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Potr. Vědy, *11*, 1993 (6) : 441-536

The Faculty is an important member of the integrated University of Chemical Technology in Prague, and should remain as such. For further development, it is necessary to emphasize the responsability of the Faculty for both the educational and scientific programmes, including public and foreign relations. On the other hand, the co-operation with other faculties and institutes of the University would be desirable for the fulfilment of various complex activities.

Similarly as in other European countries, the curricula are presented for students on three levels: B.Sc., M.Sc. and Ph.D. The M.Sc. (in our country, Ing. - Engineer) is preferred in the Czech Republic. The study takes at least five years (and often more), and is explained in more detailed form in the paper: "Teaching process at the Faculty of Food and Biochemical Technology, and its optimization" by Doc. Čepička in the same copy of this journal. In addition to these programmes, one to two years courses are offered to graduate students of other faculties or from other universities, advanced courses (e.g. summer schools), courses for employed graduates (with the preference of shorter courses or distance education), the Third Age University, and other programmes are included.

Our aim is to create a flexible educational system, attractive to students, and related to the educational systems of other European universities in the field of Food Science and Technology. Such a system would enable increased mobility of students and teachers, mutual recognition of degrees (nostrification), etc. The particular curriculum could be modified every year to adapt to the development in EU and in other countries. In our country, the requirements of our food industry, research institutes, institutions and management companies for our graduates should be observed and complied with. The possibility of employment of our graduates is an important factor as well.

In addition to several Food Technology and Engineering Programmes, a few Food Science and other sciences (Biochemistry, Organic Chemistry, Microbiology) programmes already exist. New science programmes will be offered to our students, such as logistics, informatics, etc., which are important in a modern industralized society. Other programmes of interest will be Food Marketing, Food Management, Bioengineering, and Applied Biology. Special courses in Machinery and Economics are under considerat ion.

The education should not consist of the accumulation of information and data, but it should be based on the creative work of students with the maximum application of computer techniques. Traditional lectures should be mainly replaced by seminars, workshops and laboratory courses. Some programmes will be taught in English for foreign students, mostly from developing countries.

In the research work, our Faculty will continue the traditional topics, where there is good research experience. New trends will be followed with respect to research capacities and instrumental equipment available. Our research priorities are close to

those of EU countries, i.e. healthy human nutrition, food quality assessment, environmental aspects, and biotechnology. The increasing effectiveness of the dissertations (prolonging the Ph.D. study by one year is considered) and the continuation of the M.Sc. thesis as a Ph.D. thesis will positively influence the research results. The work on international research projects is organized, at least partially, in the form of visits of foreign scientists in Prague and our scientists in the partner country.

Until now, the research was mainly funded by the Government, but other sources are looked for. Domestic and international research grants become more and more important, but they are difficult to obtain for individual scientists. Therefore, research teams are formed with other faculties and colleges and with research institutes. In such a way, expensive instruments and specialized scientists are better utilized.

The Faculty was relatively successful in receiving grants for research projects. Among 18 grant holders and coordinators, eight obtained a grant from the Grant Agency of the Czech Republic, nine grants were received from the internal grant agency of the Ministry of Education, and another grant came from the Ministry of Environment Protection. Several COST, FLAIR and 2 TEMPUS projects were received.

Until now, the Faculty has not enough space, but if some more laboratories become available, they would be converted into specialized commercial laboratories for the production of biochemical preparations, testing of additives (metabolic studies, cancerogenic and mutagenic tests, etc.), for the production of monoclonal antibodies, sophisticated analytical methods, including microbiological analysis.

Foreign relations of our Faculty are an important part of both teaching and research activities. The priority area is the integration of the Faculty into the net of European universities and research institutes, and the participation on diverse EU projects. Building of an efficient computer network contributed to it efficiently. Personal contacts are of primary importance in these activities, and several foreign teachers and scientists participated in the education at all levels, mainly in the graduate studies. Two foreign teachers were recently appointed Professors at our Faculty. External teachers from Institutes of the Czech Academy of Sciences and from the Czech industrial companies participate in the education as well.

The coordination of public relations will be facilitated by the foundation of an Association of representatives of the Faculty, Food Industry, industrial companies and research institutes. This Association will form a consulting body of the Faculty for both teaching and research.

Prof. Pavel Kadlec, D.Sc.
Dean-Elect of the Faculty

Prof. Jan Pokorný, D.Sc.
Dean of the Faculty

FACULTY OF FOOD AND BIOCHEMICAL TECHNOLOGY

Potrav. Vědy, *11*, 1993 (6) : 443-444

TEACHING PROCESS AT THE FACULTY OF FOOD AND BIOCHEMICAL TECHNOLOGY AND ITS OPTIMALIZATION

The Faculty of Food and Biochemical Technology has - owing to the number of students - become the largest faculty of the University of Chemical Technology within the last few years. It is nowadays the largest chemical and food engineering university in the Czech Republic and also the oldest one on the Continent.

Concerning its engineering education the faculty issues from the nearly 200-years lasting traditions of teaching chemical engineering and from a somewhat shorter tradition of teaching the courses, dealing with food and biochemical processes. The significant changes in industry as well as in social life during the last four years and mainly deficiencies of the former education system under the Socialist Government have led to the necessity of improving the teaching process at the Faculty. An increasing effort to confirm the education in accordance with European standard has required a new arrangement and enlargement of the list of different types of education, an optimalization of the internal contents of study, and an introduction of special lines of study within study fields. Since the school year of 1994/1995 seven fields and nineteen special lines of study are organized by the Faculty.

***Engineering studies** take five years and the curriculum - in a similar way as at the other faculties of the University - includes fundamental chemical, mathematical and biological studies as well.*

Towards the end of the third year of studies our students are obliged to sit for the comprehensive examination. The following last two years of studies comprise the student's special line, the aim of which is to explain principles of special technological and engineering processes. The studies are completed by the state examination and by the defense of the degree thesis. The graduates receive the academic degree "Engineer" (Ing.) corresponding to that of "Master of Science" (M.Sc.).

Study field: Fermentation Chemistry and Bioengineering
- *Brewing and Malting Technology*
- *Fermentation Chemistry and Technology*
- *Applied Biology and Bioengineering*

Study field: Chemistry and Technology of Saccharides
- *Sugar, Starch, Chocolate and Confectioneries*
- *Cereals, Breads, Snack Products, and Fodders*

Study field: Milk and Fat Technology
- *Milk and Fat Technology*
- *Detergent Technology*

Study field: Food Preservation and Meat Technology
- *Food Preservation*
- *Meat Technology*
- *Packaging Technology*

Study field: Food Chemistry and Analysis
- *Chemistry and Food Analysis (Quality Assessement)*
- *Ecology of Food Chain*
- *Nutrition and Catering Technology*
- *Chemistry of Natural Substances*

Study field: Basic and Applied Biochemistry
- *Biochemistry*
- *Enzyme Engineering*
- *Biomedical Engineering*

***Bachelor studies** take three years and are organized parallelly to engineering studies. We are preparing specialists for the production field of food industry, for similarly oriented fields, for inspection and control institutions, trade and state municipalities. After passing differential examinations it is possible to change bachelor studies to engineering studies. Bachelor studies are completed by passing a state examination with defense of school-leaving thesis and graduates receive the academic title "Bachelor" (Bc.). They are organized in one study field and two special lines.*

Study field: Food Technology and Food Quality Control
- *Food Technology and Management Studies*
- *Food Quality Control*

***Studies according to individual study plan** enables employed undergraduates to complete bachelor or engineering studies. The individual study plans are approved by the Dean of the Faculty.*

***Postgraduate studies** are meant for the graduates of engineering studies and are internally organized providing scholarships (take usually three years) and in the case of external postgraduate (usually five years) without granting scholarship. Postgraduate studies are completed by passing the state examination with defense of dissertation Thesis and awarding the academic-scientific title of "Doctor" (Dr.), which is equivalent to*

Ph.D. Postgraduate studies at our Faculty are organized in the following study fields:
- *Biochemistry*
- *Biotechnology*
- *Microbiology*
- *Chemistry and Food Analysis*
- *Food Technology*
- *Organic Chemistry of Natural Substances*

Address your application for all types of studies to Dean's office (Faculty of Food and Biochemical Technology, Technická 5, 166 28 Praha 6 - Dejvice). Further details are available at Dean's office as well.

Doc. Ing. Jaroslav Čepička, CSc.

Potrav. Vědy, *11*, 1993 (6) : 445-453

HPLC DETERMINATION OF ALLIIN AND ITS TRANSFORMATION PRODUCTS IN GARLIC AND GARLIC-CONTAINING PHYTOMEDICAL PREPARATIONS

Jan VELÍŠEK, Rudolf H. de VOS [1], Antony SCHOUTEN [1]

Institute of Chemical Technology – Department of Food Chemistry and Analysis, Technická 5, CZ-166 28 Praha 6, Czech Republic;
[1]TNO Institute for Biotechnology and Chemistry, Utrechtseweg 48, 3700 AJ, Zeist, The Netherlands

An HPLC method is proposed for the determination of alliin in garlic and garlic preparations. The method involves simple homogenization of the sample followed by centrifugation and pre-column derivatization with o-phthaldialdehyde/tert. butylthiol. The chromatographic system with either ultraviolet or fluorometric detector consists of a separation on a Spherisorb 5 ODS-2 column and either gradient or isocratic elution with phosphate buffer/methanol mixture as a mobile phase. Similar procedure using the ultraviolet detector, the same column and isocratic elution with the same mobile phase is proposed for the simultaneous determination of allicin and its transformation products such as ajoenes, vinyldithiins, sulfides and oligosulfides. The methods have been applied with success to garlic and various garlic preparations such as garlic essential oil, dragées and capsules containing garlic and garlic constituents and have enabled rapid, sensitive and reproducible separation and determination of a full range of chemical constituents in the samples analyzed. The method would become an efficient tool for raw material control and for quality control of food and phytomedical preparations containing garlic.

garlic; alliin; allicin; ajoenes; vinyldithiins; diallyl disulfide; diallyl trisulfide; HPLC

Allium vegetables have served as vegetables, condiments and medical plants since ancient times. Their chemical composition is of interest in phytochemistry, plant-insect relationships, chemotaxonomy, flavour industry, quality control of food and phytochemical preparations, pharmacognosy and medicine.

Garlic (*Allium sativum L.*) has a prominent position among *Allium* vegetables. Studies into the physiological and therapeutic properties of garlic (and other members of the genus *Allium*) revealed the positive effects of dietary garlic on atherosclerosis and coronary (ischemic) heart disease (blood coagulation parameters, platelet aggregation, fibrinolytic activity, blood and tissue lipids) and blood sugar level. Antimicrobial (antibacterial and fungicidal) properties of garlic are

well documented. Several chemical constituents of garlic are known to possess anticarcinogenic properties by either preventing the formation of carcinogens from precursor substances, inhibiting carcinogenesis as blocking agents or suppressing the expression of neoplasia (F e n w i c k, H a n l e y, 1985; C a r a g a y, 1992).

A number of data exist on the basic chemical composition of garlic, the principal reaction mechanisms of formation and degradation of its physiologically active constituents. The most important chemical constituent of garlic bulbs is the sulfur amino acid alliin, (+)-S-(2-propenyl)-L-cysteine sulfoxide. Methyl and propyl analogues have been identified as the minor constituents. A certain proportion of alliin probably occurs in the bound form (as a peptide). Under the influence of C-S lyases (alliinase, EC 4.4.1.4), alliin gives rise to di(2-propenyl)thiosulfinate (allicin). Spontaneous decomposition of the latter compound leads to the formation of a variety of aliphatic and heterocyclic sulfur-containing compounds. Many of these compounds posses lower relative toxicities (retaining their antimutagenic and antitumorigenic properties) than their precursors (e.g. allicin) (F e n w i c k, H a n l e y, 1984).

Several instrumental (chromatographic) methods have been introduced for the determination of alliin, allicin and its transformation products.

GLC employing non-polar silicones as stationary phases and FID or specific detectors is a valuable method in the study of separation and quantitative evaluation of volatile, thermally stable garlic constituents, such as those found in distilled garlic essential oils. Diallyl disulfide, a common component of garlic distilled oils, however rearranges at elevated temperatures to thiophenes (S i n h a et al., 1992) and allicin decomposes to several products, such as ajoenes, vinyldithiins and sulfides (B l o c k et al., 1986).

HPLC methods allow the separation and quantitative determination of alliin as well as of unstable allicin and its transformation products. These methods also have their limitations, especially in incomplete separation of some garlic constituents, low detector response of some minor components and identifying the separated compounds by means of mass spectrometry. Ion pairing HPLC and reversed phase HPLC methods using spectrophotometric, fluorometric or electrochemical detector allow separation and quantitative determination of alliin (M o c h i z u k i et al., 1988; Z i e g l e r, S t i c k e r, 1989; I b e r l et al., 1990b; A u g e r et al., 1993) which is chromatographed on octadecyl bound phases as such or after pre-column derivatization with fluorescamine, o-phthaldialdehyde or phenylisothiocyanate reagents. Octadecyl bound phases and an ultraviolet or a diode-array detector can be used for the direct determination of allicin, its analogues and transformation products (J a n s e n et al., 1987; I b e r l et al., 1990a,b; L a w s o n et al., 1991; L a w s o n, H u g h e s, 1992; B l o c k et al., 1992).

The increasing interest in garlic and its biologically active constituents brought about the need for a simple and rapid method to evaluate fresh garlic as well as various garlic products and garlic-containing preparations. Therefore aim of the present work was to simplify the already described HPLC methods employed for

the determination of alliin, allicin and its transformation products and, with the use of only one chromatographic column, simple binary solvent system and one detector, to employ this method generally for the analysis of fresh garlic, garlic products and preparations.

MATERIAL and METHODS

Material: Fresh garlic was obtained from the local market. Garlic oil (De naturwinkel Erika, Utrecht, NL) and samples of dragées and capsules (Consumentenbond, Den Haag, NL) were commercial products.

Chemicals, Reagents and Solutions: Alliin was synthesized according to the procedure described by Stoll and Seebeck (1951), allicin, ajoenes and vinyldithiins were prepared according to Iberl et al. (1990a). Phthaldialdehyde was obtained from Boom, Meppel, NL, 2-methyl-2-propanthiol was obtained from Merck, Darmstadt, D, o-(carboxymethyl)hydroxylamine hemihydrochloride and norleucine were obtained from Fluka, Buchs, CH, diallyldisulfide and allylmethylsulfide were obtained from Janssen Chimica, Geel, B, diallylsulfide was obtained from Sigma, St. Louis, MO, USA and diisobutyldisulfide was obtained from K & K.

Derivatization (OPA) reagent: 140 mg of o-phthaldialdehyde were dissolved in 5 ml of methanol and 0.1 ml of 2-methyl-2-propanthiol and 50 ml of phosphate buffer (pH 9.5) were added.

o-(Carboxymethyl)hydroxylamine (OCMHA) solution: 0.11 g in 100 ml of water (solution 1), or 0.22 g in 100 ml of water (solution 2).

Norleucine (internal standard) solution: 0.5 g in 100 ml of water.

Diisobutyldisulfide (internal standard) solution: 0.01 mg in 100 ml of acetonitrile.

Buffers: 7.8 g of sodium dihydrogen phosphate were dissolved in 1000 ml of water and the resulting solution was adjusted with 40% (w/w) sodium hydroxide to either pH 9.5 or 6.5. Buffers of pH 6.5 were diluted with water (1 part of buffer, 9 parts of water) and used as a mobile phase for HPLC.

Instrumentation: The HPLC system was a Waters (Milford, MA, USA) chromatograph consisting of a 6000A solvent delivery system, system controller 600E, autosampler 712Wisp, 470 scanning fluorescence detector with excitation at 230 nm and emission at 420 nm. For UV detection, Bruker variable wavelength monitor operated at a wavelength of 337 nm or 220 nm was used. The chromatographic column was a Chrompack (Middelburg, NL), 250 mm x 1/4 inch (o.d.), 4.6 mm (i.d.) stainless column with Spherisorb 5 ODS-2, 5 µm, thermostatted at 30 °C and joined to a Waters guard-pak precolumn containing Resolve C18 inserts. The mobile phase used for the separation of alliin consisted of A, phosphate buffer (pH 6.5), and B, methanol. The gradient program used is listed

in Table I. The flow rate was 0.8 ml/min. The mobile phase used for the separation of allicin and its transformation products was the same phosphate buffer (pH 6.5)/methanol (20 : 80, v/v), at a flow rate of 0.8 ml/min.

I. Gradient program for HPLC analysis of alliin

Eluent	Time in min					
	0	10	20	25	35	45
Solvent A [%]	90	50	10	10	90	90
Solvent B [%]	10	50	90	90	10	10

Procedures: Fresh garlic cloves were peeled and 2-2.5 g were placed in a 100 ml beaker containing 20 ml of OCMHA (solution 1) and 2 ml of norleucine solution. The sample was cut to small particles, homogenized (using Ultra-Turrax) and the resulting suspension was centrifuged. Aliquots of 0.1 ml were derivatized with 0.9 ml of OPA reagent and after 30 min of standing at room temperature analyzed by HPLC.

For the analysis of dragées and capsules, about 2 to 6 g of these preparations were weighed to a 100 ml beaker and the sample was further processed as the fresh garlic samples.

Allicin arising by the action of alliinase in aqueous garlic homogenates was determined in 5 ml aliquots. These aliquots were mixed with OCMHA solution (solution 2) and directly analyzed by HPLC.

Capsules containing edible oil extracts of garlic constituents were cut and about 0.2 g of oil was weighed to a centrifugation vial, 1 ml of diisobutyl disulfide (internal standard) solution was added, the vial content shaked and centrifuged. The separated upper acetonitrile layer was analyzed by HPLC.

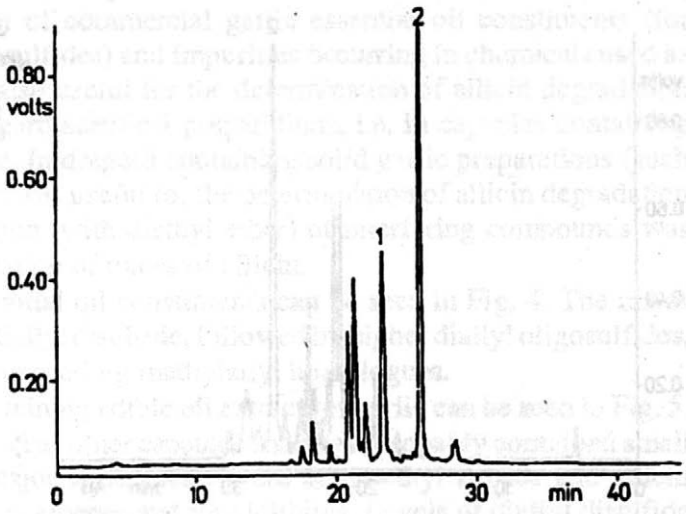
Capsules containing solid preparations were opened and about 0.5 g of their content was weighed to a 50 ml beaker. Dragées were first crushed to a powder and about 0.5 g of this powder was weighed to a 50 ml beaker. After the addition of 1 ml of OCMHA solution (solution 1) and 1 ml of diisobutyl disulfide solution (internal standard), the suspension was mixed, after 10 min of standing centrifuged and the supernatant analyzed by HPLC.

RESULTS and DISCUSSION

The known quantities of synthesized alliin, allicin and its transformation products enabled both qualitative and quantitative evaluation of the characteristic compounds of fresh garlic as well as of garlic preparations.

1. Determination of alliin of fresh garlic cloves (fluorometric detector)

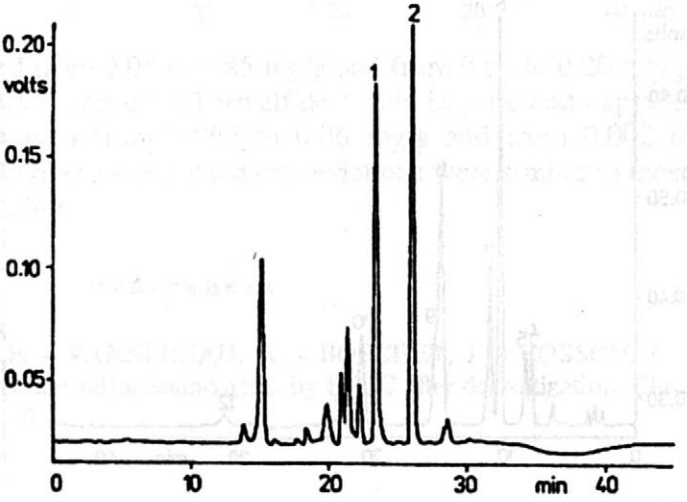
1 = alliin, 2 = norleucine (internal standard)

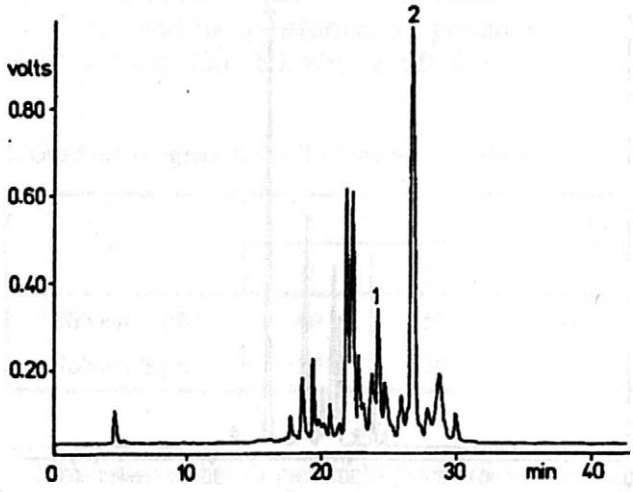


The reaction of alliin with OPA reagent, leading to the formation of the corresponding isoindole derivative, enabled very sensitive determination by either fluorometric detector (Fig. 1) or spectrophotometric detector at 337 nm (Fig. 2). The resulting reaction product was sufficiently stable (at least up to 12 hours of storage at room temperature). Gradient elution gave very good separation of alliin from its analogues and other primary amines, but isocratic elution could also be employed. As it can be seen (Figs. 1 and 2), the fluorometric detector is about three times more sensitive than the spectrophotometric detector. In both cases, the limits of alliin determination were low enough and the method was sufficiently sensitive for analysis of fresh garlic, which contained alliin at the level of about 6 mg/g. The recovery ranged from 97 to 102 % with a coefficient of variation of 0.8 %. The

2. Determination of alliin in fresh garlic cloves (spectrophotometric detector)

1 = alliin, 2 = norleucine (internal standard)





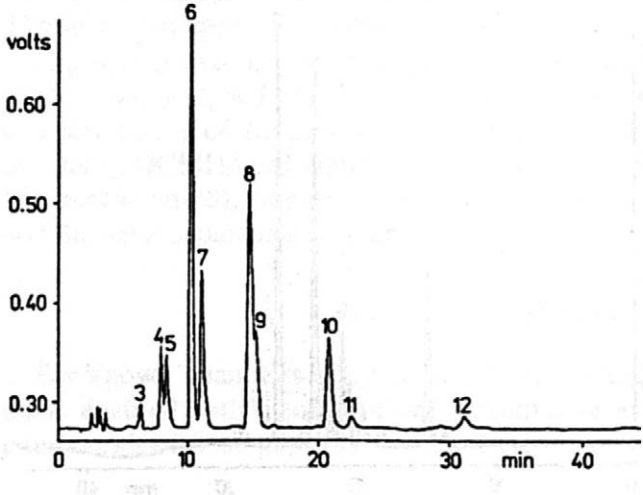
3. Determination of alliin in dragées (fluorometric detector)

1 = alliin, 2 = norleucine (internal standard)

method even allowed the determination of residual alliin in aqueous garlic homogenates stored for 30 min at room temperature. The alliin content of this homogenate was 0.01 mg/g (coefficient of variation 6.1 %). Pharmaceutical garlic preparations contained alliin at a level up to 3 mg/g. An example of alliin determination in dragées containing 0.68 mg of alliin per 100 g (1080 μg per dragée) is documented in Fig. 3.

Alliin is readily converted to allicin by alliinase during crushing fresh garlic samples or dissolving in water pharmaceutical preparations possessing alliinase activity. Thus, the OEMHA solution was used to inactivate the enzyme. Alternatively, methanol possessing the same inhibitory effect was used instead of the OEMHA solution.

The used HPLC method was also useful for the separation of allicin in aqueous garlic homogenate, purified allicin isolated from aqueous garlic homogenates by



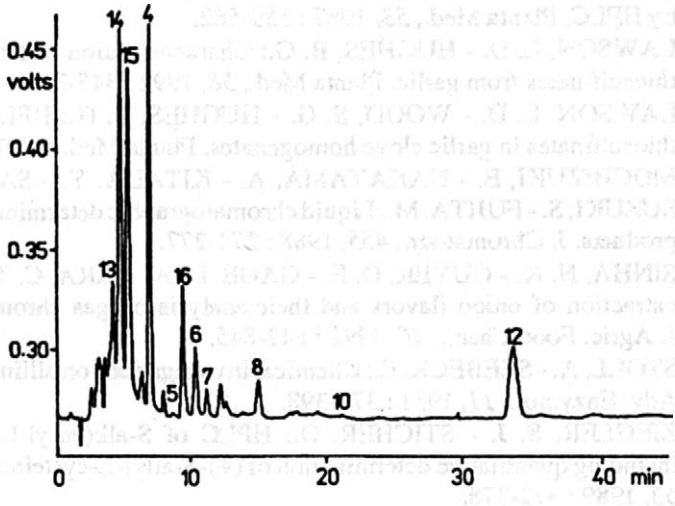
4. Determination of garlic essential oil constituents (spectrophotometric detector)

3 = allylmethyl sulfide, 4 = allylmethyl disulfide, 5 = diallyl sulfide, 6 = diallyl disulfide, 7 = allylmethyl trisulfide, 8 = diallyl trisulfide, 9 = allylmethyl tetrasulfide, 10 = diallyl tetrasulfide, 11 = allylmethlyl pentasulfide, 12 = diisobutyl disulfide (internal standard)

extraction with diethyl ether and synthesized allicin. The same method was also suitable for the separation of commercial garlic essential oil constituents (for example sulfides and oligosulfides) and impurities occurring in chemicals used as internal standards. It was also useful for the determination of allicin degradation products in commercial pharmaceutical preparations, i.e. in capsules containing edible oil extracts of garlic. In dragées containing solid garlic preparations (such as dried garlic), the method was useful for the determination of allicin degradation products. Previous extraction (with diethyl ether) of interfering compounds was necessary for the determination of traces of allicin.

Separation of garlic essential oil constituents can be seen in Fig. 4. The major component of this oil was diallyl disulfide, followed by higher diallyl oligosulfides, diallyl sulfide and the corresponding methylallyl homologues.

Analysis of capsules containing edible oil extracts of garlic can be seen in Fig. 5. The analyzed sample, as well as other capsules analyzed, probably contained small amounts of allicin. The major constituents were allylmethyl sulfide and allicin decomposition products, i.e. ajoenes and vinyldithiins. Levels of diallyl disulfide



5. Determination of Knofluvit capsule constituents (spectrophotometric detector)

5 = diallyl sulfide, 6 = diallyl disulfide, 7 = allyl-methyl trisulfide, 8 = diallyl trisulfide, 10 = diallyl tetrasulfide, 12 = diisobutyl disulfide (internal standard), 13 = alliin, 14 = ajoene, 15 = 2-vinyl-[4H]-1,2-di-thiin, 16 = 3-vinyl-[4H]-1,2-dithiin

and diallyl trisulfide ranged from 0.06 to 0.85 mg/g and from 0.06 to 0.20 mg/g, respectively. Diallyl disulfide and diallyl trisulfide levels in pills and capsules containing dried garlic ranged from 0.002 to 0.06 mg/g and from 0.002 to 0.31 mg/g, respectively. Recoveries and standard deviations were similar to those observed when analyzing alliin.

References

AUGER, J. - MELLOUKI, F. - VANNEREAU, A. - BOSCHER, J. - COSSON, L. - MANDON, N.: Analysis of *Allium* sulfur amino acids by HPLC after derivatization. Chromatographia, *36*, 1993 : 347-350.

BLOCK, E. - AHMAD, S. - CATALFAMO, J. - JAIN, M. K. - APITZ-CASTRO, R.: Antithrombotic organosulfur compounds from garlic: structural, mechanistic and synthetic studies. J. Amer. Chem. Soc., *108*, 1986 : 7045-7055.

