr. Alena Kubátová, CSc., from the Department of Botany, Charles University of Prague, for the identification of mold strains.

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Received December 9, 1995

Antifungální účinky metabolitů produkovaných kmenem *Lactobacillus acidophilus, CH5*

Byla zjištěna produkce antifungálních látek v supernatantu obsahujícím acidocin CH5 (ACS), který byl získán po kultivaci kmene *Lactobacillus acidophilus,* CH5 v MRS bujonu.

ACS potlačoval růst tří kvasinkových kmenů (ze čtyř testovaných) zařazených do rodu *Kluyveromyces* a *Candida*. Dále bylo zaznamenáno potlačení růstu mycelia a inhibice tvorby spor u pěti kmenů plísní (ze 13 testovaných) rodu *Penicillium* (3), *Cladosporium* (1) a *Alternaria* (1). Jako nejcitlivější se projevil kmen *Penicillium* sp., DMF 0006, který byl izolován ze zkaženého taveného sýra. Inhibice tohoto kmene byla sledována také v kapalném komplexním médiu. K potlačení růstu došlo při použití 2,5 a 5 % obj. ACS.

antifungální aktivita; *Lactobacillus* sp.; plísně; *Penicillium* sp.; acidocin CH5; kvasinky

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Potrav. Vědy, *15*, 1997 (1) : 49–67

ISOLATION OF INULIN FROM CHICORY ROOT*

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Abstract: This paper deals with methods used for the extraction, isolation, refining and manufacturing of inulin from chicory. The introductory study completes by way of performed experiments the fundamental physical and chemical properties of technical and pure solutions of inulin and chicory extracts. The study also discusses the necessary kinetic data concerning crystallization, extraction and filtration process. There are applied recent procedures – for example: cross-flow membrane filtration, film evaporation, spray drying and cooling crystallization by means of computer. For the determination of inulin, other saccharides and further compounds occurring in extracts of chicory roots analytical methods were developed which apply HPLC and ITP (isotachophoresis). In conclusion of work the variant design of scheme demonstrating technological process of the isolation of inulin and fructooligosaccharides is mentioned.

Inulin; fructooligosaccharide; chicory root; solubility; extraction

Inulin is one of reserve oligosaccharides in plants, for example chicory (*Cichorium intybus*), artichoke (*Helianthus tuberosus*), leek, asparagus, topinambur, jakon, onion, garlic and others (Loo, 1995). Chicory (*Cichorium* sp.) ranks in tribe Asteraceae, as grower rootcrop. The species which provides valuable root-chicory (*Chicory intybus* var. *sativum*) is cultivated especially, further *Chicory endevia* – the species gives etiolysated blades. Chicory is a biennial plant which in the first year forms the root and the ground leaf rosette. The chicory root contains (Gibson et al., 1994) 26% of dry substances and 74% of water. In dry substance 16% of inulin, 1.5% fruc-

* The work is supported by the Grant Agency NAZV of the Ministry of Agriculture of Czech Republic. The part of the work dealing with cooling crystallization by means of computer is supported by the Grant Agency of the Institute of Chemical Technology in Prague.

tose, 1% proteins, 0.3% lipids, 1.3% inorganic non-sugars and insoluble fibre are contained.

Chemically inulin is polymer of fructose (β-D-fructan) with a degree of polymerization (DP) 2-60 in which molecules of fructose are linked by bonds β(1-2) (Roberfroid, 1993). The length of chain and the degree of polymerization depends on its plant origin. For example, chicory inulin is branched with short side chains. The chain is terminated by the glucose unit or it may contain small amounts of fructan side chains without a glucose part.

The manufacturing process of inulin in principle consists of chicory cossette extraction by means of hot water, analogous to beetsugar technology, obtained extract is refined, evaporated and the final product of inulin is prepared during drying in spray drier (Roberfroid, 1993; Chochola, 1996).

The Properties and Applications of Inulin

Inulin is first of all a significant source of dietary fibre which has a favorable influence on gastrointestinal activity (Tomomatsu, 1994; Roberfroid, 1993). Human organism does not dispose of enzymes splitting the β(1-2) bond and thus the split of inulin chain into fructose is not possible. Non-hydrolysed inulin is not absorbed in the gastrointestinal tract. It is fermented by help of microflora in the large intestine and its metabolites gained during this process are used as an energetic source for organism. The energetic value of inulin is very low, 4 kJ/g approximately. Inulin is also known for its selective bifidobacterial effect, i.e. it encourages the bifidobacterial activity and inhibits the breeding of *Salmonella* and *E. coli* bacterium. Inulin participates in cholesterol blood values.

The most important attributes of inulin are neutral and insipid sweet flavor, neutral color and smell, water solubility, ability of co-creating the texture, gelatinization features and foam stabilization (Franck-Frippiat, 1995). With use of special techniques it is possible to obtain creams from inulin and the texture of creams is similar to fat texture. They are used as fat and sugar compensation. The recommended addition of inulin to bread and pastry is 5–15%, to dressing and milk products 5%, to chocolate 10–40%, to ice-creams 8%. Inulin is also used for fructose and fructose solution production at enzymatic hydrolysis. Fructose syrups are appropriate for sucrose addition in the processing industry – such as preserved fruit and juice.

ANALYSIS OF CHICORY ROOTS

For analytical purposes two following methods have been applied above all:

a) High performance liquid chromatography – HPLC – was modificated and approved for the determination of the amount of inulin and other carbohydrates.

b) Isotachophoretic method – ITP – a recent progressive procedure has been used for the determination of ash content or cations and anions.

Sample Preparation

The representative sample is prepared from thin slices of chicory roots. From a well-mixed sample 20.00 g is weighed for the analysis and then 100 cm^3 of distilled water is heated up to 80 °C, added and mixed by the laboratory mixer for one minute. The content is quantitatively introduced into 200 cm^3 volumetric flash, made up to the mark. The solution is filtered through a filter paper and the obtained filtrate diluted ten times, further filtered through a membrane filter (pore diameter 0.4 μm) and injected onto a chromatographic column.

Basic Parameters of HPLC Analysis

Precolumns: 3 x 30 mm: HEMA-BIO 1000 SB 10 μm a HEMA-BIO 1000 Q 10 μm
Column: stainless steel 250 x 8 mm OSTION LGKS 0800 in calcium form
Mobile Phase: deionized water
Flow Rate: 0.4 ml/min
Temperature: 80 °C
Detection: refractometric index

The Principle of the Isotachophoretic Method (ITP)

Isotachophoresis is a separating analytical method used for the analyzing of the ionogenic mixtures in the D.C. electric field. In the course of one analysis it is more possible to separate either cations or anions. The different ion mobility of analyzed compounds inside an electric field is the principle of separation. This method puts two electrolytes to use:

- leading electrolyte: it contains the most mobile ions in regard of the analyzed ion mobility,
- terminal electrolyte: it contains ions with the smallest mobility.

