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# Editorial

*Dear friends,*

*You have just received the first issue of scientific periodical Potravinářské vědy with a new, at a glance obviously different layout of a larger standard size. This size change has already occurred once since the foundation of this periodical in 1981; it was due to technical reasons; this second, and I believe the last change has been made to comply with formal requirements for the layout of prestigious foreign periodicals.*

*The periodical Potravinářské vědy is a unique scientific periodical in this country, dealing with a large range of issues from the food sector. It was one of the reasons for its foundation since the periodical of this type was missing here. The periodical Průmysl potravin published at that time dealt with different subjects, its mission was also different, so it could not meet requirements for a scientific periodical. When Potravinářské vědy started to be published, the Czechoslovak professional community in the food sector warmly welcomed this periodical, which was documented, among other things, by the fact that there has ever been no lack of papers submitted to the board of editors, on the contrary, the number of issues had to be increased from four to six issues per year throughout the years.*

*The board of editors have always tried not only to improve the professional standard of the periodical but also to enhance its reputation in the professional community in this country as well as in foreign countries. The periodical is registered in prominent world databases (Food Science and Technology Abstracts, Agrindex of FAO (AGRIS database), Dairy Science Abstracts, Chemical Abstracts, PASCAL – CD-ROM (INIST), WLAS, TOXILINE PLUS, Czech Agricultural Bibliography), it is however intelligible that many authors try to publish their papers in periodicals monitored by the prestigious world database Current Contents. Following our first application to include Potravinářské vědy in this database filed in 1992, some changes in presentation have been made after we received the reply of C.C. editorial office.*

*The number of papers published in English has increased substantially, there are some English issues published from time to time, papers written in the Czech or Slovak language are accompanied by long summaries in English. The above-mentioned change in size, formal improvements inside the periodical as well as its new layout were adopted carefully in order to approach the formal standard of prominent scientific periodicals to the largest extent possible.*

*The scientific standard of the subjects treated, i.e. of particular papers, is equally, or even far more important. I believe that the periodical meets all ambitious requirements in this aspect. Activities of the Scientific Board of Czech and foreign experts contribute to this goal. This changes have suggested that the present name of this periodical is not apt and therefore the board of editors have decided to change it to* ***Czech Journal of Food Science.***

*A new application to include this periodical in Current Contents is currently prepared to be filed, along with documentation of changes in the periodical. The importance and position of this periodical would be enhanced at an international scale in the case of a positive reply.*

*I believe that the periodical concerned in its new shape will be popular with both the authors of scientific papers and readers since it will facilitate rapid acquaintance with results of scientific papers at a worldwide level.*

*Ing. Zeno Šimůnek, CSc.*
*Head of Editorial Board of the periodical*
*Czech Journal of Food Science*

*Vážení přátelé,*

*dostáváte do ruky prvé číslo vědeckého časopisu Potravinářské vědy v nové, na prvý pohled nápadně odlišné úrovně, ve větším, standardním formátu. Od svého založení v roce 1981 prošel časopis již jednou touto změnou, která byla vyvolána technickými důvody; tato druhá a věřím, že poslední změna byla provedena proto, aby časopis i svým vzhledem odpovídal formálním požadavkům renomovaným zahraničním časopisům.*

*Časopis Potravinářské vědy je jediným vědeckým časopisem v naší republice zaměřeným na širokou problematiku potravin. To byl i jeden z důvodů jeho založení, neboť časopis tohoto charakteru u nás chyběl. V té době vydávaný Průmysl potravin byl jinak zaměřen, měl i jiné poslání a nemohl plnit požadavky kladené na vědecký časopis. V době zahájení vydávání Potravinářských věd byl časopis velmi dobře přijat československou vědeckou obcí v oblasti potravin, o čemž mimo jiné svědčila i skutečnost, že redakční rada neměla nikdy problémy s nedostatkem příspěvků, naopak, v průběhu let bylo přikročeno k zvýšení počtu čísel ze 4 na 6 ročně.*

*Stálou snahou redakční rady je nejen zvyšovat vědeckou úroveň časopisu, ale i jeho renomé mezi vědeckou veřejností u nás i v zahraničí. Časopis je evidován ve významných světových databázích (Food Science and Technology Abstracts, Agrindex of FAO (AGRIS database), Dairy Science Abstracts, Chemical Abstracts, PASCAL – CD-ROM (INIST), WLAS, TOXILINE PLUS, Czech Agricultural Bibliography),.je však pochopitelné, že mnozí autoři se snaží uplatnit své příspěvky v časopisech sledovaných prioritní světovou databází Current Contents. Po naší první žádosti o zařazení časopisu Potravinářskéh vědy do této databáze v roce 1992, bylo na základě odpovědi redakce CC přikročeno k určitým změnám v prezentaci.*

*Podstatně se zvýšil počet publikací v anglickém jazyce, některá čísla jsou celá v angličtině, práce v češtině nebo slovenštině jsou opatřeny anglicky psaným obsáhlým souhrnem. Rovněž výše zmíněná změna formátu a vnitřní formální úpravy i uspořádání časopisu byly provedeny s cílem co nejvíce se přiblížit formální úrovni předních vědeckých časopisů.*

*Stejně, ale mnohem důležitější, je vědecká úroveň obsahu, tj. jednotlivých příspěvků. Domnívám se, že i po této stránce časopis splňuje tyto náročné požadavky. K tomu přispívá i vědecká rada, ve které jsou zastoupeni naši i zahraniční odborníci.*

*Uvedené změny sebou nesou i změnu názvu časopisu na* ***Czech Journal of Food Science*** *(původní název zůstane pro zachování kontinuity a pro lepší orientaci čtenářů zachován jako podtitul na obálce časopisu.*