BLOCK, E. - NAGANATHAN, S. - PUTMAN, D. - ZHAO, S.-H.: Allium chemistry: HPLC analysis of thiosulfinates from onion, garlic, wild garlic (ramsoms), leek, scallion, shallot, elephant (great-headed) garlic, chive, and chinese chive. Uniquely high allyl to methyl ratios in some garlic samples. J. Agric. Food Chem., *40*, 1992 : 2418-2430.

FENWICK, G. R. - HANLEY, A. B.: The genus *Allium* - part 2. CRC Crit. Rev. Food Sci. Nutr., *22*, 1984 : 273-377.

FENWICK, G. R. - HANLEY, A. B.: The genus *Allium* - part 3. CRC Crit. Rev. Food Sci., Nutr. *23*, 1985 : 1-73.

CARAGAY, A. B.: Cancer-preventive foods and ingredients. Food Technol., *46*, 1992 : 65-68.

IBERL, B. - WINKLER, G. - KNOBLOCH, K.: Products of alliin transformation: ajoenes and dithiins, characterization and their determination by HPLC. Planta Med., *56*, 1990a : 202-211.

IBERL, B. - WINKLER, G. - MÜLLER, B. - KNOBLOCH, K.: Quantitative determination of allicin and alliin from garlic by HPLC. Planta Med., *56*, 1990b : 320-326.

JANSEN, H. - MÜLLER, B. - KNOBLOCH, K.: Allicin characterization and its determination by HPLC. Planta Med., *53*, 1987 : 559-562.

LAWSON, L. D. - HUGHES, B. G.: Characterization of the formation of allicin and other thiosulfinates from garlic. Planta Med., *58*, 1992 : 345-350.

LAWSON, L. D. - WOOD, S. G. - HUGHES, B. G.: HPLC analysis of allicin and other thiosulfinates in garlic clove homogenates. Planta Med., *57*, 1991 : 263-270.

MOCHIZUKI, E. - NAKAYAMA, A. - KITADA, Y. - SAITO, K. - NAKAZAWA, H. - SUZUKI, S. - FUJITA, M.: Liquid chromatographic determination of alliin in garlic and garlic products. J. Chromatogr., *455*, 1988 : 271-277.

SINHA, N. K. - GUYER, D. E. - GAGE, D. A. - LIRA, C. T.: Supercritical carbon dioxide extraction of onion flavors and their analysis by gas chromatography-mass spectrometry. J. Agric. Food Chem., *40*, 1992 : 842-845.

STOLL, A. - SEEBECK, E.: Chemical investigations on alliin, the specific principle of garlic. Adv. Enzymol., *11*, 1951 : 377-398.

ZIEGLER, S. J. - STICHER, O.: HPLC of S-alk(en)yl-L-cysteine derivatives in garlic including quantitative determination of (+)-S-allyl-L-cysteine sulfoxide (alliin). Planta Med., *55*, 1989 : 372-378.

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J. Velíšek, R.H. de Vos, A. Schouten (Vysoká škola chemickotechnologická – Ústav chemie a analýzy potravin, Praha; Biotechnologický a chemický ústav TNO, Zeist)

Stanovení alliinu a produktů jeho transformace metodou HPLC v česneku a přípravcích obsahujících česnek

Byla vypracována HPLC metoda stanovení alliinu v česneku a přípravcích obsahujících česnek. Metoda zahrnuje homogenizaci vzorku, odstředění a derivatizaci alliinu reakcí s o-ftalaldialdehydem v přítomnosti terc. butylthiolu. Chromatografický

systém je tvořen buď spektrofotometrickým, nebo fluorimetrickým detektorem a kolonou s reverzní stacionární fází Spherisorb 5 ODS-2. Gradientová nebo isokratická eluce se provádí směsí fosfátového pufru o pH 6,5 s methanolem. Podobný postup za použití spektrofotometrického detektoru, téže kolony i stacionární fáze (isokratická eluce) byl vypracován pro simultánní analýzu allicinu a produktů jeho transformace jako jsou ajoeny, vinyldithiiny, různé sulfidy a oligosulfidy. Metoda byla použita pro analýzu uvedených látek v čerstvém česneku a v různých přípravcích obsahujících česnek nebo chemické složky česneku, jako jsou česnekový esenciální olej, dražé a kapsule obsahující sušený česnek nebo česnekové extrakty rostlinnými oleji. Metoda umožňuje rychlé, citlivé a reprodukovatelné stanovení uvedených sloučenin a zdá se, že by mohla nalézt uplatnění jak v kontrole surovin, tak i v kontrole potravin a přípravků obsahujících česnek.

česnek; alliin; allicin; ajoeny; vinyldithiiny; diallyldisulfid; diallyltrisulfid; HPLC

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Potrav. Vědy, *11*, 1993 (6) : 455-460

CUCUMBER CELL CULTURE AS A SOURCE OF ASCORBATE OXIDASE

Blanka KRÁLOVÁ, Veronika ŠEBKOVÁ, Tomáš MACEK [1], Pavla STRNADOVÁ, Tomáš VANĚK[1]

Institute of Chemical Technology – Department of Biochemistry and Microbiology, Technická 5, CZ-166 28 Praha 6;
[1]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo n. 2, CZ-166 10 Praha 6, Czech Republic

Ascorbate oxidase production was followed in callus culture of cucumber in dark and daylight. The enzyme content showed a maximum at the beginning of the stationary phase of growth, earlier and with higher activity in dark. The accumulation of the enzyme in callus and in normal fruit was compared. The substrate specificity was tested using L-ascorbate, pyrogallol, cysteine, phenylenediamine and other compounds. Only the first three of them were accepted as substrate by the enzyme studied.

ascorbate oxidase; callus culture; determination of L-ascorbate

L-ascorbate (vitamin C) is one of the essential factors for men, thus the determination of its content in raw materials and in food is a great task of food analysis. At present, L-ascorbate is often estimated by use of ascorbate oxidase (AO) (Fresenius, 1979). This enzymic method enables to determine L-ascorbate without its previous separation. The method is quick and simple. Ascorbate oxidase (L-ascorbate:oxygen oxidoreductase, EC 1.10.3.3) belongs to the class of "blue oxidases" with at least four copper atoms in the molecule. It catalyzes the oxidation of L-ascorbate to L-dehydroascorbate (Beránková, Kovandová, 1985; Messerschmidt, Huber, 1991)

$$2\ \text{L-ascorbate} + O_2 \xrightarrow{AO} 2\ \text{L-dehydroascorbate} + 2\ H_2O$$

Ascorbate oxidase is not absolutely specific (Dayan, Dawson, 1976). Besides L-ascorbate, it usually oxidizes also o-diphenols, L-Dopa phenol, cysteine, phenylene diamine and other compounds, fulfilling the structural requirements for AO substrate, but with much lower efficiency than in the case of L-ascorbate (Stark, Dawson, 1963; Casella et al., 1985; Volk, Lansen, 1963). AO from different sources may differ in substrate specificity. Only AO, which prefers L-ascorbate as a substrate and the specificity of which is rather narrow, is convenient for analytical purposes. Methods that use AO for L-ascorbate estimation are usually based on two principles. The first determines a decrease

in the dissolved O_2 during the course of enzymic reaction (usually using oxygen electrode) (M a r c h e s i n i et al., 1974; M a t s u m o t o, O s a j i m a, 1981). The second method measures the products of reduction caused by L-ascorbate (e.g. $Fe^{3+} \longrightarrow Fe^{2+}$). Both methods compare the results with blanks where L-ascorbate was previously oxidized by AO (S k a l t s a et al., 1987). AO is usually isolated from plant material, mainly from fruits and vegetables. The enzyme is present in these materials at relatively high amounts. The use of natural sources for the isolation of AO has a lot of disadvantages, to which seasonal harvest, dependence on the weather during vegetation and high expenses belong. Therefore we were looking for the AO activity in plant cell cultures, available during the whole year (M a c e k et al., 1991). As cucumber (*Cucumis sativa*) is most often the material of choice for AO isolation, we have chosen cucumber cell culture as a producer of the desired enzyme for our experiments.

MATERIAL and METHODS

The cucumber callus culture, strain OK5, derived in 1986 from sterile seedlings, was cultivated in 100ml Erlenmeyer flasks covered with aluminium foil on solid medium according to M u r a s h i n g and S k o o g (1962) containing per 1 litre: inositol 0.1 g, sucrose 30 g, plant growth regulators (2,4-dichlorophenoxyacetic acid 10^{-6} mol/l and kinetine 10^{-6} mol/l), agar DIFCO 8 g, pH was adjusted to 5.7 and the medium sterilized (0.12 MPa, 120 °C) for 25 minutes. Two to three weeks old cucumber callus culture was used As inoculum. After inoculation the flasks were placed in conditions required for each specific experiment (light, darkness), at 26-28 °C.

For extraction, the callus was removed from solid medium, 2 ml of sodium phosphate buffer (0.1 mol/l) pH 7.0 were added to 1 g of fresh biomass and the cells were disintegrated by grinding. The homogenized mixture was then centrifuged and supernatant taken as the enzyme extract. Activity of AO was measured at 30 °C from the original rate of oxygen uptake in the course of L-ascorbate oxidation. The oxygen uptake was measured using an oxygen electrode in reaction mixture containing sodium phosphate buffer pH 7.0, 0.1 mol/l (4 ml) and the sample (0.2 ml). The reaction was started with the addition of 0.05 ml of L-ascorbate (39 mg) and EDTA (30 mg) in 1 ml of the above given buffer. The oxygen uptake was recorded automatically.

The protein content was estimated photometrically and calculated from the following equation:

$$(1.45 \times A_{280}) - (0.74 \times A_{260}) = \text{mg proteins per 1 ml of sample}$$

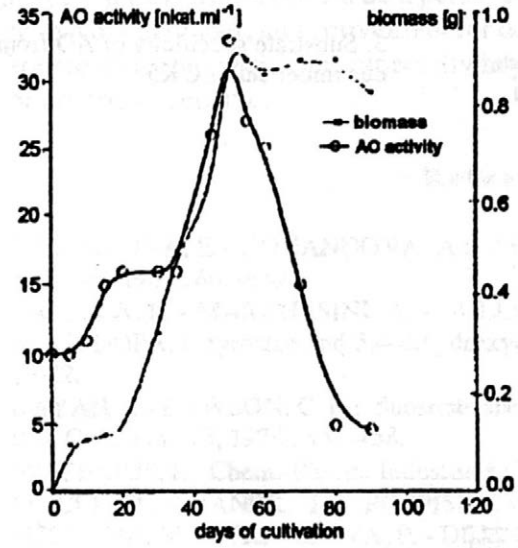
where: A_{280} and A_{260} are absorbancies at 280 nm and 260 nm, respectively

Dry matter of cells was estimated after drying the cells (100 °C) to constant weight.

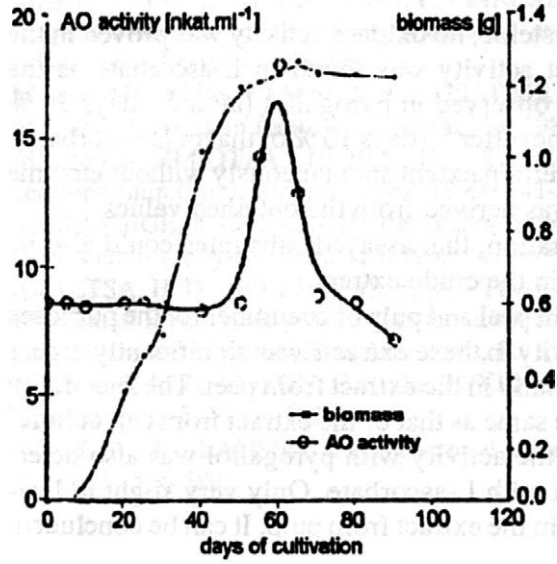
Fresh cucumbers were bought in the local market for measuring the AO activity of cucumber pulp and peel.

RESULTS and DISCUSSION

To find the optimum conditions for ascorbate oxidase production, we cultivated cucumber cell culture strain OK5 in different light conditions. The production of AO under the given conditions in dependence on the time of cultivation is given in Fig. 1 (in darkness) and Fig. 2 (in daylight).



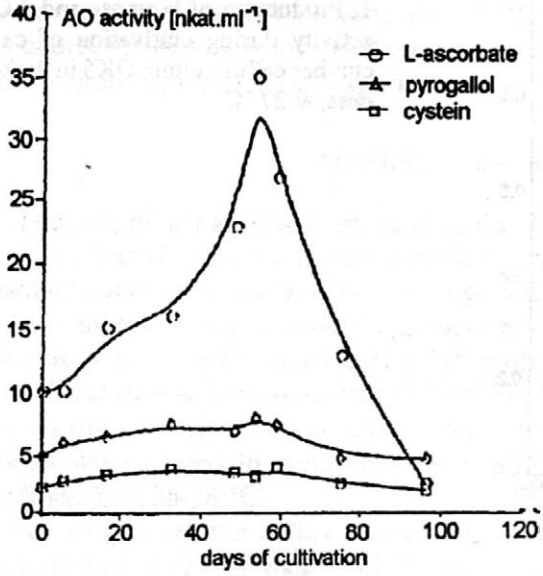
1. Production of biomass and AO activity during cultivation of cucumber callus culture OK5 in darkness, at 27 °C



2. Production of biomass and AO activity during cultivation of cucumber callus culture OK5 in daylight, at 27 °C

The results indicated that the cultivation of cucumber cell culture in darkness was more effective than in light regarding the AO production. The maximum of AO activity was achieved after 50 days of cultivation, in light 10 days later, though the growth of biomass was faster in light. In both cases the production of AO started after the growth of biomass had ceased. Shortly after reaching the maximum, the activity of AO started to decrease; the decrease was steeper in darkness.

In raw extracts from cucumber cell culture cultivated in darkness the substrate specificity of AO was measured using L-sorbitol, hydroquinone, galactose, cysteine, pyrogallol, p-phenylene diamine and L-ascorbate as substrates (Fig. 3).



3. Substrate specificity of AO from cucumber callus OK5

Except L-ascorbate, pyrogallol and cysteine, no oxidase activity was proved in the tested substrates. Though the highest activity was found in L-ascorbate as the substrate, the activity found was also observed in pyrogallol (after 50 days 26 % of that of L-ascorbate) and with cysteine (after 50 days 10 % of that of L-ascorbate). As pyrogallol could be oxidized to a certain extent spontaneously without enzyme presence, the non-enzymic reaction was derived from the obtained values.

It is necessary to take into consideration, that assayed substrates could also be oxidized with other enzymes present in the crude extract.

The AO activity was also measured in peel and pulp of cucumber for the purposes of comparison (Table I). The AO activity in these extracts was significantly higher than in extract from cell culture, especially in the extract from peel. The specificity of the extract from peel was nearly the same as that of the extract from cell culture: beside the activity with L-ascorbate, the activity with pyrogallol was also determined, which was about 30 % of that with L-ascorbate. Only very slight utilization of p-phenylendiamine was found in the extract from pulp. It can be concluded,

I. Activity of AO, content of protein and dry matter in cucumber (*Cucumis sativa*) fruit

Sample	AO activity			Protein of mg.ml^{-1} raw extract	Dry matter in % of biomass
	nkat.ml^{-1} of raw extract	nkat.mg^{-1} of protein	nkat.g^{-1} of dry matter		
Peel	1262.1	296.8	20046.1	4.2	6.3
Pulp	174.4	96.9	2419.9	1.8	7.2

that even cell cultures could be a perspective source of AO with specificity similar to that of cucumber, and convenient for determination of L-ascorbate. This enzyme source could be significant especially in periods when cucumbers are not available or are too expensive.

References

BERÁNKOVÁ, E.- KOVANDOVÁ, A.: Askorbátoxidasa a její analytické využití. Chem. Listy, *79*, 1985 : 601-612.

CASELLA, L. - MARCHESINI, A. - GULLOTI, M.: The interaction of ascorbate oxidase with L-DOPA, L-tyrosine and 3,4-dihydroxycinnamic acid. Inorg. Chim. Acta, *107*, 1985 : 19-22.

DAYAN, J. - DAWSON, C. R.: Substrate specifity of ascorbate oxidase. Biochem. Biophys. Res. Commun., *73*, 1976 : 451-458.

FRESENIUS, E.: Chem.-Pharm. Industrie KG, Bad Hamburg, FRG Patent 2824694, 1979.

MACEK, T. - VANĚK, T. - POSPÍŠILOVÁ R. - KRÁLOVÁ, B. - ŠEBKOVÁ, V. - ŠŮCHOVÁ, M. - STRNADOVÁ, P. - DEMNEROVÁ, K : Plant cells cultured *in vitro* as an alternative source of some oxidase. Biol. Chem. Hoppe-Seyler's, *372*, 1991 : 711.

MARCHESINI, A. - MONTUORI, F. - MAESTRI, E.: Application and advantages of the enzymatic method for the assay of ascorbic and dehydroaschorbic acids and reductones. J. Food Sci., *39*, 1974 : 568-571.

MATSUMOTO K.- YAMADA, K. - OSAJIMA, Y.: Ascorbate electrode for determination of L-ascorbic acid in food. Anal. Chem., *53*, 1981 : 1974-1979.

MESSERSCHMIDT, A.- HUBER, R. : The blue oxidases, ascorbate oxidase, laccse and ceruloplasmin.Eur. J. Biochem., *187*, 1990 : 341-352.

MURASHIGE, T. - SKOOG, F.: Revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant., *15*, 1962 : 473-497.

SKALTSA, H. D. - PHILIANOS, S. M. - TZAKON, O. A. - KOUPPARIS, M. A.: Specific differential spectrophotometric determination of ascorbic determination of ascorbic acid in plants using ascorbate oxidase. Anal. Lett., *20*, 1987 : 1679-1691.

STARK, G. R. - DAWSON, C. R.: In: BOYER, P. D. - LARDY, H. - MYRBACK, K. (Eds.): The Enzymes. 8. 1963 : 207.

VOLK, W. A. - LANSEN, J. L.: Bacterial ascorbic acid oxidase. Biochem. Biophys. Acta, *67*, 1963 : 576-580.

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Potrav. Vědy, *11*, 1993 (6) : 455-460

B. Králová, V. Šebková, T. Macek, P. Strnadová, T. Vaněk (Vysoká škola chemicko-technologická, Praha; Ústav organické chemie a biochemie AV ČR, Praha)

Buněčné kultury okurky jako zdroj askorbátoxidasy

Stanovení L-askorbátu, jednoho z esenciálních faktorů lidské výživy, v surovinách a výrobcích potravinářského průmyslu je důležitým úkolem potravinářské analytiky. Jeho stanovení v původním materiálu bez předchozí separace je možné pomocí enzymu askorbátoxidasy (AO). AO je přítomna v řadě rostlin, nejčastěji se však získává z oplodí okurky (*Cucumis sativa*). Vzhledem k tomu, že rostlinné materiály, zejména okurka, jsou suroviny velmi drahé a cenné i v lidské výživě, a kromě toho jsou dosažitelné jen v omezeném vegetačním období, zabývali jsme se vyhledáním alternativního zdroje tohoto enzymu, kde by omezení nepřipadala v úvahu. Studovaným zdrojem byla kalusová buněčná kultura okurky, kultivovaná na tuhé půdě.

Produkce AO v kalusové kultuře okurky byla sledována po dobu 120 dní, a to ve tmě i na světle. Maximální produkce enzymu byla zaznamenána na počátku stacionární fáze růstu kultury, na světle asi po 60 dnech kultivace, ve tmě po 50 dnech kultivace. V době maximální produkce byla aktivita AO u buněk kultivovaných ve tmě téměř dvojnásobkem aktivity získané na světle. Enzymový extrakt získaný z kalusových kultur pěstovaných ve tmě prokazoval specifitu k L-askorbátu, ale reagoval také v malé míře s pyrogallolem (asi 26 % aktivity s askorbátem) a cysteinem (10 %). Enzymový extrakt z plodu okurky byl několikanásobně aktivnější než kalusový extrakt, avšak specifita obou enzymů byla obdobná – kromě L-askorbátu byla zjištěna schopnost utilizovat pyrogallol (asi 30 % aktivity s askorbátem) a ve zcela nepatrné míře i p-fenylendiamin.

askorbát oxidasa; buněčné kultury; stanovení L-askorbátu

Potrav. Vědy, *11*, 1993 (6) : 461-469

A CONTRIBUTION TO THE STUDY OF RIPENING OF SOFT SMEAR CHEESES

Milada PLOCKOVÁ, Jana CHUMCHALOVÁ, Šárka PAVLÍKOVÁ

Institute of Chemical Technology – Department of Milk and Fat Technology, Technická 5, CS-166 28 Praha 6, Czech Republic

The ripening process of three types of soft smear cheeses manufactured in the processing plant were studied. Three various types of smear cultures were used in curing process: A - commercially used type, B - the mixture of yeasts + the mutant strain of *Brevibacterium linens*, Laktoflora 205, C - the mixture of yeasts + *Brevibacterium linens*, Laktoflora 205 + the mixture of micrococci. The cheeses made using of B and C culture types seemed to possess the better organoleptic and microbiologic properties and their ripening process (especially cheese C) was shorter and more intensive in comparison with the cheese produced using culture A.

Brevibacterium linens; micrococci; mutant strain; organoleptic properties; protein breakdown; ripening; soft smear cheese

Soft cheeses with a smear on the surface are popular due to their short ripening period, relatively easy digestibility and high flavour properties. From the factors affecting the ripening process in smear cheeses are important namely: water content, size of cheese, manner of curing and development of desirable microorganisms on the surface, i.e. microbiological composition of smear (R e p s, 1987).

The most often occurring microorganisms of the cheese smear are the yeasts *Kluyveromyces* sp. and *Candida* sp. and the bacteria *Brevibacterium* sp., *Arthrobacter* sp. and *Micrococcus* sp. Occasionally is present the mould *Geotrichum candidum*.

The first microorganisms which develop on the surface are the yeasts which lower the acidity by lactic acid oxidation. They stimulate the growth of the bacterial population by vitamin production (P u r k o, 1951) and they contribute to the ripening process by proteolytic enzymes (S z u m s k i, 1962).

The most numerous microorganisms in smear are coryneform bacteria from the genus *Brevibacterium* which depending on the composition of culture medium, age and physiologic state, presence of oxygen and light synthesize orange pigment (R e p s, 1987). *Brevibacterium linens* tolerates high NaCl concentrations (10 to 12 %) and can grow when the pH value of surface reaches 5.85. During growth *Brevibacterium linens* synthesizes highly active proteolytic and lipolytic enzymes

(R e p s, 1987). The synthesis of several intracellular and more active extracellular proteolytic enzymes with proteinase (T u c k e y, S a h a s r a b u d h e, 1957) and aminopeptidase (S o r h a u g, 1981; F o i s y, 1973, 1978) activities was published.

The role of micrococci is less significant but not negligible. However, it is not the quantity but the type of micrococci that is of practical significance since not all micrococci influence the organoleptic properties of cheese (R o b e r t s o n, P e r y, 1961). M a r t h (1963) and N a t h, L e d f o r d (1972) proved the proteolytic and lipolytic activity in *Micrococcus* sp. and M o r e n o and K o s i k o w s k i (1973) found the breakdown of β-casein by *Micrococcus freudenreichii*, *M. caseolyticus* and *M. candidus*.

The presence of moulds on the surface of smear cheese seems to be accidental and correct curing does not permit the development of moulds on the cheese surface. The most common mould on the surface of smear cheese is *Geotrichum candidum*. It is capable of growth at 15 - 25 °C, at up to 10% NaCl and at the broad range of acidity (optimum at pH 4) (R e p s, 1987). Besides the lactic acid oxidation it can produce several proteolytic and lipolytic enzymes (P u r k o et al., 1951; C h e n et al., 1972). *Geotrichum candidum* cannot be classified as a component of the microflora of smear cheeses, but it may develop on the surface simultaneously with yeasts and its presence may have an influence on the cheese ripening process.

MATERIAL and METHODS

The ripening process of soft smear cheeses manufactured in a processing plant at Ostrožská Nová Ves using three types of smear cultures was tested.

The composition of cultures:

A. Commercially used smear culture Laktoflora consisted of:

- 2 portions of *Brevibacterium linens*, Laktoflora 980,
- 1 portion of mixture of micrococci (*Micrococcus roseus*, Laktoflora 204, *Micrococcus* sp., Laktoflora 205 at ratio 1 : 1),
- 1 portion of mixture of yeasts (*Candida famata*, Laktoflora 262, *Candida utilis*, Laktoflora 159, *Kluyveromyces marxianus* var. *lactis*, Laktoflora 252 at ratio 1 : 1 : 1).

  Total concentration of c.f.u. $4.5.10^9$ per ml, ratio bacteria : yeasts = 3.2 : 1.

B. Experimental culture:

- 3 portions of *Brevibacterium linens*, Laktoflora 201 (mutant strain with higher proteolytic activity presented by C i k á n e k et al. (1989),
- 1 portion of mixture of yeasts (see culture A).

  Total concentration of c.f.u. $6.3.10^9$ per ml, the ratio bacteria : yeasts = 3.5 : 1.

C. Experimental culture:

– 2 portions of *Brevibacterium linens*, Laktoflora 201,

– 1 portion of mixture of micrococci (see culture A),

– 1 portion of mixture of yeasts (see culture A).

Total concentration of c.f.u. $4.7.10^9$ per ml, the ratio bacteria : yeasts = 3.4 : 1.

The mixture of yeasts and the mixture of micrococci were used from the original packaging of the producer (Milcom, Ke dvoru 12, Praque 6, CZ). *Brevibacterium linens*, Laktoflora 980 and 201 were maintained on the surface of nutrient agar enriched with 1 % biotine and 0,1 % of water mineral solution containing 1 % $FeSO_4.7H_2O$, 0,5 % $Na_2MoO_4.2H_2O$, 0,5% $MnSO_4.4H_2O$, 0,5% $ZnSO_4.7H_2O$, 0,5 % $NiCl_2$, 0,15 % $CuSO_4.5H_2O$, 0,15 % $CoCl_2.6H_2O$, 0,3 % H_3BO_3, 0,1 % chelaton I, pH 6.75 - 7.0. Time of incubation was seven days at 23 °C at the light place. Then the cells were suspended in sterile physiological saline to obtaine the cell concentration 10^9 - 10^{10} c.f.u.

The experimental production trials of soft smear cheeses (weight 60 g, fat in solids 40 %) were conducted in three series in the Ostrožská Nová Ves dairy. Presented results are average values from three trials corresponding to the three experimental productions. During the production and ripening processes The Guideline Technology Procedure for production of soft smear cheeses was respected. The only variable parameters were the smear cultures of A, B or C types used. After the brining procedure, approx. 200 pieces of cheese from each experimental group were cured separately using smear cultures A, B or C. Curing (treating the surface of cheeses by suspension containing 2 % NaCl, 0.1 % cheese colour Bixa Orelana, 0,6 % concentrated suspension of smear culture of type A, B or C) was made three times during the ripening process: 24 h, 48 h and 96 h after production. From each series (A, B, C), five pieces of cheese were sampled in these intervals: 24 h after production before curing, 48 h after production before curing, 72 h after production, 96 h after production before curing, six, seven and ten days after production, wrapped into plastic bags and kept at -20 °C untill they had been analyzed.