The sample is situated between these two electrolytes. Required preparation of samples is identical as for HPLC.

Basic Parameters of ITP Analysis

Cations: Driving current: 80 μA
Leading electrolyte: 7.5mM sulfuric acid + 7 mM 18-Crown-6
Terminal electrolyte: 10mM lithium citrate

Anions: Driving current: 80 μA
Leading electrolyte: 10mM HCl + 22mM ε-aminocaproic acid + 0.1% HMPC
Terminal electrolyte: 5 mM caproic acid

PHYSICAL AND CHEMICAL PROPERTIES AND KINETIC DATA OF INULIN TECHNICAL AND PURE SOLUTIONS AS WELL EXTRACTS OF CHICORY

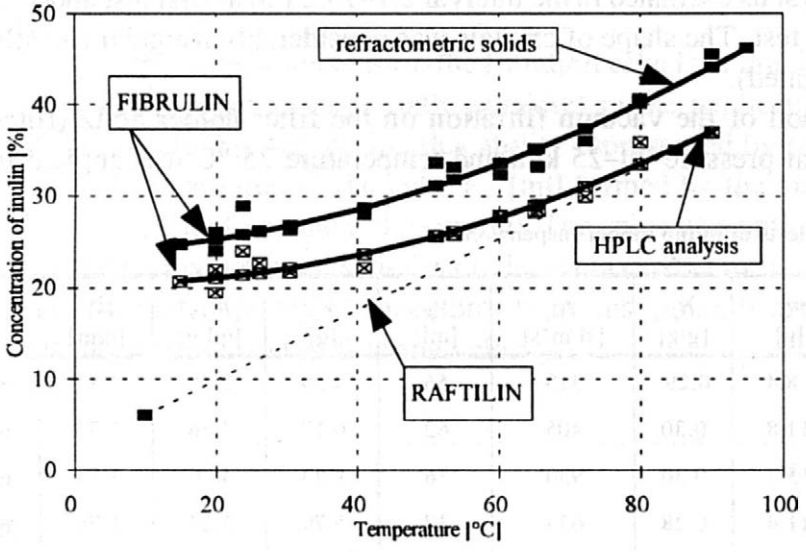
It is necessary to know the basic physical and chemical properties of technical inulin solutions and other kinetic data for the application of a number of technological operations (filtration, evaporation, crystallization). Several data originate from the literature (Anonym, 1995) and in need they were verified by the experiments. The remaining data have been acquired by our own practice.

Solubility in Water

An essential factor among the basic properties of inulin is the solubility in pure and technical solutions. It is a necessity for the design and realizing of technological procedures. The determination of solubility was performed in a temperature range from 20 to 90 °C by sample of FIBRULIN (Teeuwen, 1992) which contains 97% of inulin and 2.5% of water.

Six experimental series were carried out to determine solubility. In the first there was an aqueous suspension of inulin (50 g inulin + 100 g water) agitated in the thermostat gradually at temperatures 20–80 °C (interval 10 °C) till the gain of equilibrium between solid and liquid phases. The procedure was checked by refractometric method. Subsequently a sample of suspension was taken at each temperature. The mother liquor was separated by the vacuum filtration.

For the determination of inulin and other saccharides (saccharose, glucose and fructose above all) the method of HPLC was applied. The others experiments were realized in the same way (suspension 75 g of inulin and 100 g of water). The range of temperatures in this case was from 30 to 90 °C. The results are shown graphically in Fig. 1 where the values from literature are also presented (Anonym, 1995). The divergences between the obtained values and literary ones are influenced principally by the difference in used inulin samples – different molar mass (various chain length) of inulin molecules.



1. Solubility of inulin in water

Temperature dependence of inulin solubility (Fig. 1) was described by parabolic relationship. Coefficients of equations were evaluated from experimental data by the method of least squares:

a. Refractometric solids: S_{ref} (%):

$$S_{ref} = 23.7 + 0.0371.t + 0.0021.t^2$$

b. Content of inulin (determined by HPLC): S_{hplc} (%):

$$S_{hplc} = 20.3 - 0.0033.t + 0.0021.t^2$$

where: t – temperature in °C

The Cooling Crystallization, Separation, Filtration and Yield of Inulin

The solutions obtained from the first and second determination of solubility were used for the preliminary crystallization tests. The solutions located into the Deward container slowly, gradually, spontaneously cooled down. At the same time the nucleation and subsequent growth of the formed crystals emerged. The first cooling test lasted 17 hours and temperature was reduced from 80 to 23 °C. The second test passed in time-phase 72 hours under the temperature fall from 90 to 20 °C. The obtained microcrystals of inulin were observed by the microscope, tooled by the calibrated scale lattice. The size of crystals oscillated in the interval 2.2–13.2 μm at first test and 6–22 μm at second test. The shape of crystals was considerably irregular (mostly oval and elongated).

A method of the vacuum filtration on the filter holder Seitz (filter area 8.8 cm^2) at pressure 20–25 kPa and temperature 25 °C was applied for the

I. Filtration tests of inulin model suspensions

No.	m_s [g]	X_s [g/g]	t_F [sl/m^2s]	v_F [ml]	V_c [g]	$m_{c,s}$ [ml/g]	X_c [mm]	L [μm]
1	8.4	0.29	319	56	4.39	2.43	1.81	6–20
2	11.8	0.30	405	62	6.17	3.56	1.73	6–20
3	15.7	0.30	930	36	8.23	4.76	1.73	6–20
4	11.4	0.28	633	37	5.70	3.23	1.76	6–20
5	10.9	0.29	544	43	5.70	3.13	1.82	6–20
6	11.1	0.28	894	24	5.14	3.06	1.68	2–10
7	12.0	0.27	1181	20	5.70	3.42	1.67	2–10
8	10.9	0.27	1214	16	4.77	2.91	1.64	2–10

m_s – weight of filtered suspension sample

X_s – suspension concentration – content of solid phase in suspension: $X_s = m_{c,s} / m_s$