The edge part of cheese was defined as an average sample of 3mm edge layers obtained from five cheeses, the central part was the rest after cutting the edge part of five cheeses. During the ripening the pH values (pH meter Radelkis OP-211/1, glass electrode) and changes in proteins of edge and central parts of cheeses were investigated. The free amino acid nitrogen content N_A (R e i t e r et al., 1969), 12 % TCA soluble nitrogen N_{TCA} (D u l l e y, 1976) and pH 4.6 soluble nitrogen N_S (O'K e e f f e et al., 1976). were expressed as relative percentage of total nitrogen content N_T estimated by Kjehldahl method (Kjehltec Auto Plus 1030 Analyzer). After seven and ten days of ripening, microbiologic analysis (total plate count according to ČSN 56 0083, coliforms according to ČSN 56 0085, moulds and yeasts according to ČSN 56 0087) and sensory analysis (ranking with statistic

interpretation according to Friedman test - ISO 8587, 1988) were performed. The significance of the calculated F criterium:

$$F = \frac{12}{JP.(P+1)} . (R_1^2 + R_2^2 + \ldots + R_p^2) - 3J.(P+1)$$

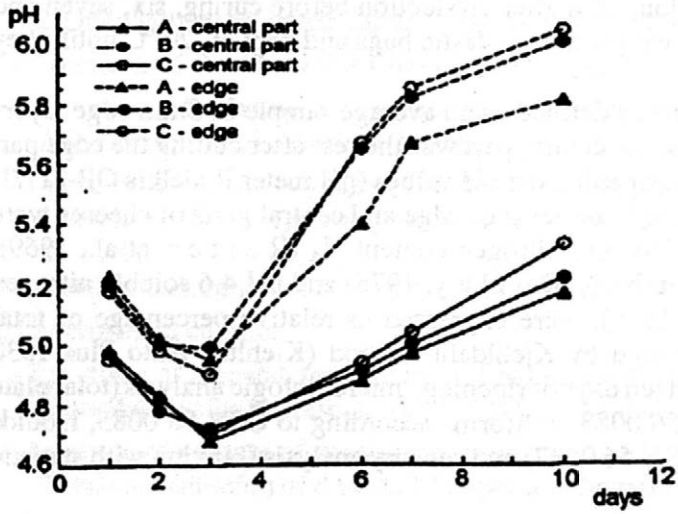
where: J - number of accessors
P - number of samples
$R_1, R_2..R_p$ - ranking sums for particular samples

was determined by comparing it with the tabulated critical value of Friedman test for $\alpha = 0.05$ and $\alpha = 0.01$.

The sensory analysis was carried out under standard conditions (ISO 6658, 1985).

RESULTS and DISCUSSION

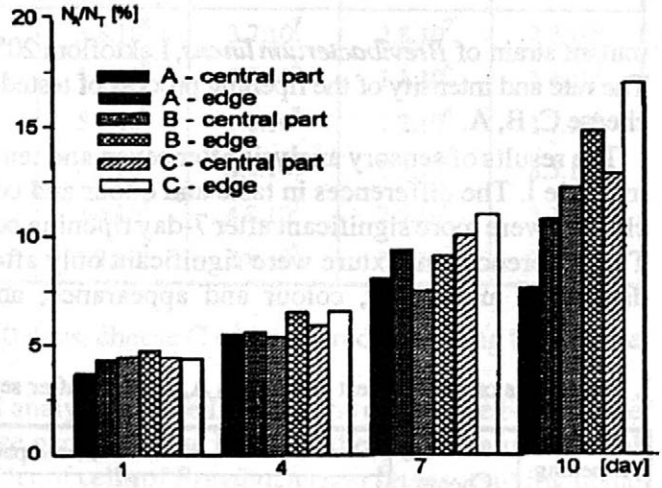
The changes in pH values of the edge and central parts of soft smear cheeses during the production and ripening process are presented in Fig. 1. It can be concluded from the results that pH values increase more quickly especially in the edge part of cheese cured by culture B and C. The pH increased particularly by lactic acid oxidation (produced by mesophilic and thermophilic dairy starters used in the previous technology step) and by diffusion of alkaline products (resulting from protein breakdown) from the surface to the central part of cheese. The rate of pH changes influenced the rate of *Brevibacterium linens* growth, its biochemical activity and the rate of surface orange smear development.



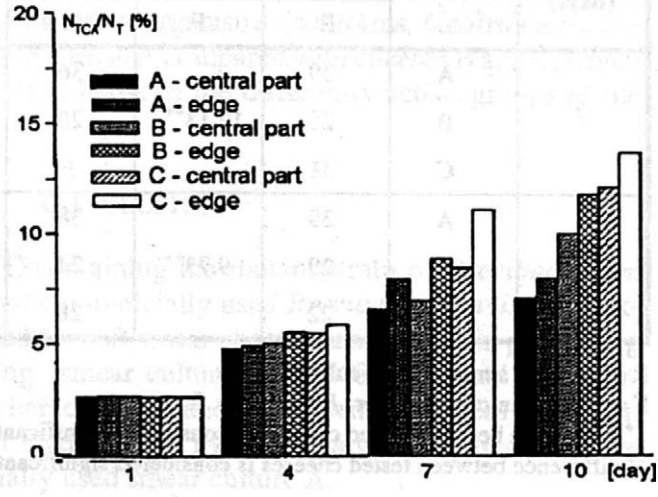
1. Changes in pH at the edge and central parts of cheeses treated with smear culture A, B, and C during ripening

The changes in the concentration of various nitrogen compounds due to protein breakdown are caused mostly by the action of proteolytic enzymes of smear culture. The contribution of native milk proteases, proteases of psychrotrophic microorganisms contaminating the raw milk and proteases of microorganisms recontaminating the cheese after production is probably lesser.

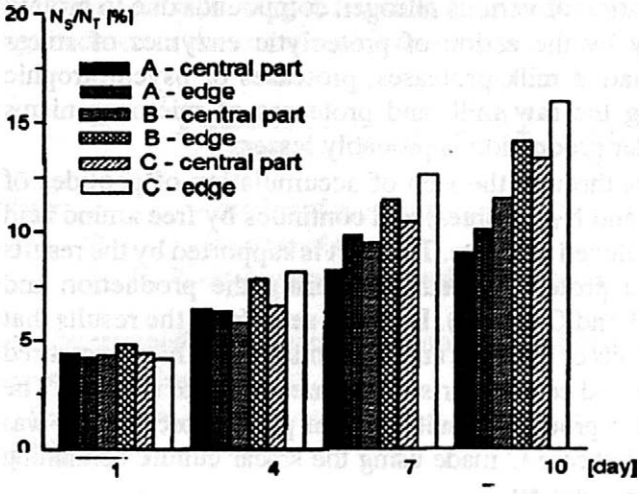
The protein breakdown goes through the step of accumulation of peptides of various MW (expressed as N_S and N_{TCA} values) and continues by free amino acid liberation (N_A) from earlier produced peptides. This fact is supported by the results of experimental testing of the protein degradation during the production and ripening of smear cheeses A, B and C (Fig. 2). It can be seen from the results that the changes in relative proportions of peptides and free amino acids have increased during the ripening at the edge and central parts of all three types of cheeses. The great amount of low molecular products resulting from protein breakdown was produced during the ripening of cheese C made using the smear culture containing



2a. Changes in free amino acid nitrogen (N_A) as a percentage of total nitrogen (N_T) at the edge and central portions of cheeses treated with smear culture A, B, and C during ripening



2b. Changes in nitrogen soluble in 12% TCA (N_{TCA}) as a percentage of total nitrogen (N_T) at the edge and central portions of cheeses treated with smear culture A, B, and C during ripening



2c. Changes in nitrogen soluble at pH 4.6 (N_S) as a percentage of total nitrogen (N_T) at the edge and central portions of cheeses treated with smear culture A, B, and C during ripening

mutant strain of *Brevibacterium linens*, Laktoflora 205 and the mixture of micrococci. The rate and intensity of the ripening process of tested cheeses decreased in the order: cheese C, B, A.

The results of sensory analysis after seven and ten days of ripening are presented in Table I. The differences in taste and odour and colour and appearance of tested cheeses were more significant after 7-day ripening compared with 10-day ripening. The differences in texture were significant only after 7-day ripening. After seven days taste and odour, colour and appearance, and texture of cheese B were

I. The results of ranking test of cheeses A, B and C after seven and ten days of ripening

Ripening period (days)	Cheese type	Tested parameters					
		Taste, odour		Colour, appearance		Texture	
		R	F	R	F	R	F
7	A	39		36		38	
	B	20	12.13^{++}	20	10.13^{++}	23	7.60^{+}
	C	31		34		29	
10	A	39		38		32	
	B	29	9.73^{++}	24	6.93^{+}	30	0.53
	C	22		28		28	

R = ranking sums for particular samples
F = Friedman criterium for $J = 15$, $P = 3$
$^{+}$ difference between tested cheeses is considered significant at a level of significance $\alpha = 0{,}05$
$^{++}$ difference between tested cheeses is considered significant at a level of significance $\alpha = 0{,}01$

II. The results of microbiologic analysis of cheeses A, B and C after seven and ten days of ripening

Ripening period (days)	Cheese type	Cheese part	Total plate count	Moulds yeasts	*Geotrichum candidum*	Coliforms
			(cfu. g^{-1})			
7	A	edge	$1.6.10^9$	$2.8.10^8$	$1.5.10^7$	$5.6.10^6$
	A	centre	$9.4.10^7$	$7.2.10^6$	$4.6.10^6$	$4.6.10^4$
	B	edge	$2.4.10^9$	$7.5.10^7$	$2.8.10^6$	$3.6.10^5$
	B	centre	$4.8.10^8$	$3.4.10^5$	$7.4.10^4$	$6.9.10^4$
	C	edge	$1.8.10^9$	$1.3.10^8$	$1.6.10^6$	$8.5.10^4$
	C	centre	$9.6.10^7$	$2.3.10^5$	$1.1.10^5$	$8.9.10^3$
10	A	edge	$3.8.10^8$	$3.7.10^7$	$2.8.10^7$	$2.8.10^6$
	A	centre	$1.1.10^8$	$1.5.10^6$	$1.1.10^6$	$1.4.10^3$
	B	edge	$5.2.10^8$	$1.8.10^7$	$1.2.10^6$	$1.7.10^5$
	B	centre	$8.5.10^7$	$6.4.10^5$	$3.5.10^4$	$8.5.10^4$
	C	edge	$7.6.10^8$	$4.3.10^7$	$2.5.10^6$	$3.2.10^5$
	C	centre	$1.2.10^8$	$3.8.10^5$	$2.1.10^4$	$2.7.10^4$

appreciated as the best. After 10 days, cheese C was prefered according to the taste and odour.

The results of microbiologal analysis (Table II) show the difference betveen the cheeses A, B and C. At the edge part of cheese B and C, the higher values of total plate count (including the number of cells of *Brevibacterium linens* resulting in the more intensive colour of surface smear) were found compared with cheese A. The number of surface contaminating microorganisms (coliforms, *Geotrichum candidum*) was significantly higher in cheese A compared with cheeses B and C, which confirms the theory of competition between the differently active groups of the microorganisms.

CONCLUSION

The smear cultures B and C containing the mutant strain of *Brevibacterium linens*, Laktoflora 205 instead of commercially used *Brevibacterium linens*, Laktoflora 980 can be recommended for soft smear cheese manufacture in processing plants. The cheeses made using smear cultures B and C possessed better organoleptic and microbiologic characteristics and their ripening process was about two to three days shorter and significantly more intensive compared with the cheeses treated with commercially used smear culture A.

References

CHEN, M. H. - LEDFORD, R. A.: Proteolytic activity of *Geotrichum candidum*. J. Dairy Sci., *55*, 1972 : 666.

CIKÁNEK, D. - BŘEZINA, P. - PLOCKOVÁ, M. - KOPEČNÝ, J. - POKORNÁ, L.: Mutantní kmen pro výrobu tvarohových sýrů *Brevibacterium linens*. AO 266 794, 1989.

DULLEY, J. R.: The utilization of cheese slurries to accelerate the ripening of Cheddar cheese. Austr. J. Dairy Technol., *31*, 1976 : 143-148.

FOISSY, H.: Einfluss der Bebrütungszeit und des Anzuchtmediums auf das elektrophoretische Proteinasen-Aktivitätsprofil von *Brevibacterium linens*. Milchwiss., *28*, 1973 : 692-695.

FOISSY, H.: Aminopeptidase from *Brevibacterium linens*: Production and purification. Milchwiss., *33*, 1978 : 221-223.

O'KEEFFE, R. B. - FOX, P. F.- DALY, C.: Contribution of rennet and starter proteases to proteolysis in Cheddar cheese. J. Dairy Res., *43*, 1976 : 97-107.

MARTH, E. H.: Microbiological and chemical aspects of Cheddar cheese ripening. J. Dairy Sci., *48*, 1963 : 869-871.

MORENO, V. - KOSIKOWSKI, F. V.: Peptides, amino acids and amines liberated from 3-casein by micrococcal cell-free preparations. J. Dairy Sci., *73*, 1973 : 39-44.

NATH, K. R. - LEDFORD, R. A.: Caseinolytic activity of micrococci isolated from Cheddar cheese. J. Dairy Sci., *55*, 1972 : 1424-1427.

PURKO, M. - NELSON, W. O.: The associative action between certain yeasts and *Bacterium linens*. J. Dairy Sci., *34*, 1951 : 699-702.

REITER, B. - SOROKIN, Y. - PICKERING, A. - HALL, A. J.: Hydrolysis of fat and protein in small cheeses made under aseptic conditions. J. Dairy Res., *36*, 1969 : 65-76.

REPS, A.: Bacterial surface-ripened cheeses. In: FOX, P. F.: Cheese: Chemistry, Physics and Microbiology. Vol. 2. London and New York, Elsevier Applied Science 1987 : 151-184.

ROBERTSON, P. S. - PERY, K. D.: Enhancement of flavour of Cheddar cheese by adding a strain of micrococci to the milk. J. Dairy Res., *28*, 1961 : 245-249.

SORHAUG, T.: Comparison of peptide hydrolases from six strains of *Brevibacterium linens*. Milchwiss., *81*, 1981 : 137-139.

SZUMSKI, S. A. - CONE, J.: Possible role of yeast endoproteinases in ripening surface-ripened cheeses. J. Dairy Sci., *45*, 1962 : 349-353.

TUCKEY, S. L. - SAHASRABUDHE, M. R.: Studies in the ripening of Limburger cheese. J. Dairy Sci., *40*, 1957 : 1329-1332.

ČSN 56 0083. Potravinářské výrobky. Stanovení celkového počtu mezofilních aerobních a fakultativně anaerobních mikroorganismů. 1987.

ČSN 56 0085. Potravinářské výrobky. Stanovení počtu koliformních bakterií plotnovou metodou. 1987.

ČSN 56 0087. Potravinářské výrobky. Stanovení počtu kvasinek a plísní. 1987.

ISO 6658. Sensory analysis - Methodology - General guidance. Geneve, International Organization for Standardization 1985.

ISO 8587. Sensory analysis. Methodology. Ranking. Geneve, International Organization for Standardization 1988.

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M. Plocková, J. Chumchalová, Š. Pavlíková

(Vysoká škola chemicko-technologická – Ústav technologie mléka a tuků, Praha)

Zrání sýrů s mazem na povrchu

Byl sledován zrací proces měkkých sýrů s mazem na povrchu vyrobených v provozních podmínkách mlékárny Ostrožská Nová Ves s použitím tří typů mazových kultur: A - komerčně používaný typ; B - mutantní kmen *Brevibacterium linens*, Laktoflora 205 a směs kvasinek; C - mutantní kmen *Brevibacterium linens*, Laktoflora 205, směs kvasinek a směs mikrokoků. Sýry vyrobené s využitím kultury B a C měly lepší organoleptické a mikrobiologické parametry a jejich zrací proces byl asi o dva až tři dny kratší a výrazně intenzivnější než u sýrů vyráběných pomocí komerčně používané mazové kultury A.

Brevibacterium linens; mikrokoky; mutantní kmen; organoleptické vlastnosti; rozklad bílkovin; zrání; měkké sýry s mazem

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THE CHEMICAL COMPOSITION AND CASEIN FRACTIONS OF GOAT MILK

Pavel BŘEZINA, Marta PRŮŠOVÁ, Šárka PAVLÍKOVÁ, Milan MAROUNEK [1], Jiří ŠTĚTINA

University of Chemical Technology – Department of Milk and Fat Technology, Technická 5, CZ-166 28 Praha 6 ;
[1] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, CZ-104 00 Praha 10-Uhříněves, Czech Republic

Milk of Czech White goats was sampled from February to April 1993, pasteurized and analyzed to compare the composition of goat and cow milks. The comparison of 12 characteristics (contents of total solids, fat, protein, lactose, ash, density, titratable acidity, pH, free fatty acids, Ca, Fe and ascorbic acid) of six pooled samples revealed no major differences between the milk of both ruminant species. Thirty additional individual samples of goat milk were obtained from the same herd. Milk was from the evening milking. The pasteurization of these samples was omitted. Electrophoretic fractionation of casein indicated a low content of α_s-casein in the goat milk in comparison with the cow milk. There was a large goat-to-goat variation in this respect.

goat milk; composition; electrophoresis; casein

There is a growing interest of farmers, technologists and physicians in goat milk. Goat milk differs from cow milk in some parameters, e.g. in proportion of the individual casein fractions, in the technological properties, etc. Goat milk is suitable for persons who suffer from cow milk allergy (B ř e z i n a, 1992). Goat milk contains on the average 12.3 % of total solids (3.4 % of protein, 3.8 % of fat, 4.3 % of lactose and 0.8 % of minerals).

The proteins of goat milk were thoroughly studied. The amino acid compositions of goat and cow milk proteins are similar. There are, however, some differences in proportions of the milk protein fractions (J e n n s e n et al., 1980). Whereas in cow milk the ratio of casein fractions $\alpha_{s1} : \alpha_{s2} : \beta : \kappa$ is typically 4 : 1 : 3 : 1, the α_{s1}-fraction is absent or non-significant in goats. The β-fraction is the principal component of goat casein. The total casein content of goat milk varies from 2.14 to 3.18 % (2.28 - 3.27 % in cow milk).

The content of α_{s1}- casein varies from 0.3 to 1.12 % in goat milk and from 0.99 to 1.56 % in cow milk. These data were published by S t o r r y et al. (1983). The low content of the α_{s1}-fraction in goat casein can be utilized for the detection of adulteration of goat milk with cow milk. Even a small addition of cow milk to goat

milk can be detected by means of the α_{s1}-spot after gel electrophoresis of milk proteins. M o r a - G u t i e r r e z et al. (1991) followed the content of α_{s1}-casein in the milk of French-Alpine and Anglo-Nubian goats. The concentration of α_{s1}--casein varied from 0.12 to 2.70 g/l, being similar in both breeds. However, in the authors' opinion, the breed can influence this parameter. There was a considerable animal-to-animal variation in their experiments. Similar findings were presented by K ř í ž e k (1992) and C h i a n e s e et al. (1992).

The α_{s1}-fraction content is a very important parameter for cheese production. Cheeses produced from milk with the higher content of α_{s1}-casein have mainly a harder consistency. Goat milk is coagulated by the rennet more rapidly than cow milk. The coagulate is softer and its particles are smaller (R e m e u f et al., 1986; C h i a n e s e et al., 1992). A number of authors have studied nutritional properties of goat milk (e.g. M a r e e, 1985; C a m p, 1987; F a n t o v á, 1989; P o d h o r s k ý, 1992).

The aim of this work was to study the composition of goat milk and the proportion of casein fractions in individual and pooled samples of milk of Czech white goats.

MATERIAL and METHODS

Six pooled samples of goat milk were obtained from an "Orlice" cooperative farm at Týniště nad Orlicí. There were 200 Czech White goats in one herd. All goats were in the second lactation. Samples were taken once a week from February to April 1993. Milk was pasteurized and sampled one day after milking from the retail package (dark bottles). Thirty individual samples of milk were obtained from the same herd by hand milking (evening milking). These samples were not pasteurized.

The acidity of milk was determined by a Soxhled-Henkel method (C v a k et al., 1992) and by measuring the pH values. Total solids were estimated by drying at 105 °C, without sand, until constant weight was achieved (Č e r n á et al., 1987). Total nitrogen was determined by means of a Kjeltec Auto Plus 1030 Analyzer. The content of protein was computed from the content of N using a coefficient 6.38. Ash, calcium, lactose, fat, and free fatty acids were determined according to Č e r n á et al. (1987). Iron was determined by means of atomic absorption spectrophotometry and ascorbic acid by titration with 2,6-dichlorphenol-indophenol (D a v í d e k et al., 1981). The casein portion of milk proteins was isolated at pH = 4.6 and subjected to starch gel electrophoresis (Č e r n á et al., 1987). Electrophoregrams were quantified by a densitometric method (Vitatron MPS instrument, type 940.800 with a recorder). Amounts of the individual casein fractions were proportional to areas of the corresponding peaks. The amino acid composition of milk proteins was estimated in acid hydrolyzed samples by ion-exchange chromatography. The automatic amino acid analyzer (model T 339, Mikrotechna, Prague) was utilized for this purpose.

RESULTS and DISCUSSION

The results of analyses of six pooled samples of goat milk are shown in Table I. Table I also presents results of analyses of cow milk from the dairy factory Radlice (Prague). Pooled samples of cow milk were obtained in the same period. The total solids of goat milk were slightly lower than the total solids of cow milk. Borš et al. (1985) found in milk of Czech white goats 13.1 % of total solids on the average. Our finding was lower (11.9 %), probably due to different feeding of goats. The percentage of fat was in the range of 3.4 -4 .0. The remaining analyses presented in Table I gave results similar to the findings reported by Voutsinas et al. (1990) and Gajdůšek et al. (1993). The protein content of goat milk was lower than that of cow milk. Our results agree with the data of Storry et al. (1983). Borš et al. (1985) found 3.37 % of protein in goat milk and Gajdůšek et al. (1993) found 2.74 % of protein. The concentration of calcium

I. Composition of goat and cow milk (Analyses of pooled samples)

Parameter	Goat milk							Cow milk
	Sample No.						Mean	
	1	2	3	4	5	6		Mean
Total solid (%)	12.66	11.99	11.86	11.52	11.52	12.09	11.94	12.89
Fat (%)	4.0	3.7	3.5	3.4	3.6	3.6	3.6	4.1
Protein (%)	3.29	3.19	3.09	2.92	2.99	3.11	3.10	3.38
Lactose (%)	4.5	4.4	4.4	4.7	4.6	4.8	4.6	4.6
Ash (%)	0.79	0.78	0.71	0.77	0.80	0.80	0.77	0.79
Density ($g.l^{-1}$)	1030.0	1030.8	1030.4	1029.2	1029.7	1030.4	1030.1	1029.4
pH	6.62	6.72	6.8	6.77	6.71	6.68	6.72	6.68
Acidity (SH)	6.7	7.1	7.0	6.7	6.5	6.7	6.8	6.7
FFA[1] ($mmol.kg^{-1}$)	6.6	5.8	11.6	8.5	7.5	8.3	8.1	7.5
Ca ($g.l^{-1}$)	1.43	1.30	1.28	1.46	1.33	1.39	1.37	1.32
Fe ($g.l^{-1}$)	ND	0.55	0.40	0.38	0.24	0.36	0.39	0.26
Ascorbic acid ($mg.l^{-1}$)	ND	ND	15.8	16.2	16.9	16.5	16.3	20.1

[1] Free fatty acids
ND not determined

was similar to the data published by B o r o š et al. (1985), who found from 1.32 to 1.37 g Ca per l, whereas G a j d ů š e k et al. (1993) found only 0.90 g Ca per l. B o r o š (1986) found 0.24 - 0.27 mg Fe in 1 l of goat milk. Our values were higher (Table I). It should be noticed in this respect that the low concentration of iron in goat milk is often criticized by physicians and may negatively influence its therapeutical use. Ascorbic acid (vitamin C) is not a stable component of milk. Its concentration in goat milk was somewhat lower than that in cow milk. B o r o š (1986) presented values of 15.0 and 21.1 mg per 1 l of goat and cow milk, and M a r e e (1985) found 20.0 mg of ascorbic acid per 1 l of milk in both animal species.

Table II presents the amino acid composition of milk proteins in six pooled samples of goat milk. The values presented are similar to those published in the literature (e.g. J e n n e s et al., 1980).

II. Amino acid composition of pooled samples of goat milk (value expressed in g per 100 g of protein)

Amino acid	Sampe No.						Mean
	1	2	3	4	5	6	
Asp	6.70	6.55	6.52	6.13	6.94	6.68	6.56
Thr	4.21	4.16	4.22	3.94	4.32	4.24	4.18
Ser	3.47	3.56	3.40	3.45	3.50	3.63	3.50
Glu	19.94	20.22	20.13	19.23	20.19	20.73	20.07
Pro	10.49	11.06	9.29	10.11	7.60	11.65	10.03
Gly	1.46	1.50	1.38	1.40	1.38	1.52	1.44
Ala	2.52	2.53	2.43	2.30	2.44	2.56	2.46
Val	6.41	6.41	6.54	6.11	6.52	6.66	6.44
Met	2.77	2.48	2.68	2.36	2.48	2.50	2.55
Ile	4.50	4.01	4.55	4.90	4.05	4.54	4.43
Leu	9.03	7.98	8.90	9.02	7.86	8.84	8.61
Tyr	7.24	6.83	7.13	7.53	8.00	4.27	6.83
Phe	7.13	6.67	6.86	7.15	7.60	7.14	7.09
His	3.64	5.07	5.15	5.86	6.19	5.62	5.26
Lys	7.34	7.61	7.30	7.26	7.81	7.46	7.46
Arg	3.14	3.36	3.52	3.24	3.12	1.95	3.06

1. Electrophoretic patterns of casien samples, obtained form goat and cow milks

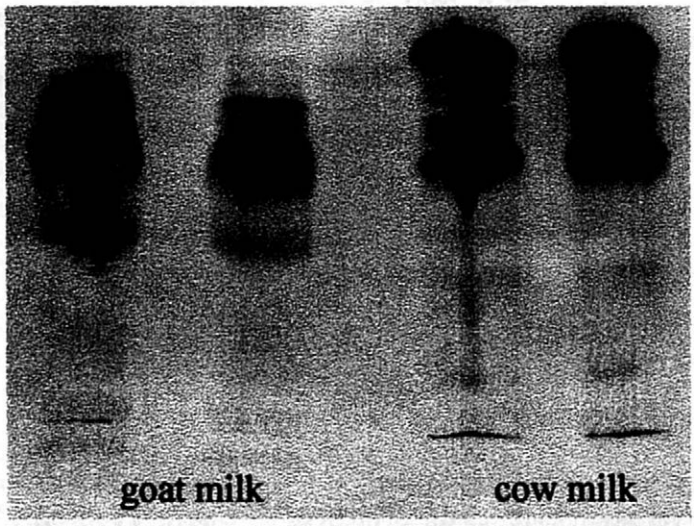
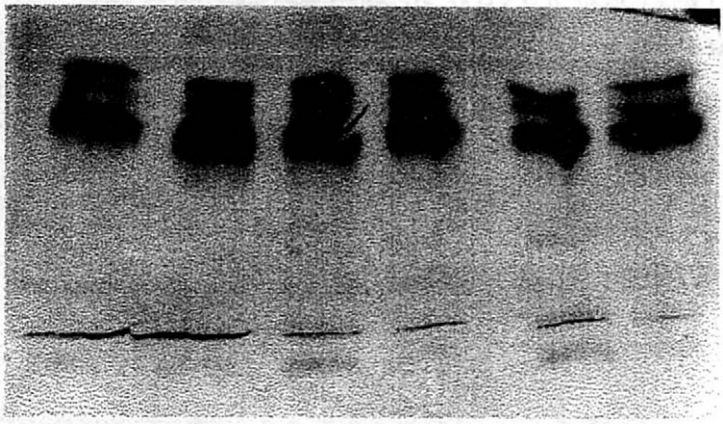


Fig. 1 presents the electrophoretic patterns of samples of casein obtained from goat milk and cow milk. As expected, the α_s-casein band was very weak in goat casein in comparison with that in cow casein. The electrophoretic mobility of this fraction was low. The α_s-casein represented 16 - 35 % and 43 - 48 % of the total casein in goat and cow milk, respectively. The ratio of casein fractions α_s/β was 0.28 - 0.64 and 0.95 - 1.78 for goat and cow milk, respectively (S t o r r y et al., 1983). Our findings, presented in Table III, confirm the observations that have been published in the literature.

III. Percentage of main fractions of casein of goat and cow milk (Analyses of pooled samples)

Fraction	Goat milk							Cow milk
	Sample No.						Mean	Mean
	1	2	3	4	5	6		
α_s	26	22	20	20	36	31	26	56
β	64	66	71	68	53	62	64	33
κ	10	12	9	12	11	7	10	11
Ratio α_s/β	0.41	0.33	0.28	0.29	0.68	0.50	0.41	1.70

Analyses of thirty individual samples of goat milk enabled us to divide goats into three groups with respect to the α_s/β ratio. In the first group (17 % of samples) the α_s/β ratio was lower than 0.50. In the second group it varied from 0.50 to 0.75. In the remaining samples the α_s/β ratio was higher than 0.75. It can be seen from Fig. 2 that the separation of κ-fraction of casein was poor. Problems in this respect



2. Electrophoretic patterns of casein samples, obtained from the individual samples of goat milk

were reported also by B o r o š (1986). There was a considerable animal-to-animal variation as far as the content of α_s-casein is concerned, in spite of the fact that all goats were of the same breed. The β-casein was the principal fraction of milk proteins of goats.

Due to the growing demand for goat milk and goat milk products, the commercial goat milk production is increasing world-wide. The real need for goat milk of a good quality is higher than the current supply. There has been a growing interest in the composition of goat milk and its technological and nutritional properties. This paper (i) presents principal parameters of composition of milk of Czech white goats and (ii) demonstrates that the main characteristics of goat and cow milk are very similar.

References

BOROŠ, V.: Kandidátská disertační práce. Nitra 1986. - Vysoká škola poľnohospodárska.

BOROŠ, V. - KRČÁL, Z. - ŠTEVONKOVÁ, E.: Změny ve složení kozího a ovčího mléka v průběhu laktace. Živoč. Výr., *30*, 1985 : 549-544.

BŘEZINA, P.: Kozí mléko - perspektivy využití a technologického zpracování. Prům. Potr., *43*, 1992 : 453-455.

CAMP, A. T.: Why isn't goat milk used more widely by people allergic to cow milk? Dairy Goat J., *65*, 1987 : 21-22, 29.

CHIANESE, L. - MAURIELLO, R. - GARRO, G. - NICOLAI, M. A. - CAPUCCIO, U. - GOGLIANDRO, E. - ADDETO, F.: Caratterizzazione della caseina in aleune razzi caprine. In: Il latte e i formagii di capra - possibilita applicative dei risultati sperimentali 90. Regio Instituto Zootechnico, Bella 1992.

CVAK, Z. - PETERKOVÁ, L. - ČERNÁ, E.: Chemické a fyz.-chemické metody v kontrole jakosti mléka a mlékárenských výrobků. Praha, VÚPP - SPI 1992.

ČERNÁ, E. - CVAK, Z.: Analytické metody pro mléko a mlékárenské výrobky. Díl I. Praha, VÚPP - STI 1987.

DAVÍDEK, J. et al. : Laboratorní příručka analýzy potravin. Praha, STNL 1981.

FANTOVÁ, M.: Význam kozího mléka ve výživě. Chovatel, *28*, 1989 : 9-10.
GAJDŮŠEK, S. et al.: Složení a vlastnosti kozího mléka. In: XX. Seminář o jakosti potravin a potravinářských surovin živočišného původu. Brno, VŠZ 1993.
JENNES, R.: Composition and characteristics of goat milk. Review 1968-1979. J. Dairy Sci., *63*, 1980 : 1605-1630.
KŘÍŽEK, J.: Chov koz. Farm, Praha 1992.
MAREE, H.P. : Goat milk and its use as a hypo-allergenic infant. Food. Dairy Goat J., *63*, 1985 : 16, 48-50.
MORA-GUTIERREZ, A. - KUMOSINSKI, T. F. - FARRELL, L. H. : Quantification of α_{s1}-casein in goat milk from french-alpine and anglo-nubian breeds using reversed-phase high performance liquid chromatography. J. Dairy Sci., *74*, 1991 : 3303-3307.
PODHORSKÝ, M.: Zdravotní přednosti ovčího a kozího mléka. In: Sbor. Sem. se zahraniční účastí. Trenčín, 1992 : 19-23.
REMEUF, F. - LENOIR, J. : Relationship between the physicochemical characteristics of goat's milk and its rennet stability. In: Bulletin IDF, Document, *202*, 1986.
STORRY, J. E. - GRANDISON, A. S. - MILLARD, D. - OWEN, A. J. - FORD, G. D.: Chemical composition and coagulating properties of renneted milks from different breeds and species of ruminant. J. Dairy Res., *50*, 1983 : 215-229.
VOUTSINAS, L. - PAPPAS, Ch. - KATSIARI, M.: The composition of Alpine goat's milk during lactation in Greece. J. Dairy Res., *57*, 1990 : 41-51.

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P. Březina, M. Průšová, Š. Pavlíková, M. Marounek, J. Štětina (Vysoká škola chemicko-technologická – Ústav technologie mléka a tuků, Praha; Ústav fyziologie a genetiky hospodářských zvířat AV ČR, Praha)

Chemické složení a frakce kaseinu kozího mléka

Kozí mléko a z něj získané výrobky představují vhodný doplněk naší stravy. Česká republika vždy patřila k zemím s tradičně vysokou úrovní chovu koz, jak co do počtu, tak hlavně co do užitkovosti. Významným důvodem zájmu o kozí mléko je i možnost jeho využití jako léčebného prostředku proti řadě nemocí, případně jako náhradu za kravské mléko pro jedince trpící alergií na tento druh mléka.

Všeobecně je uváděna lepší stravitelnost kozího mléka, a to z důvodu menší velikosti tukových kuliček a menších, jemnějších bílkovinných micel než v mléce kravském. Z hlediska zdravotních účinků kozího mléka, ale rovněž z pohledu jeho technologického zpracování je významná především jeho bílkovinná složka. Pozornost se zaměřuje na zastoupení frakcí kaseinového komplexu.

V práci bylo sledováno fyzikálně-chemické složení směsného kozího mléka (šest vzorků z období únor až duben 1993) od sledovaného stáda koz plemene česká bílá. Stanovení bylo provedeno po tepelném ošetření vzorků (85 °C po dobu několika sekund) a složení bylo porovnáváno s průměrným vzorkem kravského mléka. Je možné

konstatovat, že ve 12 sledovaných ukazatelích (titrační kyselost, pH, hustota, obsah sušiny, tuku, bílkovin, laktosy, popelovin, volných mastných kyselin, vápníku, železa a kyseliny askorbové) se oba druhy mléka kvantitativně výrazně neliší.

Dále bylo odebráno od stejného stáda 30 individuálních vzorků kozího mléka při večerním dojení s cílem sledování zastoupení jednotlivých kaseinových frakcí. Hodnoceno bylo syrové mléko pomocí elektroforézy na škrobovém gelu a výsledky byly porovnány s mlékem kravským. U kozího mléka byl zaznamenán výrazně nižší obsah frakce α_s-kaseinu a navíc i značné rozdíly v rámci sledované série vzorků.

kozí mléko; složení; elektroforéza; kasein

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CATEGORY ORDINAL SCALES WITH UNCOMPLETE VERBAL DESCRIPTORS

Jan POKORNÝ

University of Chemical Technology – Department of Food Chemistry and Analysis, Technická 5, CZ-166 28 Praha 6, Czech Republic

Texture attributes of flat crisp bread, produced by extrusion process, were evaluated by sensory profiling, and described using category ordinal scales provided with verbal description of odd-number points while the even-number points remained without verbal description. The points without verbal description were used significantly less frequently by a group of trained selected assessors than the points provided with verbal description.

extrusion; texture; sensory profiling; category ordinal scales

In spite of the great progress of sensory analysis, studies on many topics of the methodology are still lacking. Recently, we investigated the optimum number of points in ordinal category scales used for hedonic sensory ratings (P i l k o v á et al., 1992). Category scales, which are used very often both in the assessment of sensory quality and in sensory profiling, are usually accompanied by verbal description of individual points of the scale. The verbal description could be assigned either to every point or only to some points of the scale, for instance we used such a scale for the texture rating of crisp extruded bread (P o k o r n ý et al., 1991; S t r e j č e k et al., 1993). In this paper, we present our observations how much the description of only some points of the scale affects the distribution of results.

MATERIAL and METHODS

Samples of flat crisp bread were obtained by extrusion of wheat grits and rye bran as reported previously (H o l a s, P o s k o č i l o v á, 1986; P o s k o č i l o v á, H o l a s, 1985; P o k o r n ý et al., 1987). The samples were stored under defined humidity, and the effect of storage on texture attributes was determined, as described earlier (P o k o r n ý et al., 1991).

The texture profile method reported earlier (P o k o r n ý et al., 1991; S t r e j č e k et al., 1993) was used for the sensory evaluation in this study. Samples were evaluated under standard conditions (ISO, 1985a), in a standard test room (ISO, 1988), using a group of selected assessors (ISO, 1989). The following 9-point category ordinal scales (ISO, 1985b) were used:

The determination of the overall texture quality:

1 = the texture is excellen,t
2
3 = the texture is very good,
4
5 = the texture is satisfactory,
6
7 = the texture is still acceptable,
8
9 = the texture is very bad.

Breaking the plate of crisp bread between the fingers:

1 = the sample is broken very easily,
2
3 = the sample is broken rather easily but using greater force,
4
5 = the sample is broken with use of great force,
6
7 = the sample is rather bent than broken,
8
9 = the sample is bent easily but not broken.

For the determination of resistance when pressed between two fingers of one hand:

1 = crushed very easily, being very crisp,
2
3 = is rather crispy,
4
5 = only a little crispy,
6
7 = not crispy, difficult to crush,
8
9 = paste-like, not crispy at all.

It was determined, how easily the plate may be bitten off:

1 = extremely easily,
2
3 = rather easily,
4
5 = not easily,
6
7 = rather with difficulty,
8
9 = with great difficulties.

Feelings during the chewing were evaluated using the scale:

1 = chewed extremely easily,
2
3 = rather easily,
4
5 = not easily,
6
7 = rather with difficulties,
8
9 = with great difficulties.

The capacity to absorb saliva during the chewing was evaluated using the scale:

1 = the morsel absorbs saliva extremely easily,
2
3 = rather easily,
4
5 = still satisfactorily,
6
7 = rather low absorbing,
8
9 = very little absorbing.

For the evaluation of swallowing of the morsel, the following scale was used:

1 = the morsel is swallowed extremely smoothly, extremely easily,
2
3 = rather easily, without any difficulties,
4
5 = not too easily,
6
7 = with some difficulties,
8
9 = with great difficulties.

The frequency of responses at different points of the scale was evaluated by extrapolation of experimental data using the probit analysis. The software Statgraphics was used.

RESULTS and DISCUSSION

We analyzed several hundreds of samples using the category ordinal scales with uncomplete verbal description, and determined the frequencies of the use of various degrees of the above scales. It is evident from the Table I that the points of the scale provided with verbal descriptions were preferred by the assessors so that more experimental responses were obtained than theoretically expected. The conclusion

I. Frequency of ratings with use of category scales provided with uncomplete description

Descriptor used	Point of the scale	Number of responses		
		observed	calculated	difference
Breaking the slice $N = 793$	1	130	79	+51
	2	152	217	-65
	3	337	280	+57
	4	148	148	0
	5	94	68	+26
	6	20	32	-12
	7	6	6	0
	8	3	4	-1
	9	3	1	+2
Resistance to compression $N = 875$	1	89	48	+41
	2	110	178	-68
	3	330	260	+70
	4	191	188	-3
	5	97	81	+16
	6	14	34	-20
	7	19	16	+3
	8	6	10	-4
	9	19	4	+15
Ease of chewing $N = 283$	1	34	25	+9
	2	57	65	-8
	3	113	100	+13
	4	34	70	-36
	5	34	27	+7
	6	2	9	-7
	7	2	3	-1
	8	3	2	+1
	9	4	1	+3
Ease of swallowing $N = 280$	1	34	24	+10
	2	57	71	-14
	3	109	94	+15
	4	34	63	-29
	5	35	28	+7

Descriptor used	Point of the scale	Number of responses		
		observed	calculated	difference
Ease of swallowing $N = 280$	6	2	12	-10
	7	2	3	-1
	8	3	2	+1
	9	4	1	+3
Overall texture quality N = 217	1	3	4	+1
	2	2	22	0
	3	8	50	+2
	4	4	46	-8
	5	7	55	+8
	6	0	14	-16
	7	0	28	+18
	8	2	1	+1
	9	1	0	+1
Total texture ratings $N = 2667$	1	191	299	+108
	2	526	438	-88
	3	880	989	+129
	4	591	468	-123
	5	272	323	+51
	6	122	54	-68
	7	52	61	+9
	8	25	16	-9
	9	8	30	+22

N = number of responses

resulting from the data is that grading scales based on a category ordinal scale (most rating scale are of this type) should be provided with verbal description at every point (step), otherwise the distribution of the results is affected, and the use of parametric statistical methods is not possible. Scales with uncomplete verbal descriptions should not be used in the sensory analyses, even when they are recommended by the international standard (ISO, 1985).

References

HOLAS, J. - POSKOČILOVÁ, H.: Extruze v cereálním průmyslu: Křehký chléb. Mlýn.-pekár. Prům., *32*, 1986 : 303-307.

PILKOVÁ, L. - POKORNÝ, J. - DAVÍDEK, J.: Vliv kategorových ordinálních stupnic na přesnost výsledku sensorické analýzy. Potrav. Vědy, *10*, 1992 : 41-48.
POKORNÝ, J. - DAVÍDEK, J. - JASNÁ, E. - VIERECKLOVÁ, M. - PÁNEK, J. - HOLAS, J. - HOUŠKA, M. - POKORNÝ, D. - KUBEŠOVÁ, A.: Vliv skladování na texturu extrudovaného křehkého chleba. Mlýn.-pekár. Prům., *33*, 1987 : 229-233.
POKORNÝ, J. - VIERECKLOVÁ, M. - JASNÁ, E. - DAVÍDEK, J. - POKORNÝ, D. - HOLAS, J.: Hodnocení sensorické jakosti extrudovaného křehkého chleba. Potrav. Vědy, *9*, 1991 : 143-153.
POSKOČILOVÁ, H. - HOLAS, J.: Výroba cereálních potravin extruzní technologií. Mlýn.-pekár. Prům., *31*, 1985 : 163-167.
STREJČEK, F. - POKORNÝ, J. - VIERECKLOVÁ, M. - POKORNÝ, D. - HOLAS, J.: Relations between sensory attributes of flat crisp bread. Potrav. Vědy, *11*, 1993 : 81-98.
ISO 4121: Sensory analysis - Grading of food products by methods using scale categories. International Organization for Standardization, Geneva, 1985a.
ISO 6658: Sensory analysis - Methodology - General guidance. International Organization for Standardization, Geneva, 1985b.
ISO 8586: Sensory analysis - General guidance for the selection, training and monitoring of assessors - selected assessors. International Organization for Standardization, Geneva, 1989.
ISO 8589: Sensory analysis - General guidance for the design of test rooms. International Organization for Standardization, Geneva, 1988.

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J. Pokorný (Vysoká škola chemicko-technologická – Ústav chemie a analýzy potravin, Praha)

Kategorové ordinální stupnice s neúplným slovním popisem jednotlivých stupňů

Plochý trvanlivý chléb vyrobený extruzní technologií z pšeničné krupice a žitných otrub byl skladován po různou dobu v prostředí o různé relativní vlhkosti. Výrobek byl pak hodnocen senzorickým texturním profilem skupinou školených hodnotitelů. Čtyři texturní charakteristiky a celková jakost textury byly hodnoceny s použitím devítibodových kategorových ordinálních stupnic, u nichž liché stupně byly doplněny vysvětlujícím slovním popisem, zatímco u sudých stupňů tento popis nebyl. Stupně opatřené slovním popisem byly skupinou hodnotitelů užity častěji než stupně bez slovního popisu. Doporučuje se proto, aby byly u podobných stupnic všechny stupně opatřeny slovním popisem, jinak je rozdělení výsledků zkresleno a statistické zpracování s použitím parametrických metod není možné.

extruze; textura; senzorický profil; kategorové ordinální stupnice

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CHANGES OF RELATIONS BETWEEN SOME CHARACTERISTICS DURING FRENCH FRYING IN LIQUID RAPESEED SHORTENING

Lenka KOUŘIMSKÁ, Jan POKORNÝ

Institute of Chemical Technology – Department of Food Chemistry and Analysis, Technická 5, CZ-166 28 Praha 6, Czech Republic

Rapeseed oil was partially selectively hydrogenated, and the solid fraction was removed. The liquid shortening was used for French frying for six consecutive days, without replenishing with fresh oil. High correlation coefficients between various physico-chemical and sensory charateristics allow the prediction, especially if expressions comprising two or three variables are used. Viscosity and trichromatic colour measurements and the content of polar compounds were found to be the most useful criteria. There were larger differences in the relations of variables when French frying in liquid rapeseed shortening was used than in solid rapeseed shortening.

French frying; liquid rapeseed shortening; quality assessment; analysis chemical and sensory; multi-variate statistical analysis

Relations between the characteristics of frying oil and fried products are important for quality assessment and for the prediction of changes in various properties during further frying operations.

In the previous paper (K o u ř i m s k á et al., 1993) we reported on deep fat frying in hydrogenated rapeseed oil in a household fryer. Changes of frying oil were only small, and the HPLC determination of polar products was found to be a suitable indicator of deterioration of the sensory value. Rheological and spectrophotometric data were also suitable.

Hydrogenated rapeseed oil used in the above experiments had a very low content of polyunsaturated fatty acids. Therefore we tested partially selectively hydrogenated rapeseed oil under the same conditions. The selectively hydrogenated oil had a low linolenic acid content but was relatively rich in linoleic acid. Linoleic acid is a precursor of fry flavour compounds (C h a n g et al., 1978a, b; P o k o r n ý, 1989), being converted into 2,4-decadienals and unsaturated lactones. For this reason, oil rich in linoleic acid would be more suitable for the household deep frying than more hydrogenated oil, free of dienoic acids. On the other hand oxidation during frying is proportional to the unsaturation degree (F i e l l i e t a z

Goethart et al., 1985; Permanyer Fabregas et al., 1986; Blumenthal et al., 1976). When using the liquid, only slightly hydrogenated product, there is a danger of rapid deterioration of oil. The application of frying oil should thus be considered from the two above aspects.

Changes during deep frying in shortening were slow because of the low unsaturation degree (Smith et al., 1986; Blumenthal et al., 1976) so that the replacement was not necessary earlier than after 100 h frying. Fluid shortenings were evaluated under institutional conditions (Handel et al., 1984).

In order to compare the application of solid shortenings and of liquid shortenings under the same conditions, changes in deep frying in liquid hydrogenated rapeseed oil were studied, and the results and suitable analytical criteria are discussed in this paper.

MATERIAL and METHODS

Material: Refined rapeseed oil was partially hydrogenated using a very selective catalyst. The hydrogenation was interrupted at early stage, and the slurry obtained was fractionated to obtain liquid hydrogenated oil. The clouding point was lower than 15 °C. The product used (commercially called Oliol, product of Palma a. s., Bratislava, Slovakia) contained 19.6 % dienoic acids and 1.7 % trienoic acids, the content of total saturated acids was 9.6 %. Commercial prefried French fries were prefried in fully hydrogenated rapeseed oil (melting point 46.3 °C), and stored in a freezer at -16.5 °C.

Frying procedure: The household fryer Philips Nova (Model 2802.00) was filled with 2.5 kg frying oil. Oil was preheated to 170 °C and a batch of 200 g prefried material was added. The temperature of frying oil was thus reduced to 130 °C, but rose to 170 °C within 5 min again. Seven consecutive fryings took place every day so that the total of 42 fryings were performed in a week. After seven fryings, oil was cooled down, and stored till the other day at the ambient temperature. Frying oil was sensorically tested on the subsequent day before the start of the next frying French fries were left to drip, and they were then tested while still warm.

Analytical methods: The acid value and the content of total polar compounds were determined according to the international method IUPAC-AOCS-DGF (IUPAC, 1987). Total oxidation products were determined using the reversed-phase HPLC (Pánek, 1989). The trichromatic colour characteristics were calculated by with use of the standard procedure (ASTM, 1991) and experimental data were obtained by measuring the absorbances between 400 and 700 nm (IUPAC, 1987). The dynamic viscosity was determined on the HAAKE Rotovisco RV 20

Viscometer at 20 °C (by courtesy of Dr. P. Štern, Institute of Hydrodynamics, Prague).

The sensory profile of French fries was determined under standard conditions (ISO, 1985) using a panel of trained assessors (ISO, 1989), and unstructured graphical scales (ISO, 1987), represented by oriented straight lines (145 mm long). The following descriptors were chosen: acceptability of appearance, colour acceptability, intensity of crunchiness, intensity of crunching sounds, acceptability of overall flavours, intensity of fried flavour, intensity of rancid off-flavour, and intensity of total off-flavours. Details are given in the previous paper (K o u ř i m s k á et al., 1993).

The programme Statgraphics was used for statistical evaluations. Correlation coefficients of the linear and semilogarithmic regressions were calculated on the basis of 42 analyses, and the squares of correlation coefficients were calculated. Among the above four expressions, only those with the highest correlation coefficient are shown in Tables. More details were published in the previous paper (K o u ř i m s k á et al., 1993).

RESULTS and DISCUSSION

The values of correlation coefficients concerning regressions between chemical and physical variables are summarized in Table I, where only the highest values of statistically significant relations are included. A close correlation was found between the dynamic viscosities at 20 °C and 35 °C, as expected, but still other factors interfere in this linear regression. Close correlations were also observed between trichromatic characteristics and between them and the dynamic viscosity. The content of polar compounds was correlated with the spectrophotometric data and with the dynamic viscosity as well. Both data correlate with the frying time, and thus, with one another as well (R e m m e n, 1988; K o u ř i m s k á and P o k o r n ý, 1994). The content of polar compounds correlated better with other variables with than the content of oxidation products, which is in agreement with the prevailing hydrolysis compared to oxidation (B l u m e n t h a l, 1987) and the surfactant theory of frying (B l u m e n t h a l, S t i e r, 1991). We have obtained similar values in our experiments with solid hydrogenated rapeseed oil (K o u ř i m s k á et al., 1993).

Correlations of the free fatty acid content (or the oil acidity) with other variables were rather low, which is in good agreement with the results obtained for other fluid shortenings (H a n d e l et al., 1984). Again, similar conclusions were obtained with solid rapeseed shortening (K o u ř i m s k á et al., 1993).

Correlation coefficients between different sensory characteristics of French fries are given in Table II. Surprisingly, the correlation between the appearance acceptability and the colour acceptability was insignificant, contrary to our previous

I. Regressions between the characteristics of frying oil ($y = a \,.\, x + b$)

Dependent variable y	Independent variable x	Correlation coefficient r	Square value r^2 (p. c.)
A	log E	-0.6093	37.1
A	G	-0.5449	29.7
A	log H	-0.5350	28.6
log B	C	0.6545	42.8
B	log E	0.8933	79.8
B	log F	-0.8443	71.3
B	log G	0.9310	86.7
B	log H	0.8262	68.3
C	log B	0.6154	37.9
log C	D	0.5891	34.7
log D	E	-0.9745	95.0
log D	log F	0.7781	60.5
log D	G	-0.9196	84.6
log D	H	-0.7387	54.6
E	log F	-0.8236	67.8
E	log G	0.9317	86.8
E	log H	0.7872	62.0
F	G	-0.9323	86.9
F	H	-0.9797	96.0
G	H	0.9228	85.2

Only the highest coefficients of regressions between the variables and their logarithms are given. A = free fatty acids, B = total polar compounds, C = oxidized products, D = trichromatic luminance, E = trichromatic dominant wavelength, F = trichromatic saturation, G = dynamic viscosity at 20 °C, H = dynamic viscosity at 35 °C

II. Regressions between the sensory characteristics of French fries (linear regression: $y = a \,.\, x + b$, $P = 0.95$, correlation coefficient: r, s = statistically significant)

Dependent variable	Correlation coefficient r for the undependent variable						
	X	W	V	U	T	S	R
Q	0.1940	0.5171	-0.4457	0.0304	0.1936	-0.2289	0.3242
R	-0.0467	0.1067	-0.3371	0.0743	0.0355	0.1507	
S	0.0908	0.2288	0.5002	0.6393^s	-0.9544^s		
T	-0.0733	-0.2878	-0.6080^s	-0.7390^s			
U	0.1271	0.4501	0.6695^s				
V	-0.1737	-0.0858					
W	0.7693^s						

Descriptors of the sensory profile: Q = acceptability of appearance, R = acceptability of colour, S = crunchinness, T = intensity of crunching sounds, U = acceptability of colour, V = intensity of fried flavour, W = intensity of rancid off-flavour, X = intensity of total off-flavours

results with solid shortening (K o u ř i m s k á et al., 1993). As expected, the correlation between the crunchiness and the intensity of crunching sounds was very high. Significant relations were found between various flavour characteristics but there were differences between the experiments with solid and liquid shortenings, respectively.

The use of two or more variables could result in expressions with very high correlation coefficients (Table III), especially in the case of chemical and physical variables. The content of free fatty acids and of polar compounds could be predicted on the basis of viscosity data. On the contrary, viscosity could be predicted on the basis of acid values and contents of polar products. The content of oxidized products was found to be related with that of polar compounds and acidities, if sufficiently complicated expressions were used.

For the prediction of the overall flavour acceptability and the intensity of fried flavour, at least other three sensory characteristics were necessary, otherwise no significant regressions could be obtained.

The principle component analysis showed that at least four principle components were necessary to explain 95 % of the variability.

The present results show that liquid rapeseed shortening differs from solid shortening when used for French frying under identical conditions.

III. Regressions containing more than two variables

	r^2
$A = -152.4 + 0.247\ G + 1.36\ H - 0.0007\ G^2 + 0.0095\ H^2 + 0.0185\ G \times H + 34.7 \log G + 80.55 \log H - 1.66\ H \times \log G - 0.251\ G \times \log H + 0.00276\ G \times H \times \log G - 0.0884\ G \times H \times \log H$	0.9855
$B = -2161 - 3.36\ G + 21.3\ H - 0.004\ G^2 + 0.0207\ H^2 - 0.0259\ G \times H + 329.8 \log G - 311.4 \log H - 10.9\ H \times \log G + 2.46\ G \times \log H + 0.0278\ G \times H \times \log G - 0.028\ G \times H \times \log H$	0.9855
$B = -2.37 + 27.5\ A + 17.4\ C - 48.0\ A^2 - 7.47\ C^2 - 62.45\ A \times C + 387.6\ A \times \log C$	0.9145
$B = -26.2 + 135.0\ A + 45.4\ C - 1924\ A \times C - 35.8\ A^2 - 7.11\ C^2 + 859\ A \times \log C - 104.9 \log C$	0.9004
$C = -0.398 - 2.54\ B + 14.7\ A^2 + 6.66\ A \times B + 23.7 \log B - 61.9\ A \times \log B$	0.7539
$C = -102.0 - 177.3\ A + 12.2\ B + 0.809\ D + 4.11\ A \times B - 1.82\ A \times D - 0.092\ B \times D + 429.2\ A^2 - 1.79\ B^2 + 0.0001\ D^2 + 140.4 \log B + 40.2 \log D - 53.0 \log B \times \log D$	1.0000
$G = -8133 + 33814\ A + 890\ C + 6263\ B - 1.54\ A^2 - 2967\ C^2 - 1.28\ A \times C + 12153\ A \times \log C - 5.46 \log B + 420.5\ A \times B - 3398\ B \times C - 1.35\ A \times \log B$	1.0000
$H = 74.9 - 1.649\ A + 1264\ B + 337.2\ C + 4392\ A^2 - 139.6\ C^2 + 1131\ A \times C - 1.44\ A \times \log C - 1.11 \log B - 902.5\ A \times B - 620.7\ B \times C + 8272\ A \times \log B + 5611\ C \times \log B$	1.0000
$U = -63.48 + 3.636\ V - 7.337\ W + 8.862\ X + 0.082\ V \times W - 0.217\ V \times X - 0.0282\ V^2$	0.7618
$V = -103.0 + 23.6\ W - 13.4\ X - 0.912\ U \times W + 0.329\ U \times X + 0.208\ U^2 + 0.227\ W^2$	0.6250

A = free fatty acids, B = total polar compounds, C = oxidized products, D = trichromatic luminance, E = trichromatic dominant wavelength, F = trichromatic saturation, G = dynamic viscosity at 20 °C, H = dynamic viscosity at 35 °C, U = acceptability of flavour, V = intensity of fried flavour, W = intensity of rancid flavour, X = intensity of off-flavours

References

BLUMENTHAL, M. M.: Optimum Frying: Theory and Practice. Piscataway, Libra, 1987.
BLUMENTHAL, M. M. - STIER, R. F.: Optimization of deep-fat frying operations. Trends Food Sci. Technol., 2, 1991 : 144-148.

BLUMENTHAL, M. M. - TROUT, J. R. - CHANG, S. S.: Correlation of gas chromatographic profiles and organoleptic scores of different fats and oils after simulated deep fat frying. J. amer. Oil Chem. Soc., *53*, 1976 : 496-501.