t_F – total filtration time

V_F – medium filtration velocity

V_c – volume of filtration cake

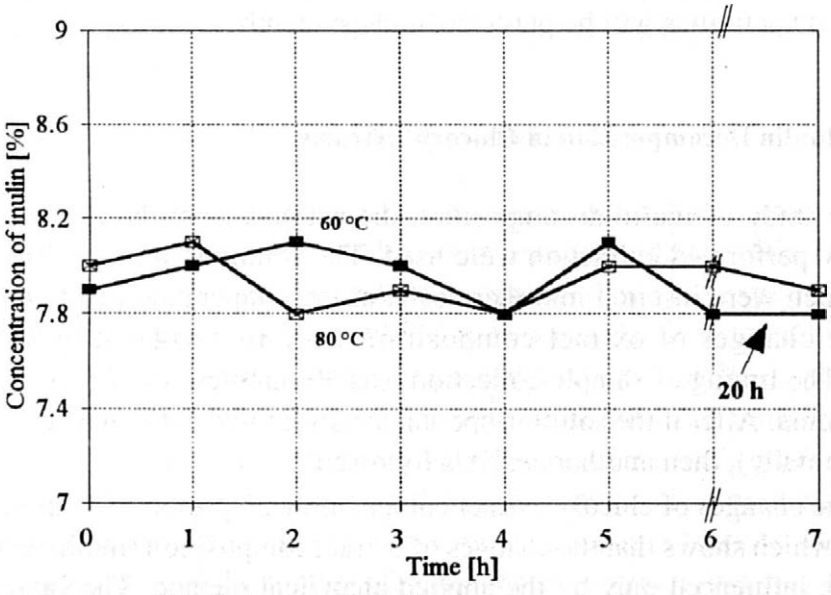
$m_{c,s}$ – weight of dry filtration cake

X_c – ability of formation of filtration cake: $X_c = V_c / m_{c,s}$

L – size of inulin crystals (particles)

separation of crystals. The results of assays performed in 8 samples are shown in Table I. The initial concentration of suspension in all 8 samples was approximately analogous (0.27–0.30 g of crystals in one gram of suspension). After granulometry the samples were divided into two groups. The larger (6–22 μm) are found in samples 1–5, the smaller (2–10 μm) in others. This dimension difference evoked changes of filtering velocity which is twice or three times higher in the first group than in the second. This conclusion is important for the controlling of crystallization procedure where a proper system of regulation keeps the process in metastable zone and hinders from the undesirable nucleation and thus prevents the unsuitable granulometry of product too.

The ability of the suspension to form the filtration cake is of crucial importance for its final properties (especially porosity) in the application in the process of filtration. We may define this ability represented by the coefficient X_c [ml/g] as volume of the cake V_c [ml] formed by the unit weight ($m_{c,s}$ = 1 g) of the filtration material (= inulin). The value of mentioned coefficient is in the second group of samples on the average 6% lower, which is a symptom of the worse quality of cake porosity in this group. It corresponds with results of measurement of filtering velocity.



2. The time course of inulin decomposition in chicory extracts

The obtained filter cakes were dried at 60 °C first when the average achieved moisture was 3%. During a drying process at higher temperature (105 °C) the decomposition of inulin occurred, which is indicated by brownishing of filter cake. The dry cakes were dissolved in water and analyzed by HPLC method. The yield of inulin by cooling crystallization oscillated in a range of 66–70% (related to the entire amount of inulin used for experiment). Balance calculation was used for the evaluation. The remaining inulin rested in the mother liquor and is available for the other technological purposes.

On the basis of these results the crystallization tests of thickened real chicory extracts after their previous purification were performed. There were various procedures of purification used. The crystals originating from chicory extracts amounted to size about 10 μm, consequently a structure of product is gelled. This phenomenon reduces the separability of crystals remarkably. Therefore we have sought for a further way in development of technological scheme, by means of the spray drying of suspension above all.

Our subsequent research works are aimed now at these topics: determination of a crystal growth rate, width of metastable zone, effects of various substances on crystallization and nucleation, application of simulation programs for achievement the optimized cooling curve and utilization of crystallizing device with computerized cooling crystallization. The results of these complement activities will be published independently.

Study of Inulin Decomposition in Chicory Extracts

For the study of inulin decomposition the extracts from the first step of laboratory performed extraction were used. The solutions were prepared in flasks which were inserted into thermostat at the temperature of 60–80 °C. The time changes of extract composition were investigated by HPLC method. The timing of sample collection was 60 minutes. Total time of test was six hours. After it the solution spontaneously cooled to the next day (i.e. 20 hours totally), then another analysis followed.

The time changes of chicory extract composition are presented graphically in Fig. 2, which shows that the changes of extract composition (inulin respectively) are influenced only by the applied analytical method. The values of inulin content did not display any falling or rising tendency.

It yields the information that temperature of 60 °C is sufficient for the stopping of enzymatic destruction. This is a positive conclusion for further technological procedures, especially with regard to energy consumption. Our investigation was carried out in primary extract without modification of pH value. Provided the isolation processes requiring modification of pH are studied the extension of the test series will become necessary.

EXTRACTION OF INULIN FROM CHICORY ROOT

The Slicing of Chicory Roots

The required quality of cossettes according to demands of extraction procedure is indicated by a length of 100g sliced chicory roots. A laboratory grater, equipped with replaceable knives, was used for the preparation of cossettes from chicory roots. We made two sorts of cossettes depending on chosen extraction procedures:

a. Fine cossettes are determined with an average length of 45 m for weighed out 100 g. In comparison with sugar beet the measured length corresponds to extraordinarily thin beet cossettes and likewise is proof of favourable mechanical qualities desirable for this process.
b. The thicker cossettes are determined with an average length of 15 m for weighed out 100 g. These findings correspond to a higher standard of sugar technology.

The Extraction of Fine Cossettes in Mixed Batch Device

Our laboratory tests were executed in the mixed batch extractor. Two realized triple-stage tests were different in temperature conditions (60 and 80 °C) only. Fine cossettes (see above) were employed in experiments. The mixture of 500 g of cossettes and 700 g of water was inserted into a heated vessel with a capacity of 3 l and agitated under revolutions 20–30 per minute.

After an extraction course of 15 minutes at adjusted temperature the cossettes were strained through a sieve, then mixed with 350 g of identically tempered water and again extracted for 15 minutes. The previous process was repeated with 350 g of water once more. After each stage we determined the yield ability – or the content of nonextracted inulin in cossettes. Our laboratory investigated the composition of juices in successive stages as well.