CHANG, S. S. - PETERSON, R. J. - HO, C. T.: Chemistry of deep fat fried flavor. ACS Symp. Ser., *75*, 1978a : 18-41.

CHANG, S. S. - PETERSON, R. J. - HO, C. T.: Chemical reactions involved in the deep fat frying of foods. J. amer. Oil Chem. Soc., *55*, 1978b : 718-727.

FIELLIETTAZ GOETHART, R. L.de - HOEKMAN, H. - SINKELDAM, E. J. - GEMERT, L. J. van - HERMUS, R. J. J.: Cooking in oil: the stability of frying oils with a high linoleic acid content. Voeding, *46*, 1985 : 300-306.

HANDEL, A. P. - HAMOUZ, F. L. - DUMPER, A. N. - KNICKREHM, M. E.: Evaluation of fluid shortenings for deep-fat frying under institutional conditions. J. Foodservice Syst., *3*, 1984 : 49-61.

KOUŘIMSKÁ, L. - POKORNÝ, J.: Use of hydrogenated rapeseed oil for French frying in household fryers. Nahrung, *38*, 1994 (in press).

KOUŘIMSKÁ, L. - POKORNÝ, J. - ŠTERN, P.: Relations between characteristics of hydrogenated rapeseed oil during French frying. Potrav. Vědy, *11*, 1993 : 217-224.

PÁNEK, J. - POKORNÝ, J. - DAVÍDEK, J.: Stanovení oxidovaného podílu jedlých rostlinných olejů kapalinovou chromatografií s vysokou účinností. Potrav. Vědy, *4*, 1989 : 253-261.

PERMANYER FABREGAS, J. J. - BOATELLA RIERA, J. - TORRE BORONAT, M. C. de la: Influence of the unsaturation on chemical alterations of heated fats. Fette, Seifen, Anstrichmittel, *88*, 1986 : 258-262.

POKORNÝ, J.: Flavor chemistry of deep fat frying in oil. In: MIN, D. B. - SMOUSE, T. H. (Eds.): Flavor Chemistry Of Lipid Foods. Champaign, IL, American Oil Chemists' Society 1989 : 113-155.

REMMEN, H. H. J. van: Beheersing en sturing van de oliekwaliteit bij industriele frituurprocessen. VMT, *12*, 1988 : 17-20.

SMITH, L. M. - CLIFFORD, A. J. - HAMBLIN, C. L. - CREVELING, R. K.: Changes in physical and chemical properties of shortenings used for commercial deep-fat frying. J. amer. Oil Chem. Soc., *63*, 1986 : 1017-1023.

ASTM: Standards on Color and Appearance Measurement. 3rd ed. Philadelphia, American Society for Testing and Materials, 1991.

ISO 4121: Sensory analysis - Grading of food products by methods using scales. Geneva, International Organization for Standardization 1987.

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

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

IUPAC: PAQUOT, C. - HAUTFENNE, A. (Eds.): Standard methods for the analysis of oils, fats and derivatives. 7th ed. Oxford, London, Edinburgh, Boston, Palo Alto, Melbourne, Blackwell Sci. Publ. 1987.

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L. Kouřimská, J. Pokorný (Vysoká škola chemicko-technologická – Ústav chemie a analýzy potravin, Praha)

Změny vztahů mezi vlastnostmi bramborových hranolků a použitého oleje během smažení v řepkovém tekutém šorteningu

Řepkový olej byl částečně selektivně ztužen a tuhý podíl odstraněn frakcionací. Takto získaný čirý kapalný šortening obsahoval jen nepatrné množství kyseliny linolenové, ale poměrně značné procento kyseliny linolové. V oleji byly dosmažovány předsmažené bramborové hranolky, a to vždy sedmkrát denně po šest následujících dní bez doplňování oleje. Hranolky byly hodnoceny senzorickou analýzou a v oleji byl stanoven obsah volných mastných kyselin, veškerých polárních produktů, veškerých oxidačních produktů, trichromatické veličiny (luminance, dominantní vlnová délka a sytost) a dynamická viskozita při dvou teplotách. Senzoricky byly hranolky hodnoceny metodou senzorického profilu o osmi deskriptorech s použitím nestrukturovaných grafických stupnic.

Výsledky byly zpracovány s použitím programu Statgraphics.

Podobně jako v předešlé práci (K o u ř i m s k á, P o k o r n ý, 1993), ve které byl použit tuhý řepkový šortening, také v tomto případě jsme zjistili významné závislosti mezi jednotlivými námi sledovanými analytickými ukazateli. Mezi fyzikálně chemickými veličinami byly zjištěny průkazné lineární, semilogaritmické nebo logaritmické vztahy (tab. I), takže lze z jedné veličiny odhadnout velikost jiné veličiny.

Mezi senzorickými ukazateli smažených bramborových hranolků byly vztahy lineární (tab. II) a průkazné byly jen některé, zvláště mezi deskriptory týkajícími se chuti.

Regresní vztahy se podstatně zlepší, jestliže se použijí dvě nebo tři analytické veličiny a do regresní rovnice se zařadí také kvadratické a logaritmické členy a interakce mezi ukazateli (tab. III). Tyto vztahy umožňují v některých případech velmi přesnou předpověď, která dovoluje omezit počet sledovaných analytických ukazatelů a předpovědět některé hodnoty, které nebyly stanovovány.

Regresní vztahy mezi veličinami se i při dodržení stejných podmínek smažení liší při smažení v tuhém řepkovém šorteningu a v kapalném šorteningu. To znamená, že zjištěné závislosti platí jen pro určitý druh tuku.

bramborové lupínky; řepkový tekutý šortening; kvalitativní určení; chemické a senzorické analýzy; multivariační statistická analýza

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QUANTITATIVE ANALYSIS OF SUGAR FACTORY JUICES BY FT-IR SPECTROSCOPY

Jitka MORAVCOVÁ, Zita VANCLOVÁ, Miroslava NOVOTNÁ[1]

Institute of Chemical Technology – Department of Chemistry of Natural Compounds and [1]*Central Laboratories, Technická 5, CZ-166 28 Praha 6, Czech Republic*

Fourier Transform Infrared (FT-IR) spectroscopy was used to determine sucrose in raw, thin and thick sugar juices. Three wavenumbers were selected for the analysis. Calibration correlations gave evidence of the validity of the Lambert-Beer law. The best results were obtained at 1056 per cm, where the standard error of prediction was 1.08 and 2.57 % (w/w) for thin juice and raw juice, respectively. All the three wavenumbers are applicable to the analysis of thick juice, giving the standard errors of prediction about 3.6 % (w/w). No sample pre-treatment is necessary and the time required to obtain an analysis report is about five minutes.

quantitative analysis; infrared; FT-IR; sucrose; analysis of sugar factory juices

Infrared spectroscopy can be conveniently applied to the quantitative analysis of sucrose in solutions and dry mixtures. Where its use is possible, infrared spectroscopy is generally faster and requires less sample handling than other techniques, the precision being potentially comparable. For powders, reflectance spectroscopy in the near-infrared region (NIR) can be the method of choice (Sverzut et al., 1987; Permanyer, Perez, 1989; Osborne et al., 1983; Suzuki et al., 1986; Giangiacomo et al., 1981) although repeatability poses some problems, and the Kubelka-Munk function must often be applied to obtain linear systems (Osborne, 1988; Alfaro et al., 1990). Attenuated total reflectance (ATR) spectroscopy in the mid-infrared can provide a solution to both problems (Dupuy et al., 1993; De Lene Mirouze et al., 1993). NIR spectroscopy in the transmittance mode was used to determine the total sugar content in natural fruit juices (Lanza, Li, 1984), in aqueous solutions (Dull, Giangiacomo, 1984; Giangiacomo, Dull, 1986), in soft drinks (Kemsley et al., 1992a) and in molasses (Dumoulin et al., 1987). For sugar factory juices, NIR spectroscopy was tested as an on-line monitoring technique (Vaccari et al., 1990) during the 1989 campaign. The potential of NIR in the analysis of high moisture sugar-containing products is however limited by the strong water absorbance in the near-infrared region which overlaps the sugar absorption bands and requires the application of complicated spectral correlation methods. In the "fingerprint" (mid-infrared) region, the bands

are narrower and the overlap problem is somewhat less severe, so that a simple mathematical approach such as Lambert-Beer calibration of peak heights plotted directly against concentrations can be applied to some systems. This method was used to determine the sucrose and fructose content in soft drinks (K e m s l e y et al., 1992b).

The *CIRCLE cell* is a useful FT-IR sampling accessory designated specifically for the analysis of liquids, excellent for determination of sample levels in aqueous solution. The use of water as a solvent in IR analysis has been avoided for many years due to its strong infrared spectrum. *CIRCLE cell* is a modification of ATR sampling technique. The internal reflection element is a cylinder with conical ends. The energy transmitted through this accessory is high, greater than 50 % when a ZnSe element is used. The zinc selenide crystal means that aqueous solution can be analyzed without any chance of the crystal becoming damaged by the action of water. In an internal reflectance element, a standing wave is established near the surface of the crystal. The amplitude of the electric field decreases exponentially with distance from the surface of the internal reflectance element. The distance is defined as the penetration depth d_p. The magnitude of d_p depends on the wavelength of the radiation, λ, the refractive index of the crystal, n_p, the refractive index of the sample, n_s, and the angle of incidence of the beam at the surface of the internal reflectance element, θ, according to the equation

$$d_p = \frac{\lambda}{2\pi n_p (\sin^2\theta - n_{sp}^2)^{1/2}}]$$

where: $n_{sp} = n / n_p \; (n_s < n_p)$

For most materials, $d_p \cong 0.1\,\lambda$ and is constant for constant values of n_s, n_p, θ, λ. In this case the Lambert-Beer law is applicable for quantitative analysis.

In this work we examined the applicability of FT-IR spectroscopy to the quantitative analysis of raw, thin and thick sugar juices.

MATERIAL and METHODS

Material: The raw, thin and thick juices were original industrial products supplied by the Sugar Factory at Modřany. They were stored at -20 °C and used as received.

HPLC: A glass jacket column (25 cm x 6 mm I.D.) packed with OSTION LGKS 0802 strong cation exchanger (H^+ form, Spolek pro chemickou a hutní výrobu, Ústí nad Labem, Czech Republic) in the Na^+ form was thermostated at 53 °C. The flow rate of deionized water was maintained at 6 ml/h by means of a Beckman 100 A pump, and analysis was monitored with an IR detector Optilab

5902 (Tecator, Sweden) interfaced to a TZ 4200 recorder (Laboratorní přístroje, Prague, Czech Republic). Capacity factors of 0.33, 0.55 and 0.69 were obtained for sucrose, glucose and fructose, respectively. The sucrose content in the industrial samples was determined by using the external calibration method for the peak heights. A set of six standard solutions with sucrose concentrations ranging from 0.2 to 1.5 % (w/w) was measured and the regression equation was calculated. The regression coefficient was 0.99989. The calibration fit was evaluated by calculating the standard error of calibration SEC:

$$SEC = \left[\frac{\Sigma(x_c - x)^2}{(n - p - 1)}\right]^{1/2} \quad [1]$$

where: x_c = calculated sucrose concentration
x = true sucrose concentration
n = number of samples
p = number of independent variables in the regression

The value obtained was 0.239 % for the mean value of 37.58 % and a variation range from 12.52 to 62.52 % (all w/w). The reproducibility of HPLC was expressed by the relative standard deviation RSD for ten replicate analyses of a thin juice sample according to the following formula:

$$RSD = \frac{100}{x_{av}} \left[\frac{\Sigma(x_i - x_{av})^2}{n}\right]^{1/2} \quad [2]$$

where: x_{av} = average sucrose concentration
x_i = sucrose concentration

The RSD value found was 1.49 %.

Defrosted samples of thick or thin juices (2.0 and 8.0 g, respectively) were weighed into 100 ml volumetric flasks and made up to the mark with water at 20 °C. Defrosted raw juice (5.0 g) in 100 ml volumetric flask was diluted with water (60 ml), alkaline lead acetate powder was added, and the mixture was diluted to volume with water at 20 °C, mixed, and filtered. Prior to HPLC analysis, the solutions were deaerated by 20min heating at a temperature 55 - 65 °C and passed through a glass microfilter (2 μm, Tessek, Czech Republic).

FT-IR spectroscopy: The *CIRCLE cell* accessory was installed in the NICOLET 740 sample compartment, aligned for maximum throughput and background spectrum of the empty cell was taken. The sample boat was then filled with distilled

water and reference spectrum was recorded. After emptying and carefully drying the boat, the standard solution or the juice was poured into the sample boat and a spectrum was recorded. Subtraction of reference spectrum from the sample resulted in the water component being subtracted out, leaving a spectrum of the remaining minor constituent of the juice. The samples were used undiluted as received.

Calibration was performed by subjecting the peak height y vs sucrose concentration x plot to linear regression processing using the least squares method. The intercept a and slope b in equation [3] as well as the correlation coefficient r were determined for each suitable wavenumber.

$$x = \frac{y - a}{b} \qquad [3]$$

The calibration results were also evaluated simultaneously at all suitable wavenumbers using the multiple wavenumber approach:

$$x = k_0 + k_1 y_1 + k_2 y_2 + \ldots + k_m y_m \qquad [4]$$

where: x = sucrose concentration
$y_1, .., y_m$ = peak heights at wavenumbers 1, .., m
$k_0, .., k_m$ = constants

Constants $k_0, \ldots, k_m$ were calculated by using QuatroPro 3.0 software. The standard error of calibration (SEC) was calculated according to (1) and the standard error of prediction SEP was used as a validity criterion to check the calibration:

$$SEP = \left[\frac{\Sigma (x_c - x)^2}{(n-1)} \right]^{1/2} \qquad [5]$$

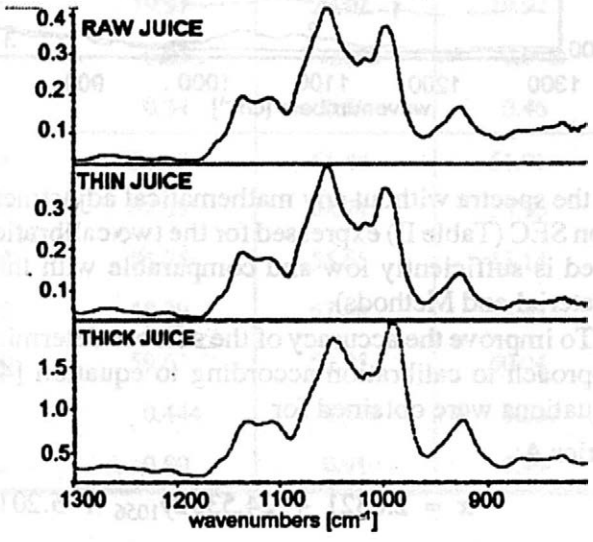
where: x = sucrose concentration in a sample as determined by the reference HPLC method
x_c = sucrose concentration as calculated by using the calibration equation
n = number of prediction samples

RESULTS and DISSCUSION

The first step in the development of the analytical method consists in selecting suitable analytical wavenumbers. This can be accomplished by comparing the spectra of pure solutions of the individual compounds in order to identify peaks

which best differentiate analyte from the other components. Therefore, the spectra of solutions containing 15 and 55 % (w/w) sucrose, respectively, contaminated with glucose and fructose (0.05 - 0.5 % of each with respect to sucrose) together with betaine (0.2 - 1.2 % with respect to sucrose) were computer-simulated using real spectra of the pure compounds, and were compared with those of the sugar factory juices (Figs. 1 and 2). The spectral contribution of components other than sucrose was found to be virtually negligible, and the standard samples could be made from sucrose solely at concentrations as expected in the sugar factory juices. The following wavenumbers were chosen for calibration: 1056 (slightly shifted to 1050 for thick juice), 1000 and 928 per cm. The number of standards dependends on the complexity of the chemical system to be analyzed; in general, $(2n + 2)$ standards are recommended for n components (D u p u y et al., 1992).

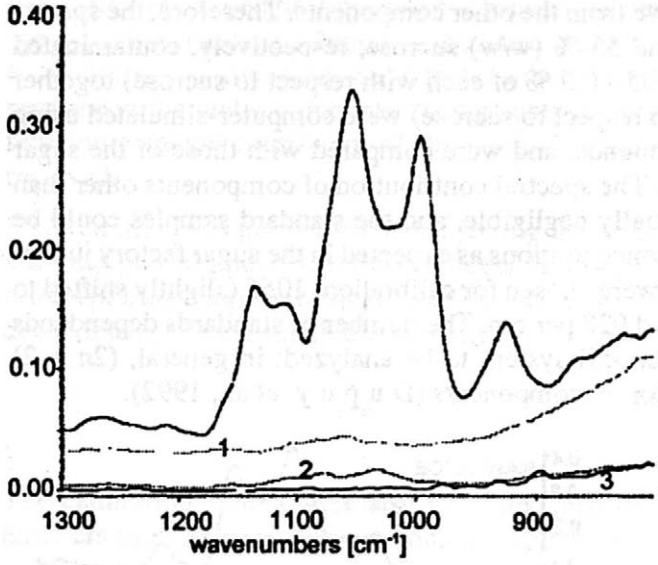
1. FT-IR spectra od industrial sugar factory juices



Two series of standard sucrose solutions whose concentrations were between 14 and 20 % (w/w) (series A, raw and thin juices) and 50 - 60 % (w/w) (series B, thick juice), were used for the calibrations. The high correlation coefficients (Table I) give evidence of the validity of the Lambert-Beer law which can be directly applied

I. Calibration parameters obtained by linear regression treatment

Parameter	Series A at wavenumber			Series B at wavenumber		
	1056	1000	928	1050	1000	928
a	-0.0354	-0.0497	-0.0247	-0.1808	-0.5923	-0.2453
b	0.0220	0.0195	0.0059	0.0258	0.0339	0.0114
r	0.9996	0.9991	0.9991	0.9858	0.9814	0.9792



2. FT-IR spectra of standard sucrose solution (15 % w/w) with respect to fructose (1), glucose (2) and betaine (3)

to the spectra without any mathematical adjustment. The standard error of calibration SEC (Table II) expressed for the two calibration series and three wavenumbers used is sufficiently low and comparable with this one of HPLC calibration (see Material and Methods).

To improve the accuracy of the sucrose determination, the multiple wavenumber approach to calibration according to equation [4] was also used. The following equations were obtained for

series A:

$$x = 2.6321 + 24.5332y_{1056} + 5.2016y_{1000} + 60.9589y_{928}$$

and series B:

$$x = 14.1576 + 20.7823y_{1050} - 1.3922y_{1000} + 43.9076y_{928}$$

As expected, the standard error of calibration SEC is approximately three times lower in the case of series A, but no change in accuracy was observed for series B (Series A: Real/Found - 14.01/14.01, 15.50/15.50, 17.01/17.03, 18.47/18.48, 20.00/19.98, SEC = 0.017 % (w/w), $100SEC/x_{av} = 0.10$ %. Serie B: Real/Found - 51.84/51.67, 54.00/54.30, 55.92/55.38, 58.00/58.32, 60.00/59.72, SEC = 0.79 % (w/w), $100SEC/x_{av} = 0.79$ %).

The objective of quantitative analysis is the determination of analyte concentration in mixtures of unknown composition. Therefore, four test samples of the three kinds of sugar factory juices were analyzed by FT-IR and the results were compared with those from HPLC analysis as the reference method and with data supplied by the manufacturer which were based on polarization measurements (Tables III).

II. Calibration and prediction results for series A and B

Series	Samples	Concentration of sucrose (%, w/w)			
		Real	Calculated at wavenumber (cm^{-1})		
			1056	1000	928
A	1	14.01	13.97	13.96	14.03
	2	15.50	15.56	15.55	15.40
	3	17.01	16.97	17.09	17.09
	4	18.47	18.52	18.37	18.45
	5	20.00	19.97	20.02	19.97
	SEC (%, w/w)	-	0.058	0.085	0.078
	100SEC/x_{av} (%)	-	0.34	0.50	0.46
B	1	51.84	51.49	51.44	51.91
	2	54.00	54.39	54.74	54.36
	3	55.92	55.75	55.65	55.14
	4	58.00	58.39	57.98	58.43
	5	60.00	59.63	59.95	60.04
	SEC (%, w/w)	-	0.444	0.511	0.557
	100SEC/x_{av} (%)	-	0.80	0.91	0.99

Although the relative error of prediction does not exceed the critical limit of 10 % (D u p u y et al., 1992), the lowest value near 6 % found for thick juice could seem to be a little too large for industrial application. On the other hand, it is still comparable with published values for the quantitative analysis of sugar solutions (K e m s l e y et al., 1992; D u p u y et al., 1992, 1993; D e L e n e M i r o u z e et al., 1993). Moreover, the polarimetric technique, which is conventionally used in industrial practice, is also characterized by high relative SEP (Tables III). Surprisingly, the application of the multiple wavenumber approach does not bring about an increase in the accuracy of analysis.

The best results are obtained at 1056 per cm, at which the prediction of sucrose in thin juice leads to an SEP of 0.17 % for the mean level of 15.75 % and variation range from 14.38 to 17.27 % (all w/w). For raw juice, the SEP is 0.46 % for the mean value of 18.00 % and variation range from 17.28 to 19.06 % (all w/w). In the case of thick juice, the remaining wavenumbers selected are also usable giving

III. Concentrations of sucrose in thin, raw and thick juice[a]

Juice		FT-IR[b]	FT-IR at wavenumberc			HPLC	Manu-facturer's data
			1056	1000	928		
Thin	1	17.17	17.15	16.91	17.27	17.37	16.5
	2	14.46	14.34	14.19	14.72	14.22	15.1
	3	14.34	14.34	14.19	14.38	14.37	14.3
	4	16.95	16.88	16.75	17.10	17.03	16.3
	SEP	0.187	0.170	0.328	0.297	-	0.831
	100SEP/x_{av}	1.19	1.08	2.12	1.87	-	5.34
Raw	1	17.60	17.74	17.45	17.44	17.28	17.4
	2	17.02	17.56	16.89	16.25	17.53	17.8
	3	17.67	18.02	17.81	17.10	18.14	16.5
	4	18.22	18.42	18.12	17.95	19.06	17.5
	SEP	0.656	0.461	0.691	1.099	-	1.318
	100SEP/x_{av}	3.72	2.57	3.93	6.39	-	7.62
Thick	1	64.28	64.15[d]	63.94	64.40	60.65	60.3
	2	55.09	55.28	55.20	55.06	54.58	54.9
	3	61.37	61.24	61.34	61.57	57.65	59.7
	4	62.77	63.23	62.99	62.34	59.27	58.2
	SEP	3.63	3.71	3.59	3.61	-	1.36
	100SEP/x_{av}	5.96	6.08	5.90	5.93	-	2.34

[a]%(w/w); [b]multiple wavenumber approach; [c](cm^{-1}); [d] 1050 cm^{-1}

SEP value about 3.60 % for the mean concentration of 58.04 % and variation range from 54.58 to 60.65 % (all w/w). The results are in good agreement with those of NIR determination of sucrose in the sugar factory juices as reported by V a c c a r i et al. (1990), where the standard deviation was about fourfold higher for thick juice than for raw and thin juices. The accuracy of the thick juice analysis could be improved by a decreasing of the concentration because absorbance of undiluted samples was above the value of 1.5 a.u. Another possibility may be given by using the first or second derivative of a spectral characteristic.

CONCLUSION

The results demonstrate applicability of the FT-IR spectroscopy in the mid-infrared region to the analysis of sugar factory juices. The samples are analyzed without any pre-treatment and only single-component calibration with a few standards is required. Subsequent analysis is fast and can be easily integrated into a control routine.

References

ALFARO, G.- MEURENS, M.- BIRTH, G. S.: Liquid analysis by dry-extract near-infrared reflectance on fiberglass. Appl. Spectrosc., *44*, 1990 : 979-986.

DE LENE MIROUZE, F. - BOULOU, J. C. - DUPUY, N. - MEURENS, M. - HUVENNE, J. P. - LEGRAND, P.: Quantitative analysis of glucose syrups by ATR/FT-IR spectroscopy. Appl. Spectrosc., *47*, 1993 : 1187-1191.

DULL, G. G. - GIANGIACOMO, R.: Determination of individual simple sugars in aqueous solution by near infrared spectrophotometry. J. Food Sci., *49*, 1984 : 1601-1603.

DUMOULIN, E. D. - AZAIS, B. P. - QUERAIN, J. T.: Determination of sugar and ethanol content in aqueous products of molasses distilleries by near infrared spectrophotometry. J. Food Sci., *52*, 1987 : 626-630.

DUPUY, N. - MEURENS, M. - SOMBRET, B. - LEGRAND, P. - HUVENNE, J. P.: Determination of sugars and organic acids in fruit juices by FT mid-IR investigation of dry extract. Appl. Spectrosc., *46*, 1992 : 860-863.

DUPUY, N. - MEURENS, M. - SOMBRET, B. - LEGRAND, P. - HUVENNE, J. P.: Multivariate determination of sugar powders by attenuated total reflectance infrared spectroscopy. Appl. Spectrosc., *47*, 1993 : 452-457.

GIANGIACOMO, R. - DULL, G. G.: Near infrared spectrophotometric determination of individual sugars in aqueous mixtures. J. Food Sci., *51*, 1986 : 679-683.

GIANGIACOMO, R. - MAGEE, J. B. - BIRTH, G. S. - DULL, G. G.: Predicting concentrations of individual sugars in dry mixtures by near-infrared reflectance spectroscopy. J. Food Sci., *46*, 1981 : 531-534.

KEMSLEY, E. K. - WILSON, R. H. - BELTON, P. S.: Potential of Fourier transform infrared spectroscopy and fiber optics for process control. J. Agric. Food Chem., *40*, 1992 : 435-438.

KEMSLEY, E. K. - ZHUO, L. - HAMMOURI, M. K. - WILSON, R. H.: Quantitative analysis of sugar solutions using infrared spectroscopy. Food Chem., *44*, 1992 : 229-304.

LANZA, E. - LI, B. W.: Application for near infrared spectroscopy for predicting the sugar content of fruit juices. J. Food Chem., *49*, 1984 : 995-998.

OSBORNE, B. G.: Comparative study of methods of linearization and scatter correction in NIR spectroscopy. Analyst, *113*, 1988 : 263-267.

OSBORNE, B. G. - FEARN, T. - RANDALL, P. G.: Measurement of fat and sucrose in dry cake mixes by near infrared reflectance spectroscopy. J. Fd. Technol., *18*, 1983 : 651-656.

PERMANYER, J. J. - PEREZ, M. L.: Compositional analysis of powdered cocoa products by near infrared reflectance spectroscopy. J. Food Sci., *54*, 1989 : 768-769.

SUZUKI, K. - McDONALD, C. E. - D'APPOLONIA, B. L.: Near-infrared reflectance analysis of bread. Cereal. Chem., *63*, 1986 : 320-325.

Potrav. Vědy, *11*, 1993 (6) : 493-502

SVERZUT, C. B. - VERMA, L. R. - FRENCH, A. D.: Sugarcane analysis using near infrared spectroscopy. Am. Soc. Agric. Eng., *30*, 1987 : 255-258.
VACCARI, G. - MANTOVANI, G. - SGUALDINO, G.: The development of near infrared (NIR) technique on line in the sugar factory. Sugar J., *4*, 1990 : 4-8.

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J. Moravcová, Z. Vanclová, M. Novotná (Vysoká škola chemicko-technologická – Ústav chemie přírodních látek a Centrální laboratoře, Praha)

Kvantitativní analýza cukrovarnických šťáv pomocí FT-IR spektrometrie

Infračervená spektrometrie (IR) je vhodnou metodou ke kvantitativnímu stanovení cukrů jak v roztocích, tak v pevných směsích. Nevyžaduje prakticky žádnou předseparaci vzorku a je srovnatelně citlivá a přesná jako jiné techniky. Se zavedením Fourierovy transformace signálu (FT-IR) a vývojem tzv. CIRCLE cely ze selenidu zinku, která umožňuje přímé měření vodných roztoků pomocí techniky zeslabeného totálního odrazu (ATR), význam IR spektrometrie ještě více vzrostl. Nezanedbatelná není ani možnost zapojení této metody jako „on-line" monitorovacího systému.

Byla studována možnost použití FT-IR spektrometrie v oblasti vlnočtu 800 až 1600 cm^{-1} ke stanovení sacharózy v surové, lehké a těžké cukrovarnické šťávě a jako referenční metoda byla použita vysokoúčinná kapalinová chromatografie na silně kyselém ionexu. Metodou nejmenších čtverců byly spočteny regresní přímky pro tři vybrané vlnočty (1056, 1000 a 928 cm^{-1}) a dvě sady standardních roztoků sacharózy ve vodě s koncentracemi odpovídajícími surové a lehké šťávě (série A) a těžké šťávě (série B). Vysoké hodnoty regresních koeficientů (0,9792 až 0,9996) potvrzují platnost Lambert-Beerova zákona a nalezené směrodatné odchylky kalibrace SEC odpovídají přesnosti referenční metody. Při analýze reálných vzorků odebraných v Cukrovaru Modřany byly nejlepší výsledky dosaženy při vlnočtu 1056 cm^{-1}. Směrodatná odchylka odhadu SEP činila pro surovou šťávu 0,46 %hm při průměrném obsahu 18,00 %hm sacharózy (rozmezí 17,28 až 19,06) a pro lehkou šťávu byla hodnota SEP 0,17 %hm při průměrné koncentraci sacharosy 15,75 %hm (rozmezí 14,38 až 17,27). Pro analýzu těžké šťávy byla nalezena hodnota SEP okolo 3,6 %hm (průměrný obsah sacharózy 58,04 %hm, rozmezí 54,58 až 60,65) pro všechny tři vybrané vlnočty. Výsledky dokazují vhodnost FT-IR spektrometrie ke stanovení sacharózy v cukrovarnických šťávách, u nichž je dosažená přesnost dokonce vyšší, než je uváděno pro stanovení cukrů v ovocných šťávách.

kvantitativní analýza; infračervená spektrometrie; FT-IR; sacharóza; cukrovarnické šťávy

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CHANGES OF INTERNAL ATMOSPHERE IN PACKAGED MEAT PRODUCTS

Lenka STAŇKOVÁ, Petr PIPEK, Tomáš RŮŽEK

Institute of Chemical Technology – Department of Food Preservation and Meat Technology, Technická 5, CZ-166 28 Praha 6, Czech Republic

The changes of pressure and composition of the atmosphere inside the vacuum packaged meat products together with the pH value and ammonium content were investigated. During the storage at 4 °C of vacuum packed sliced sausages (Junior and Jihočeský) the level of vacuum in the packets decreased and the composition of inside atmosphere changed. The rise of the internal pressure was due to the permeability of the used plastic films and to the production of gases during storage (e.g. CO_2). The content of CO_2 increased during the first period after packaging, but the rate of increase gradually became slower. An oxygen content decrease was also observed. The investigation of the mechanisms of these changes continues.

meat products; internal atmosphere; packaging; storage

The importance of packaging of meat products is related to the increasing rate of their distribution through the system of supermarkets. Packaging after slicing of sausages assures protection against recontamination and facilitates the handling during distribution and retail. On the other hand packaged sliced sausages undergo more quickly chemical and microbiological changes in comparison with relatively stable unpacked whole sausages. Their stability is not damaged by slicing, handling and vacuum treatment.

The atmosphere inside the packets of sliced sausages may change during storage. The changes are influenced by diffusion of some gases through the packaging material (usually plastic film) and by production or their uptake in interaction with sausages either physically (absorption, resp. desorption), chemically or microbiologically.

The aim of this paper is to study these changes. First the method for investigation were developed. Then pressure (level of vacuum) and internal atmosphere composition were measured during storage of packaged sliced meat products.

MATERIAL and METHODS

Sample preparation: The samples of sausages were selected from standard production at a Prague meat factory. After the removing of technological cover (casing) the sausages were sliced into 2 mm thick slices and then packed in plastic

film. The packets had standard dimensions and contained 80 g of sausage slices, in roof arrangement, per packet. After vacuum packing (level of vacuum was 9.6 kPa) the packets were kept at 4 °C. Two types of sausage were used: Junior (Lyoner type smoked and cooked sausage) and Jihočeský salám (dried smoked and cooked sausages). They were packed into two plastic combined films: PA/PE and PE/PP. Both films were characterized by the permeability for oxygen (see further text).

Methods: The packets with sausages were analyzed during 24 and 34 days, resp. The pressure inside packet (level of vacuum), the composition of inside atmosphere, the pH value and ammonium content (by Conway method) were measured.

Vacuum level in packet was measured using the controlled increasing of pressure in the environmental room. The investigated packet was placed into the pressure vessel, the pressure of which was decreased by the oil vacuum pump. The equilibration of pressure in and out of packet was indicated by a visible increasing of packet volume. The pressure at that moment was measured by mercury manometer.

Atmosphere composition inside the packet was investigated by gas chromatography. The samples of inside atmosphere were taken from packets under glycerol and were stored in special hermetically closed bottles. The gas chromatograph CHROM 4 with a TCD detector and filling columns (Porapack for CO_2 and molecular sieve for O_2) was used. The carrier gas was hydrogen.

Permeability of plastic films for gases was measured as an amount of gas that escaped from the room with atmospheric pressure through the film into the evacuated room per time unit. This amount was measured using a mercury manometer.

RESULTS and DISCUSSION

The changes of internal atmosphere were investigated during the storage of packed sliced meat products. Two types of sausages were used.

A certain volume of air remains in the packet after vacuum packaging; this volume depends on the level of vacuum. The pressure inside the packet increases from the moment of packaging due to a release of gases from the sausage and to a diffusion of gases through the plastic cover film.

There were no significant differences between both sausage types in decreasing the vacuum level; the pressure increases a little more in the case of sample packets in the combined film from PE/PP (Table I).

The internal atmosphere composition at the moment of packaging is the same as in the environmental room. This composition begins to change immediately after the closing of the packet due to chemical reactions, microbiological activity and selective diffusion of gases through the packing film.

The CO_2 content in the internal atmosphere increased immediately after packaging (it was probably released from the chemical or physical bond in sausage

I. Pressure changes inside the packets during storage of vacuum packed sausages [kPa]

Sausage	Film	Storage time [days]					
		0	5	12	18	26	32
Junior	PE/PP	9.6	9.7	10.9	14.5	16.7	19.8
	PA/PE	9.6	9.6	10.8	14.3	16.5	19.4
Jihočeský	PE/PP	9.6	9.7	10.9	14.5	16.6	19.7
	PA/PE	9.6	9.6	10.8	14.4	16.5	19.3

structure). This content increased slowly also on the following days, but the rate of increase gradually became slower. The activity of microorganisms could respond to this CO_2 production.

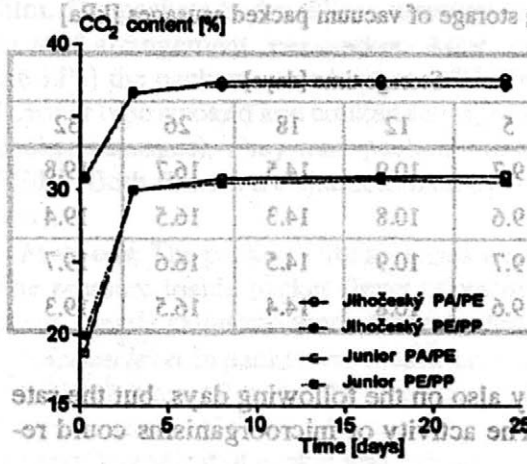
The O_2 content in the internal atmosphere decreased during the storage with an increasing content of CO_2. The rate of this decrease became smaller and the end O_2 content was about one third of initial concentration. The permeability for oxygen at the pressure difference of 0.1 MPa for the 0.1 mm thick PE/PP film (28 ml per m^2 a day) was higher that for the 0.09 mm thick film from PA/PE (14 ml per m^2 a day).

Because of partial pressure differences CO_2 may pass through the film to the environmental room (depending on the permeability of film), oxygen may diffuse in the opposite direction (Tables I and II).

The concentrations of both gases changed sooner in the case of the dried sausage Jihočeský. The two types of sausage differed in the water content, or in the water activity (that at sausage Junior was higher). The concentration of oxygen decreased

II. The internal atmosphere composition in packed sausage Junior during storage (in vol. %)

Gas	Storage time [days]				
	0	3	8	17	24
Film PE/PP					
Oxygen	16.5	12.0	9.5	5.5	5.5
CO_2	19.9	29.9	30.4	30.6	30.5
Nitrogen	63.6	58.1	60.1	63.9	64.0
Film PA/PE					
Oxygen	15.7	12.3	9.8	5.5	5.4
CO_2	18.7	29.9	30.7	30.8	30.9
Nitrogen	65.5	57.8	59.5	63.7	63.7



1. Changes of CO_2 content during storage of vacuum packed sausages

III. The internal atmosphere composition in packed sausage Jihočeský during storage (in vol. %)

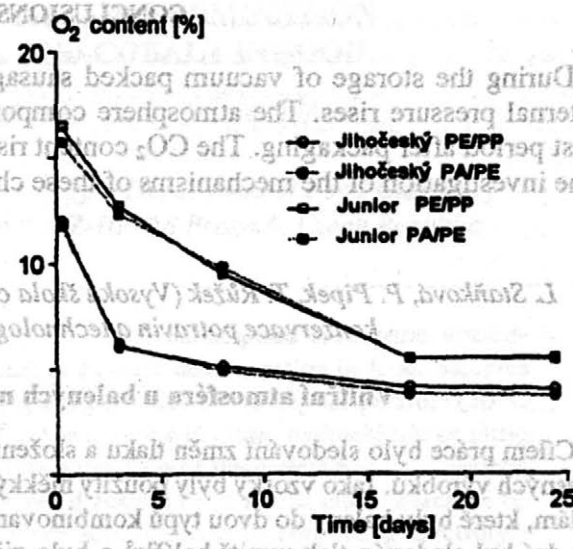
Gas	Storage time [days]				
	0	3	8	17	24
Film PE/PP					
Oxygen	12.0	6.1	5.1	4.1	4.0
CO_2	30.8	36.7	37.1	37.2	37.1
Nitrogen	57.2	57.2	57.8	58.7	58.9
Film PA/PE					
Oxygen	11.8	6.2	4.9	3.8	3.7
CO_2	30.7	36.6	37.4	37.5	37.6
Nitrogen	57.5	57.2	57.7	58.7	58.7

more quickly for the sausage Jihočeský (Fig. 1); it is probably due to the higher fat content and higher permeability of dried sausage for gases in comparison with the relatively compact structure of fine comminuted smoked sausage Junior. The lipid oxidation may occur faster in case of the dried sausage Jihočeský (the greater surface).

The release of CO_2 for the sausage Jihočeský occurred sooner; during the first day of storage the same concentrations of CO_2 were achieved, those were observed for smoked sausage Junior only after 12 weeks of storage (Fig. 2).

The differences in conditions for microbiological changes may concern the relatively lower water activity for dried sausage, that may brake the growth of microorganisms; the greater permeability facilitates the chemical reactions.

2. Changes of oxygen content during storage of vacuum packed sausages



The changes of pH value (Table IV) were very small. It can be explained by the low storage temperature (4 °C), when the changes caused by microorganisms occur slowly.

IV. pH value of the packed sausage Jihočeský during storage

Sausage	Film	Storage time [days]					
		0	5	12	18	26	32
Junior	PE/PP	6.10	6.11	6.11	6.10	6.07	6.03
	PA/PE	6.10	6.10	6.11	6.10	6.07	6.03
Jihočeský	PE/PP	5.93	5.92	5.92	5.91	5.88	5.84
	PA/PE	5.93	5.93	5.92	5.90	5.87	5.84

The ammonium content (Table V) increased in the last week of storage, there were no differences between the used films; the dried sausage Jihočeský contained more ammonium at the end of the storage period.

V. Ammonium content in packed sausage Jihočeský during storage [mg.kg^{-1}]

Sausage	Film	Storage time [days]					
		0	5	12	18	26	32
Junior	PE/PP	197	197	203	216	236	262
	PA/PE	197	197	203	216	236	262
Jihočeský	PE/PP	197	197	216	243	265	284
	PA/PE	197	197	216	243	265	284

CONCLUSIONS

During the storage of vacuum packed sausages in the used plastic films, the internal pressure rises. The atmosphere composition changes, especially during first period after packaging. The CO_2 content rises and oxygen content decreases. The investigation of the mechanisms of these changes continues.

L. Staňková, P. Pipek, T. Růžek (Vysoká škola chemicko-technologická – Ústav konzervace potravin a technologie masa, Praha)

Vnitřní atmosféra u balených masných výrobků

Cílem práce bylo sledování změn tlaku a složení atmosféry při skladování běžných masných výrobků. Jako vzorky byly použity měkký salám Junior a trvanlivý Jihočeský salám, které byly baleny do dvou typů kombinovaných fólií: PA/PE a PE/PP. Po dobu 34 dní byl sledován tlak uvnitř balíčků a bylo zjišťováno složení vnitřní atmosféry. Současně bylo měřeno pH a obsah amoniaku podle Conwaye a zjišťována propustnost použitých fólií. Tlak uvnitř balíčků byl měřen na principu vyrovnání tlaku (v postupně evakuovaném) prostoru. Vyrovnání tlaku se projevilo nadmutím fólie kolem salámu; tento tlak byl měřen rtuťovým manometrem. Složení atmosféry uvnitř balíčku bylo zjišťováno metodou plynové chromatografie; vzorek vnitřní atmosféry byl z balíčku odebírán pod hladinou glycerolu a až do vlastní analýzy byl uchováván v hermeticky uzavřené lahvičce. Bylo zjištěno, že od okamžiku zabalení se tlak uvnitř balíčku zvyšoval, což souvisí jak s uvolňováním plynů z náplně (salámu), tak i s určitou propustností použitých fólií. Mezi oběma typy salámů nebyly významné rozdíly ve snižování hladiny vakua, poněkud více se zvýšil tlak u salámů (obou druhů) zabalených do kombinované fólie PE/PP (tab. I). Vnitřní atmosféra v okamžiku těsně po zabalení odpovídá svým složením okolní atmosféře. Po zabalení salámu se ve vnitřní atmosféře okamžitě zvýšil obsah CO_2 (asi se uvolnil plyn vázaný chemicky či fyzikálně ve struktuře salámu), a pravděpodobně vzrůstal také v důsledku metabolické aktivity mikroorganismů. Se zvyšováním obsahu CO_2 byl současně zaznamenán i pokles obsahu O_2. Vzhledem k parciálnímu tlaku CO_2 v okolní atmosféře má tento plyn tendenci unikat z balíčku (v závislosti na propustnosti použité fólie), u kyslíku je tomu naopak (tab. II a III). Změny koncentrace obou plynů nastaly dříve u salámu Jihočeský, který se lišil od salámu Junior zejména obsahem, resp. aktivitou vody (vyšší u měkkého salámu Junior). Pozorované změny pH (tab. IV) a obsahu amoniaku (tab. V) byly jen velmi malé - souvisí to se skladovací teplotou 4 °C, při níž uvedené změny (způsobené zejména mikrobiálními pochody) probíhají jen velmi pomalu. Příčiny změn vnitřní atmosféry jsou dále zkoumány.

masné výrobky; vnitřní atmosféra; balení; skladování

Potrav. Vědy, *11*, 1993 (6) : 509-517

VOLTAMMETRIC DETERMINATION OF NICKEL AND COBALT IN FOOD

Richard KOPLÍK, Šárka NĚMCOVÁ

Institute of Chemical Technology – Department of Food Chemistry and Analysis, Technická 5, CZ-166 28 Praha 6, Czech Republic

Differential pulse polarography and differential pulse adsorption voltammetry were employed for nickel and cobalt determination in food. Samples were decomposed by dry ashing at a gradually increasing temperature (50 °C per hour) to maximum 450 °C. Ash extracts in dilute hydrochloric or nitric acid were additionally treated by extraction of trace elements into dithizon solution in chloroform and by wet digestion of extracted dithizonates. Final voltammetric measurements were accomplished in supporting electrolyte containing 0.5 mol/l NH_3-NH_4Cl and 0.01 % of dimethylglyoxime. The use of wet digestion instead of dry ashing for sample decomposition gives the same results. Detection limits of nickel and cobalt using the sample weight 5 g are 0.08 and 0.009 mg/kg , respectively.

nickel; cobalt; voltammetry; polarography; food

Nickel and cobalt represent nutritionally important essential trace elements (M e r t z, 1987). Moreover, nickel can also occur in environmetal samples and some foods as a contaminant (e. g. in hardened fats). In the Czech Republic nickel is included among 13 chemical elements, the maximum contents of which in food are stated by hygienic regulation (Hygienické předpisy, 1986).

Atomic absorption spectrometry is a method most often used for determination of nickel and cobalt in food (L o o n, 1980) after suitable sample preparation. Actual concentrations of nickel and namely of cobalt in biological samples are very low (as a rule below 0.1 mg/kg). Therefore it is necessary to use the highly sensitive method of graphite furnace atomization in AAS of nickel (T r u s k a et al., 1985) or cobalt (V a l d m a n, 1983). Another approach utilizes element preconcentration e. g. by extraction of metale chelates with APDC into methylisobutylketone and measurement by flame AAS (G e l m a n, 1972; B a r b e r a et al., 1985).

Voltammetric methods, namely differential pulse polarography (DPP) and voltammetry, enable very sensitive determination of nickel and cobalt. The measurements can be carried out in neutral or alkaline solutions e. g. in the electrolyte 1 mol/l NH_3-NH_4Cl. It is possible to determine up to six metal ions in this electrolyte, but potentials of DPP peaks of cobalt and zinc are very close (-1.2 and -1.23 V vs. Ag/AgCl/sat. KCl, respectively) resulting in complete overlapping of

these peaks. Furthermore, concentrations of cobalt in biological materials are mostly lower by two to three orders of magnitude than those of zinc. Therefore determination of cobalt traces at an excess of zinc is not possible in this electrolyte. Addition of dimethylglyoxime - DMG (N a r r e s et al., 1984; P i h l a r et al., 1981; A d e l o j u et al., 1984, 1985) or other oximes of dicarbonyl compounds (D o n a t, B r u l a n d, 1988; M e y e r, N e e b, 1985) to supporting electrolyte made possible substantial enhancement of sensitivity of nickel and cobalt determination as well as certain separation of cobalt peak the from interfering signal of zinc. Nickel and cobalt complexes with the respective oxime are accumulated on the surface of mercury drop by adsorption. This method is therefore known as adsorption voltammetry or adsorption stripping voltammetry (K a l v o d a, K o p a n i c a, 1989). Certain metals can be determined by adsorption voltammetry even at ultratrace levels. This article is devoted to application of differential pulse polarography (DPP) and differential pulse adsorption voltammetry (DPAV) for nickel and cobalt determination in food.

MATERIAL and METHODS

Chemicals and glassware: Except dithizone analytical grade (Sojuzchimexport, USSR) all other chemicals were products of Lachema Brno of analytical grade purity. All aqueous solutions were prepared using redistilled water.

All glassware was decontaminated before use. Cleaning procedure is described in the previous paper (K o p l í k, N ě m c o v á, 1993).

Measuring instruments and conditions: The same equipment was used for voltammetric measurements as described in the previous article (K o p l í k, N ě m c o v á, 1993).

Parameters used for nickel and cobalt determination were as follows:

- three-electrode measuring system (HMDE or SMDE, Ag/AgCl/sat. KCl electrode, platinum auxiliary electrode),
- drop area 0.7 mm^2,
- mode differential pulse,
- pulse amplitude 50 mV,
- pulse duration 100 ms,
- current sampling time 20 ms,
- initial or deposition potential -0.8 V,
- deposition time (DPAV) 30 to 180 s under stirring followed by 20 s without stirring or only 30 to 60 s without stirring,
- potential scan direction -,
- scan rate 20 mV per s (DPAV) or 5 mV per s (DPP),
- pulse frequency (DPAV) g/s,
- drop time (DPP) 2 s.

A spectrometer Varian AA 775 ABQ was used for flame atomic absorption spectrometric determination of nickel and cobalt. Both elements were measured in acetylene-air flame using 232.0 and 240.7 nm lines, respectively.

Sample decomposition: Dry ashing of samples at a gradually increasing temperature (maximum 450 °C) was applied as the main method of decomposition. Generally 5 g test portions were used and acidic ash extracts were diluted to the volume 50 ml. The final solution of ash extract was 0.3 mol/l in HCl or 0.1 mol/l in HNO_3. Some check analyses were carried out using wet digestion of samples in HNO_3-$HClO_4$-H_2SO_4 mixture. Details are given in the previous paper (K o p l í k, N ě m c o v á, 1993).

Additional sample treatment and voltammetric determination of metals: 3 ml of 30% solution of tartaric acid and 2 drops of 0.1% solution of phenolphthaleine in ethanol were added to 5 - 20 ml of ash extract in 50 ml separatory funnel. The mixture was carefully neutralized by dropwise addition of 5 mol/l ammonia solution until the solution turned light pink. Then 5 ml of dithizon solution in chloroform (c = 100 mg/l) was added and the mixture was extracted by 2 min. thorough shaking. After separation of liquid phases the chloroform layer was transferred to 50 ml Kjeldahl flask. Extraction of water phase with another portion of dithizon solution was repeated till the chloroform layer after extraction remained green. After rinsing of water phase with 5 ml of chloroform the obtained extract was added to joint extracts of metal dithizonates in Kjeldahl flask. The solvent was evaporated and dry residue was mineralized by 10 min. heating with a mixture of 2 ml of 65% HNO_3 and 0.5 ml of 70% $HClO_4$. Acids were completely evaporated by heating of the flask in hot flame. After cooling of the flask the residue was dissolved in 20 ml of supporting electrolyte (0.5 mol/l NH_3 and NH_4Cl).

5 ml of this solution was transferred to a polarographic cell and 0.05 ml of 1% dimethylglyoxime (DMG) solution in ethanol was added. After oxygen removing from this solution by 5 min. bubbling with nitrogen, nickel and cobalt were determined using DPP or DPAV under the above mentioned conditions. The quantitation was made mostly by standard addition technique.

RESULTS and DISCUSSION

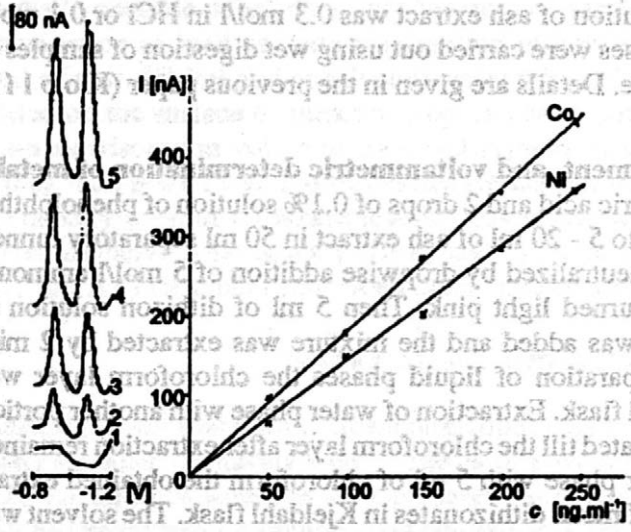
Supporting electrolyte containing 0.5 mol/l NH_3-NH_4Cl and 0.01% DMG was used for nickel and cobalt determination. Two methods of measurement were tested:

1. differential pulse polarography (DPP) at static mercury drop electrode (SMDE);
2. differential pulse adsorption voltammetry (DPAV) at hanging mercury drop electrode (HMDE).

The former is suitable for measurement of higher concentrations of both elements (approximately 0.1 µg/ml), the latter for measurement of trace and ultratrace concentrations. The potentials of the nickel and cobalt peaks in this electrolyte are

-0.92 and -1.07 V vs. Ag/AgCl/sat. KCl electrode, respectively. Voltammetric curves were recorded from -0.8 to -1.2 V.

Calibration of nickel and cobalt measurement by DPP is shown in Fig. 1. Calibration curves are linear up to 300 ng/ml for both elements. Therefore in the interval of 50 to 150 ng/ml nickel and cobalt can be determined by a standard addition method and from 50 to 300 ng/ml on the basis of calibration curve.



(1): electrolyte 0.5 mol NH_3-NH_4Cl per l, 0.01% DMG
(2): 49 ng (Ni, Co)/ml
(3): 98 ng (Ni, Co)/ml
(4): 146 ng (Ni, Co)/ml
(5): 194 ng (Ni, Co)/ml

1. Calibration curve for nickel and cobalt determination by differential pulse polarography and the shape of the respective DPP curves

DPP method may be applied for nickel and cobalt determination in real food samples. An example of nickel determination using DPP is given in Fig. 2. Owing to the alkaline medium used for final measurement, it is necessary to perform separation of trace elements from phosphates and cations forming insoluble hydroxides and salts in alkaline solutions (Ca^{2+}, Mg^{2+}, Fe^{3+}) (N a n g n i o t, 1985) for a number of samples. This was accomplished by extraction of metals dithizonates from buffered aqueous solution to chloroform and their decomposition by wet digestion. Apart from nickel and cobalt, cadmium, copper, lead, tin and zinc are also separated using the described extraction procedure. In a sample treated in this way, it is also possible to determine copper and zinc using DPP in ammonia-containing electrolyte before addition of DMG. Potentials of DPP peaks of copper and zinc in this electrolyte are -0.35 and -1.22 V, respectively.



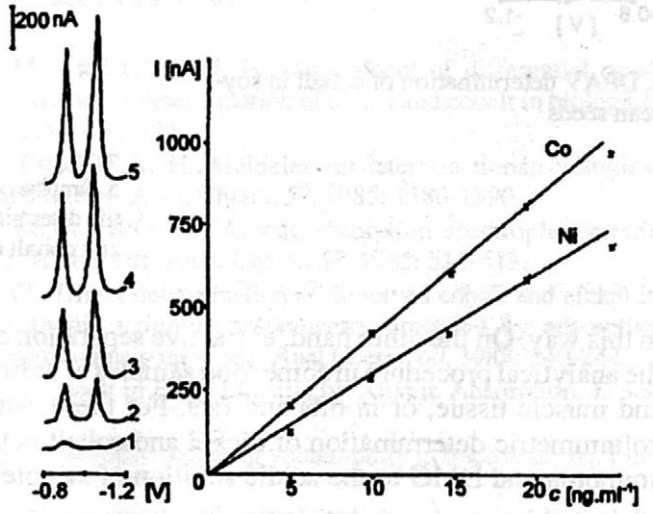
2. DPP determination of nickel in soybean seeds
electrolyte 0.5 mol per l NH_3-NH_4Cl, 0.01% DMG; initial potential -0.8 V
(1): sample; (2): (1) + 49 ng Ni/ml; (3): (1) + 93 ng Ni/ml

A convenient approach to measurement of nickel and cobalt levels lower than 50 ng/ml is the use of DPAV at HMDE. A substantial increase of sensitivity can be achieved by extending deposition time and by stirring during deposition. The height of nickel and cobalt voltammetric peaks is proportional to concentration in the interval of 0 to 200 ng/ml when deposition time 30 s without stirring is applied. The longer the deposition time (namely under stirring), the narrower is the range of linear dependence of concentration and peak height.

Calibration curves for nickel and cobalt determination by DPAV using deposition time 30 s with stirring followed by 20 s without stirring are shown in Fig. 3. The linear range is limited to 20 ng/ml and therefore this procedure is suitable only for measurement of concentrations below 10 ng/ml.

Utilization of DPAV method for cobalt determination and simultaneous determination of nickel and cobalt in food samples is illustrated in Fig. 3 and 4,

(1): electrolyte 0.5 mol NH_3-NH_4Cl per 1, 0.01% DMG
deposition time 30 + 20 s
(2): 4.9 ng (Ni, Co)/ml
(3): 9.8 ng (Ni, Co)/ml
(4): 14.6 ng (Ni, Co)/ml
(5): 19.4 ng (Ni, Co)/ml



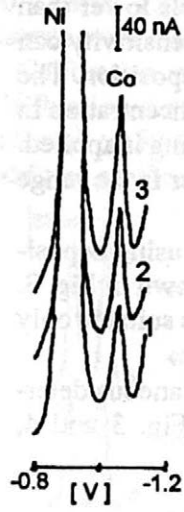
3. Calibration curve for nickel and cobalt determination by differential pulse adsorption voltammetry and the shape of the respective voltammetric curves

respectively.

The results of various procedures and methods were compared, in order to verify reliability of the proposed method. The data are summarized in Table I.