II. Results of extraction tests in batch extractor

	Weight of cossettes [g]	Content of inulin [%]	Weight of extract in cossettes [g]	Content of inulin in extract [%]
at 60 °C				
Beginning	500	19.0	700 (water)	0
after 1st stage		8.7	481	7.9
after 2nd stage		4.9	358	5.6
after 3rd stage		3.6	354	3.4
at 80 °C				
Beginning	500	1.0	700 (water)	0
after 1st stage		8.4	555	7.9
after 2nd stage		5.0	332	5.1
after 3rd stage		3.2	342	3.2

The total time of one test was two hours. Obtained results are summarized in Table II.

According to results in Table II the used method was able to extract 81 to 82% of the amount of inulin from chicory pulp. The temperature elevation did not afford a considerable growth of the extraction effect. The use of further extraction stages creates a further thinning of inulin in the extract, consequently energy consumption by the water evaporation increased. The optimum of extraction stages is to be evaluated on the basis of economic calculations.

Extraction of Cossettes in Column

A heat jacket glass column with a blinding by frit glass and tap was used for extraction. The column length was designed one meter and available diameter 29 mm. The column harmoniously charged with cossettes, then watered by the lower path and tempered at a required level (chosen values of 80–95 °C). The thermal sensor AHLBORN-ALMEMO (Germany) was used for the measurement of temperature and it was installed in the centre of the

column. Warm water (extraction medium) entered through the bottom and passed through the cossettes by pressure of the adjusted water column. In this way an intensity of liquid flow (or performance of column) was controlled. The obtained extract was sampled in the upper periphery of the column. The content of dry substances was determined by refractometer. Further analysis of inulin and other saccharide content was performed by HPLC method. The temperature inside the column varied in the range of ± 0.2 °C.

1st extraction test in the column

Cossette batch – 240 g of fine cossettes (45 m/100 g)
Inulin content in cossettes – 20.0%
Temperature of extraction – 80 °C

2nd extraction test in the column

Cossette batch – 212 g of thicker cossettes (15 m/100 g).
Inulin content in cossettes – 18.8%
Temperature of extraction – 95 °C

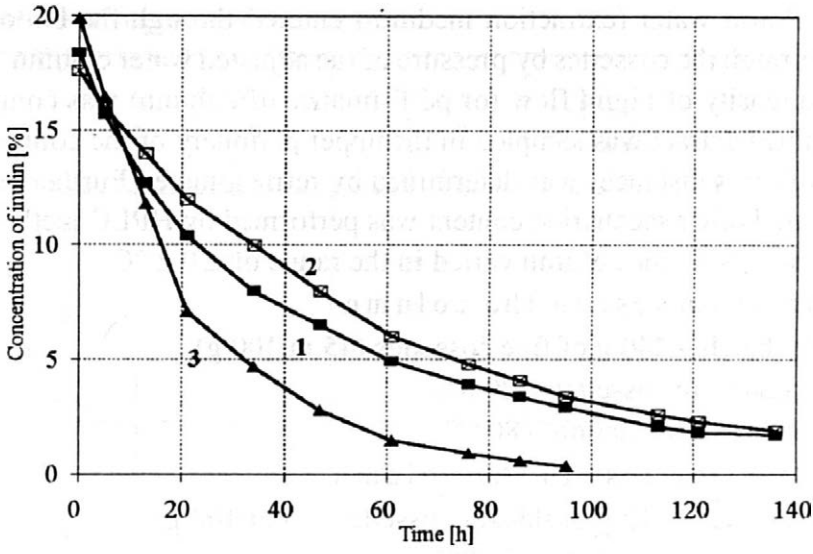
3rd extraction test in the column

Cossette batch – 239 g of fine cossettes (45 m/100 g)
Inulin content in cossettes – 20.9%
Temperature of extraction – 95 °C

The results of the test are graphically represented in Fig. 3. The speed of extraction markedly increased at a higher temperature. For example – we achieved 2% of dry substance concentration in extract within 55 minutes at temperature 95 °C observing the identical cossette quality. While at keeping a lower temperature (80 °C) dry substance concentration 2% was achieved within 115 minutes. The double prolongation of the extraction time was found out when we compared the thicker and finer cossettes under identical experimental conditions. The average value of extraction water flux amounted to 5 ml/min.

Extraction of Inulin in the Four-Column Countercurrent Extractor

The above-mentioned knowledge was taken for the source of scheme of four-column extraction equipment. Three columns are in the active stage while the change of cossette batch runs simultaneously in the fourth one. The columns are sequenced in a series and the countercurrent movement of juice and cossettes is formed by sequential switching of water inlet. Water is



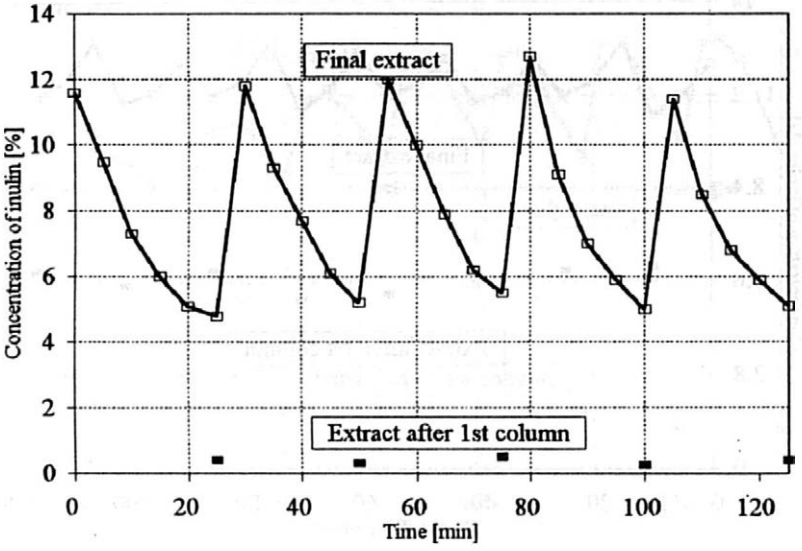
1 – Test No.1, fine cossettes, temperature 80 °C; 2 – Test No.2, thick cossettes, temperature 95 °C; 3 – Test No.3, fine cossettes, temperature 95 °C

3. The extraction diagrams of one-column extraction

pumped by the high accuracy dosing-pump PROMINENT (Germany) into the most exhausted column. The course of extraction is continuously monitored by automatic refractometer ABBEMAT (Germany) equipped with through-flow cell. The final extract is periodically sampled after the least exhausted column and further the juice after 1st column (i.e. the most exhausted c.) is measured. Experiments were carried out under following conditions:

- temperature: 60–75 °C,
- flow of extraction water: 20 ml/min,
- batch (charge) of 1 column: 250 g of cossettes + 250 g of water or juice,
- cossettes: the fine (45 m/100 g) and the thicker cossettes (15 m/100 g),
- extraction period (frequency of column replacement): 15–30 min,
- total extraction time: 45–90 min,
- juice production (extract): 60–240% cossettes.