With respect to a good agreement of results obtained using dry ashing and wet digestion, the application of proposed procedure (dry ashing, extractive separation of trace metals and voltammetric measurement) is more advantageous. Using this procedure and starting from the sample weight 5 g, detection limits of nickel and cobalt 0.08 and 0.009 mg/kg, respectively, were achieved.

A certain disadvantage of this approach consists in the necessity of trace elements separation from interfering components taking much time. Namely samples of milk, dairy products and most foods of plant origin have to be treated



electrolyte:
0.5 mol NH_3- NH_4Cl per l,
0.01% DMG;
deposition time 60 s

(1): sample
(2): (1) + 2.46 ng Co/ml
(3): (1) + 4.90 ng Co/ml

4. DPAV determination of cobalt in soybean seeds

electrolyte:
0.3 mol NH_3-NH_4Cl per l,
0.01 % DMG;
deposition time 60 + 20 s

(1): sample
(2): (1) + 4.99 ng (Ni,Co)/ml
(3): (1) + 9.01 ng(Ni, Co)/ml



5. Simultaneous voltammetric determination of nickel and cobalt in bovine liver

in this way. On the other hand, extractive separation of metals can be omitted from the analytical procedure in some food samples of animal origin such as liver, kidney and muscle tissue, or in oils and fats. For these samples, a medium suitable for voltammetric determination of nickel and cobalt is formed simply by addition of ammonia and DMG to the acidic solution of sample ash (0.2 ml of 15 mol/l NH_3

I. Results of nickel and cobalt determination in soybean seeds using various analytical procedures

Analytical procedure	Element content[a] found [mg .kg^{-1}]	
	Ni	Co
Dry ashing, extractive separation, voltammetric detn. - evaluation by standard addition	4.97 ± 0.49	0.140 ± 0.020
Dry ashing, extractive separation, voltammetric detn. - evaluation using calibration curve	4.88 ± 0.36	
Wet digestion, extractive separation, voltammetric detn. - evaluation by standard addition	5.01 ± 0.43	0.138 ± 0.026
Dry ashing, detn. by flame AAS	4.93 ± 0.28	≤ 0.5

a - mean and standard deviation (n = 5)

and 0.05 ml of 1% DMG in ethanol are added to 5 ml of sample solution in 0.3 mol/l HCl). After addition of ammonia no precipitate may appear. This simplification results in the lowering of sample contamination during analysis and therefore leads to lower detection limits (0.02 and 0.001 m/kg for nickel and cobalt, respectively).

List of abbreviations

AAS	atomic absorption spectrometry
APDC	ammonium pyrrolidinedithiocarbamate
DMG	dimethylglyoxime
DPAV	differential pulse adsorption voltammetry
DPP	differential pulse polarography

References

ADELOJU, S. B. - BOND, A. M. - BRIGGS, M. H.: Assessment of differential pulse adsorption voltammetry for the simultaneous determination of nickel and cobalt in biological materials. Anal. Chim. Acta, *164*, 1984: 181-194.

ADELOJU, S. B. - BOND, A. M. - BRIGGS, M. H.: Multielement determination in biological materials by differential pulse voltammetry. Anal. Chem., *57*, 1985: 1386-1390.

BARBERÁ, R. - FARRÉ, R. - MONTORO, R.: Atomic absorption spectrophotometric determination of cobalt in foods. J. Assoc. Off. Anal. Chem., *68*, 1985: 511-513.

DONAT, J. R. - BRULAND, K. W.: Direct determination of dissolved cobalt and nickel in seewater by differential pulse cathodic stripping voltammetry preceded by adsorptive collection of cyclohexane-1,2-dione dioxime complexes. Anal Chem., *60*, 1988: 240-244.

GELMAN, A. L: Determination of Cobalt in Plant Material by Atomic Absorption. J. Sci. Food Agric., *23*, 1972: 299-305.

KALVODA, R. - KOPANICA, M: Adsorptive stripping voltammetry in trace analysis. Pure Appl. Chem., *61*, 1989: 97-112.

KOPLÍK, R. - NĚMCOVÁ, Š.: Voltametrické stanovení kadmia, olova, mědi a zinku v potravinách. Potrav. Vědy, *11*, 1993: 371-380.

LOON, J. C. van: Analytical Atomic Absorption Spectroscopy. Selested Methods. New York, Academic Press 1980.

MERTZ, W. (Ed.): Trace Elements in Human and Animal Nutrition. Fifth Ed., Vol. 1, San Diego, Academic Press, Inc. 1987.

MEYER, A. - NEEB, R.: Bestimung von Cobalt und Nickel in einigen biologischen Matrices - Vergleich Chelat - Gas - Chromatographie und Adsorptions - Voltametrie. Fresenius Z. Anal. Chem., *321*, 1985: 235-241.

NANGNIOT, P.: Applications of polarographic and voltammetric analysis in the fields of agriculture and alimentation. Trends Anal. Chem., *4*, 1985: 155-161.

NARRES, H. D. - VALENTA, P. - NÜRNBERG, H. W.: Die voltammetrische Bestimmung von Schwermetallen in Fleisch und inneren Organen von Schlachtrindern. Z. Lebensm. Unters. Forsch., *179*, 1984: 440-446.

PIHLAR, B. - VALENTA, P. - NÜRNBERG, H. W.: New high-performance analytical procedure for the voltammetric determination of nickel in routine analysis of waters, biological materials and food. Fresenius Z. Anal. Chem., *307*, 1981: 337-346.
TRUSKA, P. - PAĽUŠOVÁ, O. - BALÁŽOVÁ, G. - URSÍNYOVÁ, M.: Nikel v životnom prostredí a v biologických materiáloch. In: Mikroelementy 85. Sbor. přednášek XIX. semináře o metodice stanovení a významu stopových prvků v biologickém materiálu. Brno 7. 5. 1985: 96-101.
VALDMAN, J.: Kobalt v našich běžných potravinách. In: Mikroelementy 83. Sbor. přednášek XVII. semináře o metodice stanovení a významu stopových prvků v biologickém materiálu. Rožnov pod Radhoštěm 3. - 5. 5. 1983: 112-117.
Hygienické předpisy, svazek 61/1986, směrnice č. 69 MZ ČSR, Praha, Avicenum 1986.

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R. Koplík, Š. Němcová (Vysoká škola chemicko-technologická, Praha)

Voltametrické stanovení niklu a kobaltu v potravinách

Pro stanovení niklu a kobaltu v potravinách se vedle spektrofotometrických metod nejčastěji uplatňuje atomová absorpční spektrometrie. Plamenová technika AAS často není dostatečně citlivá pro stanovení nižších hladin niklu a zvláště kobaltu, takže musí být kombinována s prekoncentrací analytů (G e l m a n, 1972; B a r b e r a et al., 1985). Vhodnější je tedy aplikace vysoce citlivé techniky AAS s elektrochemickou atomizací (V a l d m a n, 1983; T r u s k a et al., 1985).

Citlivostí a selektivitou se této metodě vyrovnají voltametrické metody stanovení niklu a kobaltu (N a r r e s et al., 1984; P i h l a r et al., 1981; A d e l o j u et al., 1984, 1985; D o n a t, B r u l a n d, 1988; M e y e r, N e e b, 1985). Voltametrická měření se zpravidla provádějí v diferenčním pulsním modu s použitím amoniakálních nebo acetátových elektrolytů s přídavkem oximu dikarbonylové sloučeniny.

V této práci byla pro stanovení niklu a kobaltu použita diferenční pulsní polarografie (DPP) a diferenční pulsní adsorpční voltametrie (DPAV). Vzorky potravin byly mineralizovány zpopelněním při nárůstu teploty (50 °C/h) na 450 °C. Podrobnosti jsou uvedeny v předchozí práci (K o p l í k, N ě m c o v á, 1993). Z výluhu popela ve zředěné kyselině chlorovodíkové nebo dusičné byly nikl a kobalt (spolu s Cd, Cu, Pb, Sn, Zn) po úpravě pH separovány opakovanou extrakcí chloroformovým roztokem dithizonu (c = 100 mg/l). Extrakt byl po odpaření rozpouštědla zminezalizován směsí HNO_3 - $HClO_4$ a získaný zbytek byl rozpuštěn v základním elektrolytu (0,5 mol/l NH_3 a NH_4Cl). Před měřením metodou DPP nebo DPAV bylo ke vzorku přidáno takové množství ethanolového roztoku dimethylglyoximu, aby jeho výsledná koncentrace byla 0,01 %.

Kalibrace a tvary příslušných voltametrických křivek niklu a kobaltu, získané metodami DPP a DPA,V jsou uvedeny na obr. 1 a 3. Metody se liší zejména citlivostí a použitelným koncentračním rozsahem. Příklad využití DPP pro stanovení niklu v reálném vzorku ukazuje obr. 2. Aplikace metody DPAV je demonstrována na příkladech stanovení kobaltu a společného stanovení niklu a kobaltu v nižším koncentračním oboru (obr. 4 a 5).

Spolehlivost navrženého analytického postupu (zpopelnění, extrakční separace, voltametrické měření) byla ověřena porovnáním s výsledky AAS a voltametrického stanovení při použití mokré mineralizace vzorku (tab. I). Výsledky jsou ve velmi dobré shodě. Meze detekce navrženého postupu při použití navážky 5 g vzorku činí pro nikl 0,08 a pro kobalt 0,009 mg/kg.

nikl; kobalt; voltametrie; polarografie; potraviny

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SURVEY

BIO AND CHEMILUMINESCENT SYSTEMS APPLIED TO FLOW SENSORS AND VIRAL DNA AND RNA DETECTION

*S. GIROTTI, E. FERRI, S. GHINI, M. MUSIANI [1], M. ZERBINI [1], G. CARREA [2], R. BOVARA [2], A. RODA [3], I. ZINI [4], *Pavel RAUCH [5]*

Institute of Chemical Sciences, University of Bologna; [1]Institute of Microbiology, University of Bologna; [2]Institute of Hormone Chemistry, CNR, Milano; [3]Department of Pharmaceutical Sciences, University of Bologna; [4]Institute of Human Physiology, University of Modena, Italy;

[5]Institute of Chemical Technology – Department of Biochemistry and Microbiology, Technická 5, CZ-166 28 Praha 6, Czech Republic

Immobilized enzymes are widely used to assay several analytes and enzyme activities. The use of immobilized enzymes makes these reagents recoverable, disposable and in most cases increases their stability and catalytic activity. Metabolite analysis in biological systems requires sensitive and specific methods because analytes are present at low concentrations and further dilution of biological samples is often needed to avoid interference due to matrix effects. In analytical enzymology luminescence is a well-established tool which with to amplify the analytical signal and to develop fast and sensitive methods (K r i c k a et al., 1984; C a m p b e l l, 1988). In conjunction with bioluminescent enzymes (firefly and bacterial luciferases) and chemiluminescent catalyst (peroxidase and alkaline phosphatase) (R o d a et al., 1988, 1989; D e L u c a, M c E l r o y, 1986; B e c k, K ö s t e r, 1990), we set up high-sensitive flow sensors based on the use of nylon tube coil or epoxy methacrylate (Eupergit C) column as solid support. When these flow sensors were applied to *in vivo* determination a suitable microdialysis probe inserted directly into tissues or blood allows continuous measurement of extracellular analyte levels by means of a bioluminescent flow detector system.

In the diagnosis of several microbial diseases, the use of nucleic acid hybridization techniques has led to the use of several radioactive or non isotopic labelled probes which can be immunoenzymatically visualized (T e n o v e r, 1988). It has also demonstrated that chemiluminescent enzyme substrates are the most sensitive tools available for the detection of enzyme conjugates, including the nucleic acid hybridized probe (M u s i a n i et al., 1991). The hybridized DNA and RNA probes are generally visualized by Fab fragments labelled with alkaline phosphatase (ALP) or horseradish peroxidase (HRP) (G i r o t t i et al., 1991).

* To whom the correspondence should be addressed

Here we summarize our experience using immobilized luminescent enzymes coupled with the analyte specific enzyme and using DNA and RNA chemiluminescent probes applied to viral detection.

LUMINESCENT SYSTEMS

Firefly Bioluminescence

Firefly luminescence is the most extensively studied bioluminescent system (D e L u c a, M c E l r o y, 1986; L e a c h, 1981). The light-producing reaction requires the enzyme luciferase, luciferin, Mg^{2+}, ATP, and molecular oxygen, which give an oxyluciferyl adenylate-enzyme complex in the excited state. After emission (560 nm) the ground-state complex dissociates to form enzyme, AMP, pyrophosphate (PPi), oxyluciferin, and carbon dioxide:

$$ATP + Luciferin + O_2 \xrightarrow{Luciferase} AMP + PPi + CO_2 + oxyluciferin + LIGHT$$

The concentration of other metabolites, of cofactors involved in the luminescent reaction like Mg^{2+}, or the activity of enzymes participating in coupled reaction leading to the formation or consumption of ATP can be assayed with the aid of the firefly luciferase-dependent reaction (K r i c k a et al., 1984; C a m p b e l l, 1988; R o d a et al., 1989).

Bacterial Bioluminescence

The bioluminescent enzyme system from marine bacteria (K r i c k a et al., 1984; C a m p b e l l, 1988; R o d a et al., 1989; D e L u c a, M c E l r o y, 1986) consists of an NAD(P)H : FMN oxidoreductase and a luciferase which emit light at 490 nm in the presence of FMN, NAD(P)H, a long chain aliphatic aldehyde and molecular oxygen according to reactions (1) and (2).

$$(1.)\quad NAD(P)H + H^+ + FMN \xrightarrow[\text{oxidoreductase}]{\text{NAD(P)H : FMN}} NAD(P) + FMNH_2$$

$$(2.)\quad FMNH_2 + RCHO + O_2 \xrightarrow{\text{luciferace}} FMN + RCOOH + H_2O + LIGHT$$

Various substances of biological interest and enzyme activities can be analyzed by coupling the luciferase and the oxidoreductase to a third reaction which produces or consumes NADH or NADPH.

Luminol Chemiluminescence

The amount of H_2O_2 which derives from the catalysis of a specific oxidase may be assayed be adding excess HRP and luminol (K r i c k a et al., 1984; C a m p b e l l, 1988; R o d a et al., 1989; D e L u c a, M c E l r o y, 1986) and measuring steady state chemiluminescence intensity according the scheme:

$$\text{Luminol} + 2\,H_2O_2 + OH^- \xrightarrow{\text{HRP}} \text{Aminophthalate} + 3\,H_2O + N_2 + \text{LIGHT}$$

Since the quantum yield of this reaction is very low (~10 %), the addition of enhancers such as luciferin, p-iodophenol or p-hydroxy cinnamic acid in the system further increases light emission and transforms the signal from a flash to a steady state output (T h o r p e et al., 1985). The enhancers also reduce the background light emission from the luminol-oxidant mixture, further improving the signal/background ratio.

Dioxetane Chemiluminescence

New ALP chemiluminescent substrates are the adamantyl-1,2-dioxetane phenyl phosphate (PPD) and its Cl- and Br-derivatives. In the presence of alkaline phosphatase, PPD is dephosphorylated and produces an unstable intermediate which decomposes further, emitting a glow of light in direct proportion to the amount of alkaline phosphatase (B e c k, K ö s t e r, 1990):

$$\text{PPD} \xrightarrow{\text{ALP}} [\text{PD}]^* + \text{PHOSPHATE}$$

$$[\text{PD}]^* + \text{F} \xrightarrow{\text{ENERGY TRANSFER}} \text{PD} + [\text{F}]^*$$

$$[\text{F}]^* \longrightarrow \text{F} + \text{LIGHT}$$

where the asterisks represent an excited state and F is the fluorescent acceptor (enhancer). The Cl- and Br-substituted dioxetanes given lower background and lower detection limits when compared with non derivatized ones (B r o n s t e i n et al., 1991). The new dioxetanes contain a modified hydrophobic adamantyl group to prevent aggregation, and the background chemiluminescence observed was 50 % lower than that of PPD.

IMMOBILIZED FLOW SYSTEMS

The continuous flow format of the analytical method offers greater possibilities than a single batch system because it leads to rapid and sensitive assays. The immobilized enzymes, on nylon coils or methacrylate beads, can be used for many analyses and multichannel systems can be developed. The analytical performances in terms of detection limit, linearity and reproducibility for the different methods developed are reported in Table I: as shown many analytes can be detected at picomole levels, with good precision and a wide range of linearity. Moreover, unless high sensitivity is required, they reduce the analysis time to a few seconds and need smaller sample specimens.

Apparatus

The general scheme of the system is designed to be as simple and versatile as possible. Using enzyme immobilized on nylon coils the system is schematically composed of

I. Analytical performance of the immobilized luminescent enzymes

Analyte	Range (pmol)	Detection limit (μmol/l)	Precision	
			Conc. (pmol)	RSD (%)
NADH (B)	1-2500	0.1	2.5	3.7
NADPH (B)	5-1500	1	10	5.27
α-Bile acid (B)	1-1000	0.5	2.7	5.9
3α-Bile acid (B)	1-1000	0.5	2.8	7.1
12α-Bile acid (B)	10-1000	1	5.2	6.3
Ethanol (B)	50-2500	1	100	8.3
Glycerol (B)	50-500	2	70	7.2
Acetaldehyde (B)	50-1000	2	100	6.8
L-Alanine (B)	5-500	0.5	75	7.4
Branched-chain aminoacids (B)	20-2000	0.5	75	7.8
ATP (L)	0.05-100	0.02	1.5	8.0
ADP (L)	0.6-100	0.1	15	8.2
H_2O_2 (C)	50-8000	1	150	7.2
Oxalate (C)	50-8000	1	150	9.1
ATP (M, r-LM)	1-500	0.3	10	9.3
ATP (r-LN)	0.3-100	0.06	10	5.2
L-Lactic acid (B)	1-500	0.1	7	5.2
D-Lactic acid (B)	10-500	5	50	7.8
L-Glutamic acid (B)	50-1000	10	500	3.7
L-Phenylalanine (B)	1-100	0.5	50	4.3
Magnesium (L)	0.05-6.7	0.05	0.67	4.5
Magnesium (r-LN, M)	0.01-6.7	0.01	0.99	2.0
	(U/l)	(U/l)	(U/l)	(%)
Lactate Dehydrogenase (B)	3-2000	1	151	8.8
Creatine Kinase (L)	0.1-100	0.1	17	8.2

(B): Bioluminescent bacterial system on nylon; (L): Bioluminescent firefly system on nylon; (C): Chemiluminescent luminol system on nylon; (M): Bioluminescent firefly system on methacrylate beads; (r-LM): Recombinant firefly luciferase on methacrylate beads; (r-LN): Recombinant firefly luciferase on nylon

a sampler which permits insertion of the sample within two bubbles of air or directly after a suitable time interval by a three-way valve, another two or three streams for buffer or substrates supply, the specific reactor with the analytes, specific immobilized enzyme and finally the luminescent enzyme(s) reactor, inserted directly into the luminometer. In order to achieve reproducible and accurate measurements the sample

must be separated from the working solution stream by two air bubbles to prevents axial diffusion caused by the concentration gradient which can reduce reproducibility.

Using Eupergit with immobilized enzymes the beads are generally packed into small Plexiglas column (3 cm x 2 mm i.d.) and the column is positioned inside the luminometer in front of the photomultiplier window. The same system used for coils can be employed for Eupergit column, except that in the second stream the air is replaced with buffer and the presence of air bubbles inside the column is carefully avoided. Also an Eupergit column can be coupled with a nylon coil containing not luminescent, immobilized enzymes, that can be placed before the column, outside the luminometer.

Applications

A typical example of these analytical systems is a manifold-use bacterial luciferase for L-lactate assay developed with two separate nylon coils: the first contains the specific lactate dehydrogenase (LDH) enzyme:

$$\text{L-LACTATE} + \text{NAD}^+ \xrightleftharpoons{\text{LDH}} \text{PYRUVATE} + \text{NADH} + \text{H}^+$$

and the second the bacterial bioluminescent enzymes. With this system a detection limit of 5 µmol per l was obtained. We also coimmobilized alanine aminotransferase (ALT) together with LDH:

$$\text{Pyruvate} + \text{Glutamate} \xrightleftharpoons{\text{ALT}} \text{Alanine} + \text{2-oxoglutarate}$$

ALT which withdraws pyruvate, gave higher conversion of lactate with respect to the coil with only LDH (115 - 180 %) and a detection limit of 0.1 µmol per l.

When high sensitivity is needed, such as in microdialysis analysis, the LDH/ALT coil is recommended.

A continuous-flow system was developed for the assay of magnesium (II) in serum, drugs and beverages, making use of firefly luciferase (LUC) or recombinant luciferase (r-LUC) for *Escherichia coli* immobilized both on nylon coil and on epoxy methacrylate. The sample assay rate was 20 samples per hour with no carry-over and 10 µl samples were analyzed. The detection limit of the assay was 0.05 mmol per l for LUC-nylon coil and 0.01 mmol per l for LUC-Eupergit column and r-LUC-nylon coil. No interferences from ions present in the samples (Ca^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}) were found. The nylon immobilized enzymes give a relatively high stability (one to four months), despite low recovery in terms of activity with respect to the soluble forms; Eupergit C gave better sensitivity and activity recovery but lower enzyme stability (three days to one month).

These flow systems do not require thermostation, light emission being constant in the range 18 - 28 °C; however, this can be done if an accurate control of temperature is needed, particularly when enzymatic activity is measured. On the other hand, flow-rate variations do not influence light emission over a sufficiently wide range. Variations in sample volume obviously have greater effects when a short coil is used (50 cm) since saturation values are soon reached. On the other hand, using a longer coil, the light signal remain constant over a greater range of sample volumes.

Microdialysis technique

In vivo metabolite determinations can be performed inserting "an artificial capillary" into a target area (brain area, muscle, adipose tissue, blood, etc.). The probe consists of two concentric steel cannulas covered at the tip by a dialysing membrane (molecular mass cut-off around 20 KDa); a physiological fluid is introduced through the inner cannula, flushes the inside of the membrane and leaves via the outer cannula (Ungersted, Hallstöm, 1987). This system allows sampling of endogenous substances from the extracullular space.

We applied the immobilized enzyme luminescent assay of lactate to the direct determination of this metabolite in ischemic rat brain. The microdialysis probe, with a 2 x 0.5mm dialysing membrane at the tip, was inserted into the nucleus caudatus-putamen of both normal and ischemic anaesthetized rats, connected to a microinfusion pump and continuously perfused at 2 µl/l with Ringer solution. The recovery of lactate through the dialysis membrane, tested by *in vitro* experiments with standard solutions, was around 4 - 10 %. The perfused Ringer solution was collected every 5 min (10 µl total volume) and immediately analyzed for lactate content. Results were in agreement with the values obtained in the same conditions by other measurement techniques, such as HPLC which, however, require greater injection volumes.

DNA AND RNA PROBES

We have developed dot blot hybridization immunoenzymatic assays with chemiluminescent substrates for the sensitive and quantitative detection of cytomegalovirus, B19 Parvovirus and plasmid pBR328 DNAs (Tenover, 1988). Digoxigenin-labelled probes were used and then visualized by anti-digoxigenin Fab fragments labelled with alkaline phosphatase or horseradish peroxidase (Musiani et al., 1991; Girotti et al., 1991). Digoxigenin was preferred as a nucleotide marker because problems that may arise with the use of biotin-labelled probes detected with avidin systems in sera and because its high specificity and sensitivity similar to that of radiolabelled probes without the potential health hazards, disposal problems and instability associated with radioisotopes.

Light emission can be revealed both by instant development photographic film as a qualitative test and by a luminometer as a reliable quantitative assay in clinical samples. Spots of hybridized DNA, cutted from nylon membrane, were placed in cuvettes containing the chemiluminescent mixture and directly analyzed in a luminometer, both in hybridization revealed by ALP-conjugated and by HPR-conjugated reagents.

Of the two labelling systems, the ALP system, using PPD as the chemiluminescent substrate, was more reliable and appliable. In fact, the samples can be stored dry at 4 °C for at least one week, it is not necessary to measure them immediately like for HRP conjugates; the measurements can be performed all together, not one by one because the light emission is not an unstable flash but glows for several days. The results obtained with ALP detection were more reproducible: the relative standard deviation was about 6 %, whereas that obtained with the HRP detection system was 13 %.

The sensitivity of the chemiluminescent assay is compared with that of the colometric one in Table II. Moreover, for membrane-based application (Southern blotting and

II. Sensitivity of colorimetric and chemiluminescent photographic test detection of viral and plasmid DNAs using different concentrations of digoxigenin labeled probes

Probe Concentrations	Colorimetric detection limit	Chemiluminescent detection limit
10 ng/ml	5 pg	1 pg - 500 fg
40 ng/ml	1 pg - 500 fg	200 fg - 100 fg
160 ng/ml	500 fg - 100 fg	50 fg - 10 fg

DNA sequencing) Cl-derivatives give, in comparison with PPD, faster kinetics, permitting faster exposures, and sharper images of the bands obtained for DNA sequencing blots. For both systems a key step was the use of the same kind and batch of nylon membrane, because some types are very highly charged and can cause a high background; to avoid this, each batch must be pre-tested.

CONCLUSIONS

The bioluminescent flow sensors based on enzymes immobilized on nylon tubes and Eupergit columns specifically assay several analytes and enzymes activitites (Table I) and are simple, require only minor modifications of a commercial detector, and allow the analysis of about 20 to 30 samples per hour. 700 to 900 samples can be analyzed using only a few milligrams of enzymes. Therefore the continuous-flow bioluminescent methods appear highly competitive with other methods such as radioimmunoassay, enzyme-immunoassay, high performance liquid chromatography and fluorometry which require radioactive materials, separation steps, sample manipulation or expensive equipment.

When the flow sensors are used coupled with microdialysis probe there are several advantages: a) the reaction takes place in few minutes allowing continuous analysis; b) sensitivity is in the order of picomoles; c) the microdialysis probe allows biological specimens to be drawn without proteins or macromolecules. This example can be extended to the analysis of other analytes which need to be detected continuously such as during therapy monitoring or in emergency care units.

The use of chemiluminescent dot blot DNA and RNA hybridization techniques suggest that chemiluminescent substrates can be considered useful tools for the detection of nucleic acid hybridization reactions because of their high sensitivity and specificity. The chemiluminescent dot blot hybridization systems represent simple, inexpensive and relatively rapid means of determining the viral DNA content in clinical samples, avoiding the use of systems such as a charge-coupled device (CCD), image detector, reflectance densitometer or polymerase chain reaction (PCR) technique. Although a higher sensitivity can be reached by this technique, PCR precludes any standardization of the results and needs special equipment and laboratories in order to avoid cross contamination, making PCR arduous in routine diagnostic laboratories. In our assay the use of Cl-derivative as chemiluminescent substrate of alkaline phosphatase allowed a more rapid and clear detection of DNA or RNA hybridized probes with both photographic and luminometric systems.

References

BECK, S. - KÖSTER, H.: Applications of Dioxetane chemilumiscent probes to molecular biology. Anal. Chem., *62*, 1990 : 2258-2270.

BRONSTEIN, I. - VOYTA, J. C. - ERVE, Y. vant - KRICKA, L. J.: Advances in ultrasensitive detection of proteins and nucleic acids with chemiluminescence: novel derivatized 1,2-Dioxetane enzyme substrates. Clin. Chem., *37*, 1991 : 1526-1527.

CAMPBELL, A. K. (Ed.): Chemiluminescense: Principles and Applications in Biology and Medicine. Chichester, Ellis Horwood 1988.

DeLUCA, M. - McELROY, W. D.: Enhanced chemiluminescent reaction catalyzed by horseradish peroxidase. Meth. Enzymol., Biolumin. Chemilumin., Part B., *133*, 1986 : 331-584.

GIROTTI, S. - FERRI, E. - GHINI, S. - MUSIANI, M. - ZERBINI, M. - GIBELLINI, D. - GENTILOMI, G.: Direct quantitative chemiluminescent assays for the detection of viral DNA. Anal. Chim. Acta, *255*, 1991 : 387-394.

KRICKA, L. J. - STANLEY, P. E. - THORPE, G. H. G. . WHITEHEAD, T. P. (Eds.): Analytical Applications of Bioluminescence and Chemiluminescence. London, Academic Press 1984.

LERCH, F. R.: ATP determination with firefly luciferase. J. Appl. Biochem., *3*, 1981 : 473-517.