By four-column countercurrent extractor we carried out 20 extraction tests under various conditions. Three characteristic courses of the process are depicted in Figs. 4–6. Besides dry substance content of the final extract the dry



temperature: 75 °C; flow of extraction water: 20 ml/min; cossettes: 45 m/100 g; charge of 1 column: 250 g of cossettes + 250 g of water; extraction period: 25 min

4. The diagrams of continual fourcolumn countercurrent extraction

matter of juice after 1st column (i.e. the most exhausted column) is given too. This last value indicates the losses of inulin in extracted cossettes and also efficiency of the process.

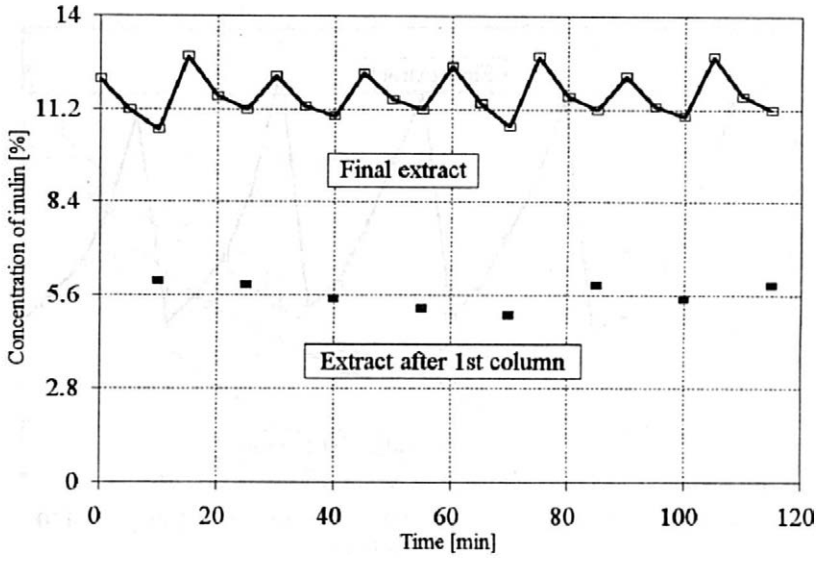
With aim to design an industrial device, we can recommended the following parameters:

- temperature: 75 °C,
- extraction time: 75 min
- batch of column: 50% of cossettes + 50% of produced juice,
- cossettes: 45 m/100 g of cossettes.

Example of results (Fig. 6):

a. Chicory root cossettes – raw materials
- content of inulin: 18.6%

b. Produced juice – final extract
- production: 60% (related to cossettes)
- dry substance: 20.2% (refractometric)
- content of inulin: 16.3%
- purity: 82%



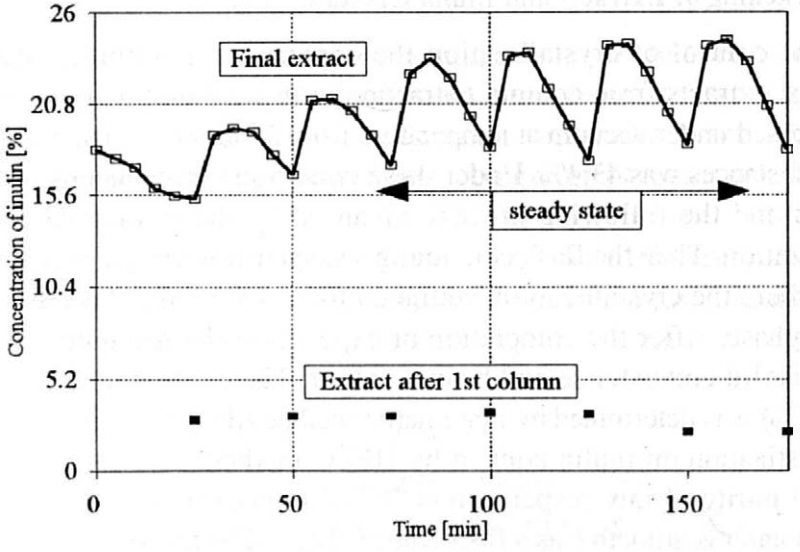
temperature: 75 °C; flow of extraction water: 20 ml/min; cossettes: 45 m/100 g; charge of 1 column: 250 g of cossettes + 250 g of water ; extraction period: 15 min

5. The diagrams of continual fourcolumn countercurrent extraction

c. Exhausted cossettes
   – residue of inulin: 0.5%
d. Juice after 1st column (i.e. the most exhausted c.)
   – dry substance: 2.2% (refractometric)
   – content of inulin: 0.9%
   – purity: 41%

The Refining and Filtration of Extracts

The purity of inulin extracts obtained from chicory could be treated in conformity with users' quality requirements. The simplest process is basic mechanical filtration with a possible addition of subsidiary filtration agents and decolorizers. Another way with an absence of chemical reagents offers the use of microfiltration and the method of cross-flow filtration. The applications of these technologies make possible to remove of fine dispersed particles. At last a chemical refining process is suitable to use all kinds of agents which are known in analogical technologies (sugar industry).



temperature: 75 °C; flow of extraction water: 20 ml/min; cossettes: 45 m/100 g; charge of 1 column: 250 g of cossettes + 250 g of juice; extraction period: 25 min

6. The diagrams of continual fourcolumn countercurrent extraction

The refining tests are currently taking place in our laboratory and their general results will be published in our next report after the conclusion of verified experiments. The refining tests are performed on various portions from column extraction. These successive substances were applied in the meantime experimentally: NH_4CO_3, CaO, $Ca_3(PO_4)_2$, $Ca(OH)_2$, MgO, $H_3(PO)_4$, selected organic acids and carbon adsorbent were tested for purpose of decoloration, adsorption and bitter compounds removal.

The partial refining effect was achieved through the use of phosphoric acid, lime milk and further organic acids (oxalic and acetic). For the removal of dispersed insoluble particles a device of French producer T.I.A. was employed successfully. The unit operates on the principle of crossflow filtration through ceramic membranes MEMBRALOX (France) with filtration area of 2,4 m^2 and pore-diameters of 50 and 100 μm.

The tests of standard pressure filtration (simulation of filter press, 400 kPa) demonstrated a requirement of filtration, barrier and necessity of filter aid agent addition. In this case we recommend to carry out another control filtration, particularly when the filtrate would be utilized directly for crystallization.

The Thickening of Extracts and Inulin Crystallization

For the control of crystallization the experimental solubility data were used. The extracts from column extraction were combined and without refining thickened under vacuum at temperature from 55 to 60 °C. The final content of dry substances was 43.4%. Under these conditions spontaneous nucleation occurred and the following process advanced by the isothermal evaporate crystallization. Then the flask containing a suspension was put into a Deward vessel where the crystallization continued for a period of 2 days through the cooling phase. After the completion of experiment the resultant suspension had semisolid consistence and brown colour. The content of dry substance ($S = 47.2\%$) was determined by gravimetric method (drying with sand at 60 °C). The investigation on inulin content by HPLC method gave result of 32.8%. The final purity of raw suspension is 70% (inulin content in dry substance) and this matter is attractive as a fiber source at cereal technology.

Our next research activity will be devoted to feasible possibilities of refining this product.

THE CONCLUSION AND DESIGN OF TECHNOLOGICAL DIAGRAM

The objective of recommended technological procedures are these two basic products:

a. The preparation of inulin in varied purity grades.

b. The preparation of fructo-oligosaccharides and fructose.

The Design of a Scheme for Isolation Technique Separating Inulin and Fructo-oligosaccharides (The Simplified Design of Production Scheme)

A. The cleaning of chicory roots

In view of the fact that chicory and sugar-beet have the analogous physical and mechanical properties we can recommend to adopt the fundamental processes and machinery from sugar technology.

B. The Slicing of Root

The application of standard equipment of sugar works is also possible here. Chicory offers better mechanical properties in comparison with sugar beet and that is why we can prefer a finer size of cossettes, reduction of the extraction time and the increase of extractor performance.

C. The Extraction

For the extraction of chicory various methods are available: batch semi-continual method or method of continual countercurrent extraction. The laboratory tests displayed adequate efficiency, high yield ability and so low losses by application of the four-column countercurrent extraction.

D. The Refining of Extract

Our experience up to now has not shown adequate efficiency of applied chemical agents (they will be further studied). Till now, mechanical filtration and separations in the following option seem to us to be more advantageous:

1st variant:

The thickening of extract without refining.

The crystallization: isothermal evaporating and cooling.

The separation of product: centrifuging and pressure filtration.

The drying of product.

2nd variant:

The microfiltration on ceramic membranes.

The separation of inulin on chromatographic column, the thickening, spray drying or crystallization.

3rd variant:

The enzymatic or acid hydrolysis of purified juice to fructo-oligosaccharides, possibly to fructose.

The division of hydrolysate on chromatographic column, isolation of oligosaccharide mixtures.

The processing of the isolated fraction: filtration with carbon adsorbent (a reduction of bitter compounds), thickening, spray drying or crystallization.

E. The Thickening

For our work the evaporator ARMFIELD (GB) with a climbing film and an isothermal vacuum evaporator were used. Both devices could be successfully applied for this purpose as well as the adequate equipment which was produced for sugar technology.

F. The Cooling Crystallization

With regard to a slow crystallization rate of inulin and consequently to a long course of this operation the automatization of the process is very neces-

sary. For computer controlled cooling crystallization an apparatus developed within the framework of the grant project at VŠCHT (Bubník et al., 1996) with use of simulation models of Bubník and Kadlec (1996) were used.

G. The Spray Drying

The spray drying could be applied to concentrated solution and also to microcrystal suspension arising by cooling crystallization or through a milling of filter cake. All hereby mentioned procedures were studied on a laboratory spray drier ARMFIELD (GB) by help of which optimal conditions of the process were projected.

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Received December 9, 1996

Potrav. Vědy, *15*, 1997 (1) : 49–67

Izolace inulinu z čekanky

Práce se zabývá metodami pro izolaci, čištění a výrobu inulinu a jeho derivátů z čekanky. Úvodní studie doplňuje pomocí vlastních experimentů základní fyzikální a chemické vlastnosti technických a čistých roztoků inulinu a extraktů z čekanky a dále i potřebná kinetická data pro řešení tepelných, krystalizačních, extrakčních a filtračních procesů. Jsou aplikovány moderní postupy, např. membránová filtrace, filmové odpařování, sprejové sušení a počítačem řízená chladicí krystalizace. Pro stanovení obsahu inulinu, jiných sacharidů a dalších látek obsažených v čekankových extraktech jsou vyvinuty analytické metody s aplikací HPLC a izotachoforézy. V závěru práce je uveden variantní návrh schémat technologických postupů na izolaci inulinu a frukto-oligosacharidů.

čekanka; inulin; frukto-oligosacharidy

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Potrav. Vědy, *15*, 1997 (1) : 69–79

CLASSIFICATION OF FOODS IN THE CZECH REPUBLIC*

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Abstract: The most important systems of food classification (Standard Classification of Products, classification systems used in the Customs Tariff, in the act on consumer taxes, in the act on value added tax, in the Czech Food Codex, in the Czech Food Composition Tables, the system used in the Statistical Year Book of the Czech Republic, the classification used in Household Budget Surveys and the system of food classification used in the Informative Catalogue) used in the Czech Republic were described and discussed. The systems of food classification were evaluated, most of all, from the point of view of nutrition and some improvements were proposed.

food, classification, system, nutrition

Food products represent a very large and varied group of products in the developed industrial countries. The classification, coding and description is necessary for good orientation and work with this group. The way of classifying and coding depends, of course, on the purpose of use. Special criteria of classification are used for the purpose of customs, other ones are used for classification foods listed in food composition tables. The main groups of foods would be the same for all purposes of classification and specification, fulfilment, and more detailed data from the aspect of special use would be added only in some parts.

The unifying of national systems is necesary due to growing international business as well. The unification in the international frame is more complicated. The optimal international system of classification is looked for. The working group on Food Description, Nomenclature and Terminology (Chairman: Dr. Jayne Ireland-Ripert, France) is interested in this field (Programme COST 99). The Czech Republic is participating in this project as well.

* The project within the frame of the programme COST 99, supported by the Ministry of Education, Youth and Sporting Activities, Czech Republic.

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Classification of Food in the Czech Republic (Survey and Discussion)

Systems of food classification in the Czech Republic are gradually changing at present. The Soviet systems and our own historical systems were used until quite recently. These systems have been gradually substituted by systems of the European Union now.