MUSIANI, M. - ZERBINI, M. - GIBELLINI, D. - GENTILOMI, G. - GIROTTI, S. - FERRI, E.: Chemiluminescent assay for the detection of viral and plasmid DNAs using digoxigenin labeled probes. Anal. Biochem., *194*, 1991 : 394-398.

RODA, A. - GIROTTI, S. - GHINI, S. - CARREA, G.: Continuous flow assays with nylon tube-immobilized bioluminescent enzymes. Meth. Enzymol., Immobil. Enzymes and Cells, *137*, 1988 : 161-171.

RODA, A. - GIROTTI, S. - GHINI, S. - CARREA, G.: Coupled reactions for the determination of analytes and enzymes based on the use of luminescence. J. Biolumin. Chemilumin., *4*, 1989 : 423-435.

TENOVER, F. C.: Diagnostic deoxyribonucleic acid probes for infectious diseases. Clin. Microbiol. Rev., *1*, 1988 : 82-101.

THORPE, G. H. G. - KRICKA, L. J. - MOSELEY, S. B. - WHITEHEAD, T. P.: Phenols as enhancers of the chemiluminescent horseradish perosidase-luminol-hydrogen peroxide reaction: application in luminescence-monitored enzyme immunoassay. Clin. Chem., *31*, 1985 :1335-1341.

UNGERSTED, U. - HALLSTRÖM, A.: *In vivo* microdialysis - a new approach to the analysis of neurotransmitters in the brain. Life Sci., *41*, 1987 : 873-876.

Využití bio- a chemiluminescenčních systémů v průtokových senzorech a detekce virových DNA a RNA

Moderní luminiscenční techniky, využívající průtokových senzorů, představují významnou analytickou metodu používanou v širokém spektru laboratoří chemicko-biologického vědního základu. V potravinářských laboratořích se uplatní např. při stanovení aminokyselin, některých alkoholů a oxokyselin, aktivit enzymů (např. fosfatasy v mléce), nebo jako detekční činidlo při enzymové imunoanalýze, resp. při diagnostice mikroorganismů pomocí hybridizačních sond. Nepřímo lze stanovit různé biogenní a toxické ionty a organofosforečné, resp. karbamátové pesticidy.

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INFORMATION



KARL NAPOLEON BALLING – FIRST INTERNATIONALLY REKNOWN PERSONALITY OF FOOD TECHNOLOGY IN THE CZECH LANDS



When at the end of the XVIIIth century, an outstanding technician - František Josef G e r s t n e r prepared a project of educational programme for the Royal Bohemian Estates Technical School in Prague[1)], from which later, after many changes, the Prague Technical University was founded, as well as the University of Chemical Technology, with its Faculty of Food and Biochemical Technology, Europe was subject to deep economic difficulties. This was mainly caused by the Napoleonic wars. The realization of the technical school was, therefore, postponed[2)]. It is not at all surprising that the personality chosen for the teaching of, as we would now call it, chemical technological disciplines, J. O. S c h e e r accepted, a year before the opening of the Prague Technical School, a Professorship offered to him by the Vienna University. He was rapidly replaced by another candidate - Karl Augustin N e u m a n n, an excellent expert in fabric technology with education in economics. K. A. N e u m a n n, besides his modest knowledge in general chemistry, had lectured in the fields of glass technology, iron metallurgy and chemical technology, with respect to textile industry.

Only Neumann's assistant and successor Ph. Mr. Josef S t e i n m a n n, appointed Professor in 1817, had included into the course on General Chemistry the very modern classes on "Plant and Animal Substances". He then also expanded the technological courses by adding courses on agricultural and fermentation chemistry, lectures concerning brewery, vinigar and spirits technologies. Even though the lectures of J. Steinmann were influenced by experience in practice, namely the production process in breweries, they had not at that time resulted in such original technological knowledge as to lead to a Professorship at the Technical School in Prague, to be considered a post worth international recognition of progress in the region of Food Technology.

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1) Official name of the School "Königlich böhmische ständische Lehranstalt zu Prague", concerning the Kingdom of Bohemia, where lectures were given in German, not in Czech.
2) Text poznámky

Only in the times of Steinmann's successor - Karl Napoleon B a l l i n g - did this happen. Born in Gabrielina Huť, near the town of Chomutov in Bohemia, in 1805 K. N. Balling was appointed lecturer for the branch of Technological Chemistry in 1833 and became Full Professor in 1835. His teaching carrier began at a time when to the economically more and more important branches of fermentation technology other branches of beet rave sugar industry were added. Even though K. N. Balling was an excellent expert in the field of metallurgy and fuel technologies, his first effort was directed at sugar technology. Already in 1838 he had published the book "Zur Rübenzuckerfabrication" (Sugar Production). Owing to the fact, that the intensive technologies of agroindustries are always based on the corresponding production of raw materials, Balling had paid appropriate attention to agricultural chemistry, especially to the fertilization of beetrave with mineral fertilizers, based on Liebigs' Law of Minimum [3)].

Already in 1842, Mr. Balling had acquainted the home public with the work of Liebig and that in the Prague periodical „Oekonomische Neuigkeiten und Ver-handlungen" (Economic News and Transactions). An earlier Balling's publication, though, is already connected with plant nutrition, i.e. a publication dealing with the chemical analyses of molds.

Issueing from his original work in the region of sugar technology, that had created Balling's international fame, some terms should be mentioned. One of them is the "Quotient of Purity" of sugar beet juices and also the densitometric scale for the determination of mass concentration of saccharose in aqueous solutions (°Bg).

Nevertheless, fermentation chemistry and technology were and remained Balling's main aim of interest. This technology consists of brewery, malt, yeast, spirit, wine and vinegar technologies and has been developed to attain a generally acknowledged scientific base. Again, only as an example of extraordinary technological ingenuity in the region of Fermentation Chemistry, the theory of attenuation should be mentioned. The bases of this theory are the original percentage (at present "concentration") of beer, still having world wide importance and utilization. Balling published his findings and observations in many original papers and monographs. Already in 1844 his publication dealt with the fermenatation of beer from cereal malts and also the Belgian way of beer brewing. Nevertheless, his famous Die Gährungschemie, published in 1845 - 1847, the third edition of four volumes which had already been published in 1865, became his most famous world-wide known work.

In addition to his applied scientific research, K. N. Balling was obviously also very active as the organizer of professionals meetings and as a University teacher. During the numerous reorganizations and transformations of the Prague Technical School (under different names), he always succeeded to assert courses in Food Technology. Thus, in 1864, when the college was reformed to the Royal Czech Polytechnic Institute, Balling

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3) Liebig's Law of Minimum: The plant regulates, resp. limits its growth with respect to the factor present in a minimum quantity, depending on the plant requirements or possibilities.

had a five hours weekly course in General Chemistry, in the third semester, including the processes of burning, fermentation, putrification, and rotting. In the following semester he again had a five hours weekly course in Technical Chemistry. This included fermentation and sugar technologies, agricultural chemistry, analytical chemistry and, among the non-food technologies, also glass technology, the production of dyes and chemicals, metallurgy, etc.

In order to legalize the consulting and expertise activities of Balling's Department, the Agricultural Chemical Station was founded in 1855, an Independent section of the Department, providing a large analytical service. This Station also issued certificates, expertizes, etc., for the various needs of industrial production. As a very active member of the Patriotic Economic Society in Prague (from the forties to the end of his life), Balling also promoted agroindustry, including the processing of flax. He also immensely contributed to the assertion of Czech industry and scientific results at World Agricultural Expositions (London 1851, Paris 1855).

Balling's importance in the process of modernizing and taking a more scientific approach to chemical and, above all, food technology was not only based on his rich activities and personality, but also on the fact, that many of his younger co-workers had reached, under his leadership, excellent results and were, therefore, later able to take over and to continue his work (later called specialization) in applied technologies. Balling himself was a German-speaking Czech, but, as some other Professors of the Prague Polytechnic Institute, he favoured the effors of some Czech speaking students and pedagogues who had promoted the teaching of chemical technologies to the Czech students in the Czech language. He had indirectly contributed to this effort, for most of his co-workers and fellow lecturers were patriots. It was thus possible that when the Polytechnic Institute was reorganized into a billingual Institution (so-called utraquist)[4], and later on May 14th, 1869 was divided into the independent Institute, where the teaching continued in the German language, and into the independent Czech Polytechnical Institute of the Kingdom of Bohemia, enough required specialists, mainly several of Balling's assistants and collegues who were able to present their lectures in the Czech language, had applied for teaching posts. In this connection it is at least necessary to mention Professor František Š t o l b a who had already been lecturing on the subjects taught by Prof. Balling at the utraquist college. Professor Vojtěch Š a f a ř í k was appointed Professor of General Chemistry in 1869, Jan D u š á n e k - Associate Professor in the field of Metallurgy, Jan K r e j č í - Associate Professor in the field of Mineralogy. Karel P r e i s, Balling's former assistant, later became a prominent Professor in the field of sugar technology. Owing to its success, The Czech Polytechnic Institute had also attracted another excellent scientist, a former student of Prof. Balling and later on his son-in-law, Antonín B ě l o h o u b e k. A. Bělohoubek specialized in Fermen-

4) Students could use the Czech language as the official language of the Institute, and they could choose between lectures given in the Czech as well as the German languages.

tation Technology and, no doubt, influenced the professional carrier of his lecturer and the later famous mycologue - Prof. Karel K r u i s.

Prof. Balling himself did not live to see the opening of the Czech Polytechnical Institute, for he died on March 17th, 1868 - 125 years ago, at the age of 63.

Prof. K. N. Balling was not only our first scientist and University teacher, who had become an internationally renown personality in Food Technology. His fame was based not only on his own research work and teaching activities, but also on achievements of his numerous students and co-workers. Both his activities and those of his school formed a sound basis for the further development of the discipline leading to the present Faculty of Food and Biochemical Technology. Therefore, the Scientific Council of the Faculty of Food and Biochemical Technology decided to create a medal with Prof. Balling's name that distinguished scientists of Food Chemistry and Technology will be awarded with.

References

KYZLINK, V.: Vývoj studia chemické technologie potravin v českých zemích a fakulta potravinářské a biochemické technologie VŠCHT v Praze. In: Sbor. VŠCHT, A26, 1983.
SKALA, L.: Malý slovník biografií. I. In: Sbor. ČSAZ, 1988, č. 125.
VELFLÍK, A: V.: Dějiny technického učení v Praze. Praha, Matice technická 1910.

Prof. Vladimír K y z l i n k , M. Eng, PhD., DrSc.

POTRAVINÁŘSKÉ VĚDY
FOOD SCIENCES

Volume *11* ● 1993

Abid Ali M., Dodok L., Halásová G.: Reologické vlastnosti ciest z cícera – Rheological characteristics of doughs of whole grain chickpea flour 143

Beran M., Paulíček V.: Characterization of biological active Cr-Compounds from yeast product Diastabil – Izolace a identifikace biologicky aktivních Cr^{III+} - sloučenin z kvasičného produktu Diastabil^{R} 259

Bokvajová A., Demnerová K., Šůchová M., Králová, B., Král M.: Inhibition of nitrate reductase activity of microbial contaminants hopped wort – Sledování nitrátreduktázové aktivity kontaminující mikroflóry pivovarských surovin a ověření možnosti její inhibice 225

Březina P., Průšová M., Pavlíková Š., Marounek M., Štětina J.: The chemical composition and casein fractions of goat milk – Chemické složení a frakce kaseinu kozího mléka 471

Čopíková J., Noveková M., Svobodová L.: High performance liquid chromatographic determination of saccharose and lactose in milk chocolate – Chromatografické stanovení sacharosy a laktosy v čokoládě 181

Dobiáš J., Čurda D., Váňa V., Žáková P.: Influence of pectic substance extraction into the brine on the texture of canned fruit – Vliv extrakce pektinových látek do nálevu na konzistenci kompotovaného ovoce 233

DodokL., Abid AliM., BuchtováV., HalásováG., Poláček I.: Zloženie aminokyselín a mastných kyselín celozrnnej múky z cícerov – Composition of amino acids and fatty acids of the whole grain flour from chickpeas 51

Doležal M., Fatai S. A., Voldřich M., Velíšek J.: Determination of cyanogenic glycosides of cassava (*Manihot esculenta* Crantz) and cassava products – Stanovení kyanogenních glykosidů kassavy (*Manihot esculenta* Crantz) a výrobků v kassavy 163

Erban V., Gottwaldová M., Špicner J., Bohačenko I.: Citlivost kvasinkových kmenů sbírky RIFIS k nystatinu – Sensitivity of yeast strains of the RIFIS collection to nystatin 311

Fargašová A.: Vplyv kulinárneho spracovania a udržiavania zeleniny na obsah dusičnanov – Influence of culinary processing and holding of vegetables on content of nitrates 63

Fiedlerová V., Shultis K., Houška M., Křišťan P.: Ascorbic acid stability in yoghurt – Stabilita kyseliny askorbové v jogurtu 265

Filip V.: Geometrical isomerization of double bonds of fatty acid methylesters – Geometrická isomerizace dvojné vazby methylesterů mastných kyselin 171

Filip V., Plocková M., Chumchalová J.: HPLC determination of lactose and glucose in culture media – Stanovení laktosy a glukosy v kultivačních médiích metodou HPCL 209

Gilarová R., Voldřich M., Demnerová K., Čeřovský M., Dobiáš J.: Gas chromatographic analysis of cellular fatty acids in the identification of lactic acid bacteria – Plynová chromatografie celulárních mastných kyselin v identifikaci baktérií mléčného kvašení 197

Havlová J., Erban V., Jičínská E.: Kontaminace a kolonizace mlékárenských provozů osmotolerantními mikroorganismy – Osmotolerant microbes: contamination and colonization of dairy plants 21

Holadová K., Hajšlová J., Kocourek V., Míšková H.: Rezidua daminozidu v jablečných produktech – Residues of daminozide in apple products 99

Holasová M., Paříkzková H., Kopecký A., Blattná J., Winterová L., Mrázek M.: Stabilita nenasycených mastných kyselin řady n-3 při kulinární úpravě makrely – Stability of 3n-polyunsaturated fatty acids during culinary processing of mackerel 335

Houška M., Rysová J., Kubešová A., Šrámek P.: Korelace senzorické a přístrojové viskozity tekutých potravin – The colerration between sensoric and instrumental viscosity of fluid food 299

Houšová J., Kristová Š., Topinka P.: Mikrovlnný ohřev: porovnání rovnoměrnosti mikrovlnného pole ve třech typech mikrovlnných zařízení pro domácnosti – Microwave heating: A comparison of microwave field uniformity in the three households microwave ovens 323

Hozová B., Černá J., Žáková L.: Využitie symbiózy propiónových baktérií s kvasinkami pri fortifikácii kyslej kapusty B vitamínmi – Utilization of propionic bacteria in symbiosis with yeasts for sauerkraut fortification with B vitamins 55

Kmínková M., Prošková A., Kučera J.: Charakterizace sorbentů Ostsorb red a Ostsorb blue pro chromatografickou separaci bílkovin – Characterization of Ostsorb red and Ostsorb blue sorbents for protein chromatographic separation 291

Koplík R., Němcová Š.: Voltametrické stanovení kadmia, olova, mědi a zinku v potravinách – Determination of cadmium, lead, copper and zinc foodstuffs 371

Koplík R., Němcová Š.: Voltammetric determination of nickel and cobalt in food – Voltametrické stanovení niklu a kobaltu v potravinách . 509

Kouřimská L., Pokorný J.: Changes of relations between some characteristics during french frying in liquid rapeseed shortening – Změny vztahů mezi vlastnostmi bramborových hranolků a použitého oleje během smažení v řepkovém tekutém šorteningu 485

Kouřimská L., Pokorný J., Štern P.: Relations between characteristics of hydrogenated rapeseed oil during french frying – Vztahy mezi charakteristikami hydrogenovaného řepkového oleje během smažení bramborových hranolků 217

Kováčová M., Príbela A., Kozárová I.: Spôsob stability antokyanínových farbív a eliminácia nežiadúcich senzoricky aktívnych zložiek bazy chabzdovej – The way of stability of anthocyanin colorants and elimination of undesired sensorically active components of danewort . . 401

Králová B., Šebková V., Macek T., Strnadová P., Vaněk T.: Cucumber cell culture as a source of ascorbate oxidase – Buněčná kalusová kultura okurky jako zdroj askorbátoxidásy 445

Ledahudcová K., Kvasnička F., Velíšek J.: Stanovení 3-amino-1,2-propandiolu v hydrolyzátech bílkovin – Determination of 3-amino-1,2-propanediol in protein hydrolysates 121

Moravcová J., Vanclová Z., Novotná M.: Quantitative analysis of sugar factory juices by FT-IR spectroscopy – Kvantitativní anlýza cukrovarnických šťáv pomocí FT-IR spektrometrie . . . . . .

Paulíček V., Beran M.: Flow-microcalorimetric determination of celloblase activity and its end inhibition by glucose – Mikrokalorimetrické stanovení celobiasové aktivity a její inhibice glukosou 275

Plocková M., Chumchalová J., Pavlíková Š.: A Contribution to the study of ripening of soft smear cheeses – Zrání sýrů s mazem na povrchu 461

Pokorný J.: Category ordinal scales with uncomplete verbal descriptors – Kategorové ordinální stupnice s neúplným slovním popisem jednotlivých stupňů 479

Pokorný J.: Food quality and its assessment in the education of food scientists – Kvalita potravin a její hodnocení v učebních programech pro potravinářské odborníky 161

Pokorný J., Velíšek J., Pánek J., Káňová J., Pařízková H., Holasová M., Koplík R., Čmolík J.: Minor lipophilic components in crude rapeseed oil – Doprovodné lipofilní složky surového řepkového oleje 189

Popadičová K., Bohačenko I.: Biologický test na kyselinu sorbovou a některé látkové složky limonád pomocí organismů *Lebistes reticulatus* a *Daphna magna* – Biological test for sorbic acid and same material components of soft drinks using the organisms *Lebistes reticulatus* and *Daphna magna* 281

Staňková L., Pipek P., Růžek T.: Changes of internal atmosphere in packaged meat products – Vnitřní atmosféra u balených masných výrobků 503

Staruch L., Bán S., Heriban V.: Vplyv udiaceho preparátu (UTP-1) na rast štartovacích kultúr pre fermentované mäsové výrobky a na rast *Escherichia coli* – The effect of smoking preparation (UTP-1) on the growth of the starter cultures for fermented meat products and on the growth of *Escherichia coli* 381

Strejček F., Pokorný J., Vierecklová M., Pokorný D., Holas J.: Relation between attributes of flat crisp bread – Vztahy mezi výsledky jednotlivých ukazatelů senzorického hodnocení křehkého chleba 81

Suhaj M.: Stanovenie sacharínu v požívatinách metódou kapilárnej izotachoforézy a derivačnej spektrofotometrie – Saccharin determination in foodstuffs by capillary isotachophoresis and derivative spectrophotometry . . 363

Šimko P., Knežo J.: Vplyv ohrevu na obsah benzo(a)pyrénu v údených mäsových výrobkoch – The effect of heating on the benzo(a)pyrene content in the smoked meat products 391

Šubrtová D., Hejtmánková A., Vaňková Z., Hubáček J.: Změny obsahu těkavých látek, solaninu a škrobu v důsledku infikace mokrou hnilobou – Changes in the contents of volatile substances solanine and starch caused by soft rot infection 31

Takácsová M., Dudášová S., Hozová B., Rajniaková A., Minarovičová I.: Zmeny lipidov pri kombinovanej metóde konzervácie potravín s aplikáciou glycínu – Changes in the lipids at use of combined method of food preservation with the glycine application . . . 111

Takácsová M., Hozová B., Rajniaková A., Minarovičová I.: Zmeny mastných kyselín lipidov vzoriek pri kombinovanej metóde konzervácie potravín s aplikáciou glycínu – Changes in the lipid fatty acids of the samples at use of a combined method of food preservation with the glycine application 409

Takácsová M., †Šorman L., Dudášová S., Rajniaková A., Hozová B.: Zmeny v zastúpení mastných kyselín lipidov vzoriek, konzervovaných kombináciou termosterilizácie a ionizujúceho žiarenia – Changes in the proportion of fatty acids of lipid samples preserved by the combination of the sterilization and of ionizing radiation 43

Urbanová E.: Hodnocení metod stanovení počtu psychrotrofních baktérií v syrovém mléce – The evaluation of the methods of determination of a number of psychrotrophic bacteria in raw milk 135

Urbanová E., Vyhnálková J., Malíková M.: Závislost počtu mezofilních a psychrotrofních baktérií u vzorků z prvovýroby mléka – Relationship between the counts of mesophilic and psyrchotrophic bacteria in samples from the milk primary production 13

Velíšek J., Vos R.H. de, Schouten A.: HPLC determination of alliin and its transformation products in garlic and garlic-containing phytomedical preparation – Stanovení alliinu a produktů jeho transformace metodou HPLC v česneku a přípravcích obsahujících česnek 445

Velíšek J., Míková K., Mašátová L.: Využití metod multivariační analýzy k hodnocení kvality bezinek – Use of multivariate methods for evaluating the quality of elderberries 353

†Zeman I., Schwarz W., Brát J., Zajíc J.: Chromatographic analyses of the products from fatty acid dimerization – Chromatografické analýzy produktů z polymerace mastných kyselin 1

PŘEHLEDY – SURVEYS

Houšová J., Topinka P.: Monitorování teploty materiálu během mikrovlnného ohřevu (problémy a možné metody) – The monitoring of time-temperature of materials during microwave heating (problems and possible methods) 349

Kalač P., Třísk a J.: Možnosti průniku polychlorovaných dibenzo-p-dioxinů a dibenzofuranů do potravních řetězců – A review of food chains contamination with polychlorinated dibenzo-p-dioxins and dibenzofurans 69

Kopecký A.: Esenciální mastné kyseliny – Essential fatty acids 415

Nývlt V.: Obrazová analýza - perspektivní metoda pomáhající při klasifikaci kvality v potravinářství – Image analysis - prospective method assisting in the quality classification in food production 341

Rauch P., Káš J.: The role of radionuclides in modern food research, analysis and controlling of technological processes – Úloha radionuklidů v moderním potravinářském výzkumu, analýza a řízení technologických procesů 243

Giroti S., Ferri E., Ghini S., Musiani M., Zerbini M., Carrea G., Bovara R., Roda A., Yini I., Rauch P.: Bio and chemiluminescent systems applied to flow sensors and viral DNA and RNA detection – Využití bio- a chemiluminescenčních systémů v průtokových senzorech a detekce virových DNA a RNA 519

Velíšek J., Ledahudcová K.: Problematika organických sloučenin chloru v potravinářských hydrolyzátech bílkovin – Survey on organic chlorine-containing compounds occurring in protein hydrolysates 149

INFORMACE – INFORMATION

Káš J., Rychtera M.: Collaboration of the Faculty of Food and Biochemical Technology, Institute of Chemical Technology Praque with the European Network – Spolupráce fakulty potravinářské a biochemické technologie Vysoké školy chemicko-technologické Praha s evropskou sítí univerzit 251

Kyzlink V.: Karl Napoleon Balling - first internationally reknown personality of food technology in the Czech lands . . . . . .

Ruiter A.: High education if food chemistry – Vysokoškolské studium v potravinářské chemii 423

ÚVODNÍK

Celba J.: 35 let trvání Výzkumného ústavu potravinářského v Praze 257

Čepička J.: Teaching process at the faculty of food and biochemical technology and its optimalization 443

Pokorný J.: Food quality and its assessment in the education of food scientists 161

Z VĚDECKÉHO ŽIVOTA

Ingr I.: Pocta profesoru Vladimíru Kyzlinkovi 158

RECENZE

Davídek J.: J. Dziuba, H. Kostyra - Biochemia zywnosci, cwiczenia i metody 42

Dostálová J.: M. L. Brown (Ed.) - Present Knowledge in Nutrition . . . 50

Kalač P.: Abdel-Fattah M. Rizk (Ed.) - Poisonous Plant Contamination of Edible Plants 134

Pokorný J.: E. Davídková - J. Dostálová - Náhrada cukru jinými sladidly 438

Pokorný J.: J. Šindelářová a kol. – Zdravotní nezávislost potravin 439

OBSAH – CONTENTS

Čepička J.: Teaching process at the Faculty of food and biochemical technology and its optimalization 443

Velíšek J., Vos R. H. de, Schouten A.: HPLC determination of alliin and its transformation products in garlic and garlic-containing phytomedical preparations – Stanovení alliinu a produktů jeho transformace metodou HPLC v česneku a přípravcích obsahujících česnek 445

Králová B., Šebková V., Macek T., Strnadová P., Vaněk T.: Cucumber cell culture as a source of ascorbate oxidase – Buněčné kultury jako zdroj askorbátoxidasy 455

Plocková M., Chumchalová J., Pavlíková Š.: A contribution to the study of ripening of soft smear cheeses – Zrání sýrů s mazem na povrchu 461

Březina P., Průšová M., Pavlíková Š., Marounek M., Štětina J.: The chemical composition and casein fractions of goat milk – Chemické složení a frakce kaseinu kozího mléka 471

Pokorný J.: Category ordinal scales with uncomplete verbal descriptors – Kategorové ordinální stupnice s neúplným slovním popisem jednotlivých stupňů 479

Kouřimská L., Pokorný J.: Changes of relations between some characteristics during french frying in liquid rapeseed shortening – Změny vztahů mezi vlastnostmi bramborových hranolků a použitého oleje během smažení v řepkovém tekutém šorteningu 485

Moravcová J., Vanclová Z., Novotná M.: Quantitative analysis of sugar factory juices by FT-IR spectroscopy – Kvantitativní analýza cukrovarnických šťáv pomocí FT-IR spektrometrie 493

Staňková L., Pipek P., Růžek T.: Changes of internal atmosphere in packaged meat products – Vnitřní atmosféra u balených masných výrobků . . . 503

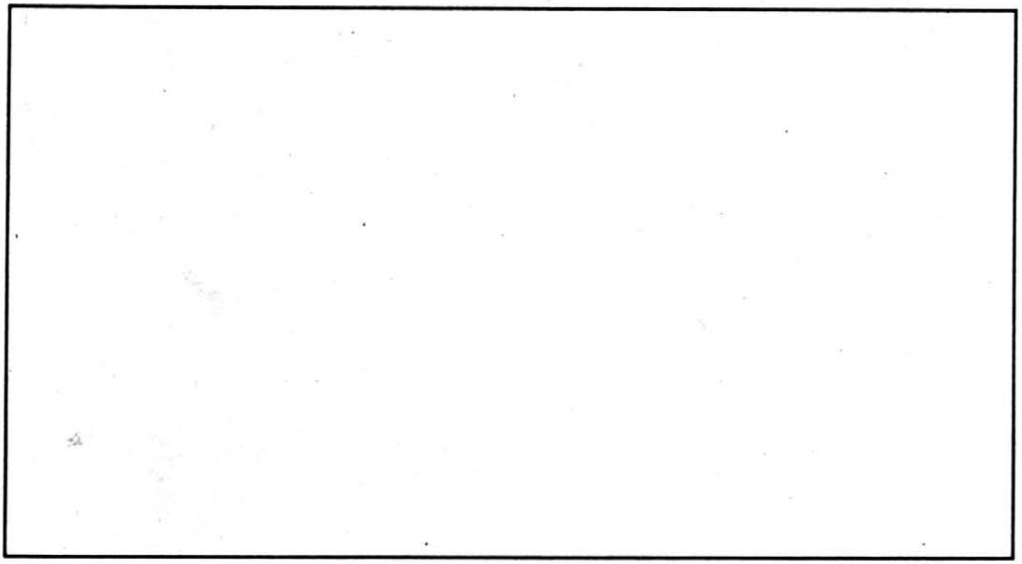
Koplík R., Němcová Š.: Voltammetric determination of nickel and cobalt in food – Voltametrické stanovení niklu a kobaltu v potravinách 509

SURVEY – PŘEHLEDY

Giroti S., Ferri E., Ghini S., Musiani M., Yerbini M., Carrea G., Bovara R., Roda A., Zini I., Rauch P.: Bio- and chemiluminescent systems applied to flow sensor and viral DNA and RNA – Využití bio- a chemiluminescenčních systémů v průtokových senzorech a detekce virových DNA a RNA 519

INFORMATION – INFORMACE

Kyzlink V.: Karl Napoleon Balling - first internationally reknown personality of food technology in the Czech lands 527



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