Several different systems of food classification of more importance and greater number of further systems of minor importance are used in the Czech Republic at present. These systems differ in various aspects, most of all in the number of food groups and subgroups, in the order of groups, in grouping individual foods, in coding etc. Main systems of food classification will be described and discussed.

Standard Classification of Product

The most important system of classification is that used by the Czech Statistical Office – Standard Classification of Product (SCP). (Standardní klasifikace produkce, 1996). This system came into force on the 1st January 1994 within the framework of harmonisation of the classification system with international standards. It is based on the European standard CPA (Classification of Product by Activities), which is obligatory for European Union countries. By its content it represents continuation of classification of product by OUN (CPC), international customs tariff (HS/CN) and the former Czechoslovak classification of product.

The classification CPA has been practically taken over, specification, fulfilment and more detailed data from the aspect of national requirements have been made only in some parts. Several modifications of terminology have been also introduced.

The decimal classification was used in the SCP. The classification of SCP has five levels. Two digits codes have been used for the first level of this system. Further classification proceeds decimally by individual steps and thus numeric codes on the fifth level have six digits. The classification is composed of 60 groups. Foods are included in the groups presented in Table I.

Group 15 consists of 9 main subgroups (Table II). The example of classification to fifth level is presented in Table III. Every product can be included only in one classification place.

I. Groups of SCP including foods

01	Products of agricultural origin and forestry
05	Fish and other aquatic products
15	Food products, beverages, delicacy

Since 1994 the Czech Statistical Office has been extending the SCP by adding the seventh level (eight digits code). This innovated SCP ought be available to users still during this year.

II. List of main subgroups of group 15

15.1	Slaughter products (fresh, chilled or frozen meat, etc.)	15.6	Mill and starch products
15.2	Fish prepared and fish products including canned fish	15.7	Feedstuffs
15.3	Fruits and vegetables, prepared and canned	15.8	Other food products
15.4	Oils and fats,animal and vegetable	15.9	Beverages
15.5	Milk products and ice		

SCP is obligatory for statistical data obtained after Act No. 278/199 Sb. on government statistics and in the cases determined by the special act. SCP has been used for the purpose of an international comparison. According to the evaluation of foreign statistical experts the Czech Statistical Office has already reached the highest harmonisation of statistical classification from among the countries performing the transformation to market economy (Drápal, 1996).

This system does not meet all requirements, however, when the nutritional value of consumed food is evaluated. E.g. milks and sweet creams with fat content of 6% w/w are included in one subgroup. It is necessary, from nutrition point of view, to classify this products to several subgroups /lower fat milk (0–2%), whole milk (3.5%) and creams (above 3.5%)/. The subgroup

III. Classification of milk products to fifth level

15	Food products, beverages, delicacy	15.51.11	Milks and sweet creams processed, fat content below 6% (w/w)
15.5	Milk products and ice		
15.51	Milk products	15.51.12	Sweet creams, processed, fat content above 6 % (w/w), non concentrated, without ingredients
15.51.1	Milks and sweet creams, processed		

15.43.10 Margarine and other vegetable fats does not meet requirements for nutritional evaluation either. This subgroup contains high fat products (100%) and low fat products (40%) as well. It is possible to find further shortcomings.

The classification system of Customs Tariff

Further important classification system is used by the Customs Tariff (Celní sazebník, 1994). It applies complex nomenclature of the customs tariff of European Union. This nomenclature has its origin in a harmonised system of description and coding of commodities (International Treaty, Brussels, 14. 6. 1983 and Memorandum on Amendment, Brussels 29. 6. 1986) using four digits number and six digits items and supplementing it by double digits numeric (eight digits items). The foods are included in chapters 2–4, 7–12 and 15–22 (Table IV).

A more detailed classification for milk products is presented in Table V.

This classification system is more convenient for nutrition evaluation than the system used in SCP.

Some products in this system can be classified, according to specific rules, into two or more classification groups. This fact is more convenient as well.

The classification system of Tax Act

The classification system applied Consumer Tax Act and in Value Added Tax Act (Sbírka zákonů, 1992).

The classification of foods in the Czech Food Codex

The Czech Food Codex (Potravní kniha, 1995) that is presently in the stage of elaboration, will use another classification system, where 41 main food groups are proposed, and where some of them do not correspond to the groups used in both classification systems described before. The proposed system is shown in Table VI.

This classification system is rather unusual from the point of view of grouping individual foods and from the point of view of the order of groups as well. The titles of some food groups are not quite clear in many cases so that it is difficult to predict, which foods are listed there, e.g. groups "Cereal

IV. Classification of foods in the Customs Tariff

Chapter	
Class I – Living animals, animals products	
2	Meat and edible offals
3	Fish and crustaceans, molluscs and other water invertebrates
4	Milk and milk products, bird eggs, natural honey, edible animal products, not mentioned at other place
Class II – Plant products	
7	Vegetables, edible plants, roots and tubers
8	Edible fruits and nuts, peels of citrus fruits and melons
9	Coffee, tea, maté and spices
10	Cereals
11	Milling products, malt, starches, inulin, wheat gluten
12	Oilseeds and oil fruits, various seeds and fruits, industrial and drug plants, straw and fodder plants
Class III – Animal and vegetable fats and oils, products of their splitting, shortenings, animal or plant waxes	
16	Products from meat, fish or crustaceans, molluscs and other aquatic invertebrates
17	Sugar and sweets
18	Cocoa and cocoa products
19	Products from cereals, flour, starch or milk, pastry
20	Products from vegetables, fruits, nuts or other parts of plants
21	Various food products
22	Beverages, alcohol liquids and vinegar

products for direct consumption and milling products from them", "Cocoa and dry mixtures", "Oilseeds for direct consumption and products from them" (edible fats and oils are also products of oilseeds but another group is named "Edible fats and oils") etc. The final version should be harmonised with the classification system of SCP. Further possibility is to accept the unified system for European Food Composition Tables after developing them accordingly.

V. More detailed classification of milk products

0401	Milk and cream, non-condensed, without added sugar or other sweeteners
0401 10	– with fat content below 1%
0401 1010	– in packing for direct use with net volume below 2 litre
0401 20	– with fat content above 1%, but below 6%
	– – below 3%

Classification system used in Food Composition Tables

Another system of classification is used in Food Composition Tables (Potravinové tabulky, 1992, 1993). This system consists of only 12 main groups (Table VII) of foods divided into several subgroups. The items in the subgroups are not coded, but the alphabetical order is used.

This system is sufficient for these tables due to the low number of items they contain. The tables should be innovated as the number of food products is too low and some products are not on the present Czech market any more.

System of the Statistical Year Book of the Czech Republic

The Statistical Year Book of the Czech Republic consists of 33 groups of foods, they are neither coded nor ordered alphabetically (Statistická ročenka, 1996). Table VIII shows this system.

The classification system used in statistics of Household Budget Surveys

The classification used in Household Budget Surveys in the Czech Republic (Statistika rodinných účtů, 1995) is more detailed (Table IX), but for better evaluation of the nutritional value of food consumed, it would be useful to classify individual groups in more detail. The main reason is, in fact, that the content of nutrients differs within some groups very much. E.g. the group "Vegetable and other fats" includes products containing very different amounts of fat (low fat margarines – 40% and hydrogenated fats – 100%), the group "Pulses" contains soybeans containing 22% of lipids and 42% of proteins and other pulses with about 2% of lipids and about 25% of proteins.

It is planned to accept the PROCOME (COICOP-HBS) Food Coding System classification (PROCOME, 1996) for the Household Budget Surveys in

VI. The classification system proposed for the Czech Food Codex

1.	Meat and meat products	22.	Sugar
2.	Poultry and poultry products	23.	Honey
3.	Game	24.	Non-chocolate candy
4.	Fish and sea animals	25.	Cocoa and dry mixtures
5.	Eggs and egg products	26.	Chocolate and chocolate candy
6.	Milk and milk products	27.	Tea, herb and fruit tea
7.	Icecreams and ice	28.	Coffee and coffee substitutes
8.	Edible fats and oils	29.	Packed water including mineral water
9.	Fresh fruits and vegetables	30.	Non-alcoholic beverages
10.	Prepared fruits	31.	Fruit, herb and malt wines
11.	Prepared vegetables	32.	Beer
12.	Dry shell fruits (nuts)	33.	Alcohol and distilled liquors
13.	Mushrooms	34.	Vinegar
14.	Potatoes, potato products	35.	Mustard
15.	Cereal products and milling products	36.	Yeasts
16.	Pulses and pulse products	37.	Spices and extracts
17.	Oilseeds and oilseed products	38.	Salt
18.	Pasta	39.	Dry soups and sauces, flavourings
19.	Bakery products	40.	Dressings and cold sauces
20.	Confectionery products	41.	Sterilised ready meals
21.	Starch and starch products		

the near future. This PROCOME system has to be modified in certain details with respect to national differences. The project COST 99 will participate in this modification.

VII. The main groups of foods in Food Composition Tables

1.	Meat, meat products, poultry, game	7.	Potatoes, mushrooms, pulses
2.	Fish, fish products	8.	Vegetables, vegetable products
3.	Milk and milk products	9.	Fruits, fruit products
4.	Eggs	10.	Mill and bakery products
5.	Fats and nuts	11.	Other foods, delicacy
6.	Sugar, candy, confectionery products	12.	Beverages

VIII. The system of the Statistical Year Book of the Czech Republic

1.	Meat total	16.	Pasta
	including: Pork	17.	Rye flour
	Beef	18.	Bread
	Veal	19.	Rice
2.	Poultry	20.	Sugar
3.	Fish, total	21.	Chocolate sweets and chocolate
4.	Fats and oils	22.	Chocolate-free sweets
5.	Lard and bacon	23.	Potatoes
6.	Butter	24.	Pulses
7.	Vegetable and edible fats and oils	25.	Vegetables
8.	Milk and milk products	26.	Fruits including: Citrus fruits
9.	Market milk	27.	Coffee (roasted)
10.	Cheese	28.	Tea
11.	Curd and cottage cheese	29.	Alcoholic beverages
12.	Eggs	30.	Spirits (40%)
13.	Cereal products	31.	Beer
14.	Wheat flour	32	Wine
15.	Rolls and pastry	33.	Non-alcoholic beverages including: Mineral water

The Informative Catalogue classification

In the Informative Catalogue products and services offered by companies are listed. Two digits codes have been used for the first level (for foods the code 20 is used). This system may be suitable for all requirements of business activities, but for food industry or nutrition point of view it has many shortcomings, and therefore it is not presented here.

The other systems of food classification

The other systems of food classification of minor importance exist as well, e.g. in the act on advertisement, in various catalogues from exhibition and fairs, in various textbooks on food commodity science, etc. A special system of classification was developed for the evaluation of the human dietary exposure of various contaminants in the Czech Republic (Ruprich, 1993, 1996).

IX. Classification of Household Budget Surveys

1. Nutrition
Food
Meat and meat products
Pork
Beef
Other meats and offals
Smoked meat products
Canned meat, other meat products
Poultry and poultry products
Fish and fish products
Fresh fish
Frozen fish
Other fish and fish products
Fats and oils
Butter
Lard and bacon
Oils
Vegetable and other fats
Eggs, milk, cheese
Eggs
Egg product
Fresh milk (liquid)
Canned and dry milk
Cheese
Other milk products
Bread, bakery products, cereal products, rice
Bread
Wheat bakery products
Pastry (flour confectionery products)
Biscuits
Wheat flour
Pasta
Other cereal products
Rice
Potatoes, vegetables and products
Potatoes
Potato products
Fresh vegetables
Frozen vegetables
Vegetable products
Pulses
Fruits and fruit product
Fresh fruits from mild climate region
Citrus fruits and other subtropical and tropical fruits
Jams
Frozen and canned products
Dry fruits (nuts, raisins, coconut meal, dates, figs, etc.)
Sugar, sweets products
Sugar
Chocolate and chocolate products
Non-chocolate sweets
Confectionery products
Cocoa, coffe, tea, other food
Cocoa
Coffee
Tea
Dry soups and sauces
Other food and delicacy
Soft drinks
Concentrated soft drinks
Soft drinks
Catering
Lunch and dinner – meal services for employees
Lunch and dinner – school meal services
Lunch and dinner – restaurant
Other expenditures in food service
2. Alcoholic drinks, tabacco
Alcoholic drinks
Beer
Wine
Spirits and other alcoholic drinks

Conclusions

Several different systems of food classification and coding are used in the Czech Republic at present. It will be desirable to unify these systems in main points and to harmonise them with the international systems. It is very important to accept the proper food classification system for the nutritional evaluation of our population nutrition state. The PROCOME (COICOP-HBS) system, modified after DAFNE with certain additional modification with respect to the national specifities, is the most convenient from the systems discussed above.

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Recieved December 4, 1996

Potrav. Vědy, *15*, 1997 (1) : 69–79

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potraviny; třídění; výživa

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