*V současné době je připravováno podání nové žádosti o zařazení do Current Contents spolu s dokumentací změn v charakteru časopisu. změny anglického názvu na . V případě kladného vyřízení by se dále zvýšil význam a postavení časopisu na mezinárodní úrovni.*

*Věřím, že v této nové formě si časopis zachová oblibu mezi autory vědeckých prací i mezi čtenáři, neboť bude umožňovat rychlé seznámení s výsledky vědeckých prací na světové úrovni.*

*Ing. Zeno Šimůnek, CSc.*
*předseda redakční rady časopisu*
*Czech Journal of Food Science*

# Effect of Nisin on Injured Cells of *Lactococcus lactis* subsp. *lactis**

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**Abstract**

Injury by heating (50 °C/10 min) or freezing (–20 °C/24 h) and rapid thawing increased the sensitivity of two nisin-resistant nisin-producing strains of *Lactococcus lactis* subsp. *lactis* to nisin. The freezing and thawing alone decreased the viability of both *Lactococcus lactis* strains less than heating. Combined action of physical treatment with nisin action decreased the viability more sensitively compared with physical treatment alone. Combination of nisin and injury by heating or freezing enhanced antibacterial effect on *Lactococcus lactis* strains in distilled water compared with LM17 broth. The reparation procedure had a low stimulative effect on the repairing of cells injured in LM17 broth, and hardly any effect on cells injured in water.

nisin; injured cells; *Lactococcus* sp.; heating; freezing

The operations used in the dairy technology such as heating, freezing and drying may influence the viability of starter culture or undesirable microorganisms. In the past it was found that the cells surviving different sublethal physical and chemical treatments may be injured. The injury found to be reversible and injured cells were able to repair when supplemented with several organic compounds such as peptides (Straka, Stokes, 1959). Bacterial injury due to heating and freezing and many other physical and chemical treatments (Ray, 1979) of pathogenic and indicator bacteria (Ray, 1986; Hartman, 1979; Netten et al., 1990) were studied intensively. Microbiologic cell injury studies of food spoilage bacteria, lactic acid bacteria and yeasts were published less often (Valdéz, Giory, 1993; Valdéz et al., 1985; Brennan et al. 1986; Williams, Russell, 1992).

It was proved that the treatments such as heating, freezing and drying may destabilize weak bonds (e.g. H-bonds, ionic bonds and hydrophobic bonds) of some macromolecules, changed their normal conformations, resulted in manifestations such as increased sensitivity to selective agents, activation of some enzymes or loss of cellular materials. The sites of damage may be some wall components, cell membrane, ribozomes and rRNA or structural DNA. The repair mechanisms include *de novo* synthesis and reorganization of some macromolecules (Ray, 1986). Changed sensitivity of Gram-negative and resistant Gram-positive bacteria to bacteriocins (Kalchayanand et al., 1992) and cationic and other stressing agents of injured spores of *Bacillus subtilis* (Williams, Russell, 1992) were studied.

It is known that nisin used in food industry as biopreservative destabilizes the membrane vesicles of Gram-negative bacteria as it does to the cytoplasmic membranes of sensitive Gram-positive bacteria (Hurst et al., 1993) and so the hypothesis exists that the injury focused on the damage of cell walls and cell membranes may influence nisin sensitivity.

The present work was undertaken to investigate the changes in nisin sensitivity of *Lactococcus lactis* subsp. *lactis* strains injured by freezing and thawing and prove if the combination of nisin and injury by heating or freezing enhanced antibacterial effect on these strains possibly used in dairy starters.

## MATERIAL AND METHODS

### Microorganisms

Two nisin producing strains *Lactococcus lactis* subsp. *lactis* R5 (NIZO, Netherlands) and *Lactococcus lactis* subsp. *lactis* LCC 702/68 (Culture Collection of Dairy Microorganisms Laktoflora, Czech Republic) were grown to the stationary phase (about 15 h) at 30 °C in LM17

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broth (Oxoid). All strains were subcultured minimally three times before use.

**Nisin resistance evaluation**

*Lactococcus lactis* subsp. *lactis* strains were grown using conditions mentioned above and after finishing the incubation either the samples for evaluation of the nisin resistance of non-injured cells were taken or the cell suspension for injury process was prepared as follows: 1 ml of each cell suspension were pippeted into Eppendorph microtubes. The tubes with *Lactococcus lactis* strains were either frozen at –20 °C for 24 h, then thawed rapidly, or heated at 50 °C for 10 min and then cooled immediately in water (4 °C). After it the tubes with LM17 broth with final concentrations of nisin (Applin & Barrett, UK) 0, 10, 100, 1000 IU/ml were inoculated by 1% (v/v) of cell suspensions of non-injured and/or injured cell suspensions of each tested strains. After 6 h incubation at 30 °C in LM17 broth (Oxoid) absorbance $A_{615}$ was evaluated and the growth of cells [%] was calculated.

**Sublethal-stress and nisin treatments**

The cells after normal cultivation for each strain as it was mentioned above were harvested by centrifugation (4000 rpm, 4 °C, 5 min) and resuspended in sterile distilled water or sterile LM17 broth to a cell concentration of about $10^5$–$10^6$ cfu/ml. The cfu after resuspendation were evaluated by use of plating. The sets of samples for each strain were prepared as follows: 0.9 ml cell suspension and 0.1 ml nisin solution to the final concentrations of nisin 0, 10, 100, 1000 IU /ml. The tubes with *Lactococcus lactis* strains were either frozen at –20 °C for 24 h and then thawed rapidly or heated at 50 °C for 10 min and then cooled immediately in water (4 °C). After treatment all samples were enumerated for colony forming units on LM17 agar (Oxoid).

**Reparation procedure**

Cells of both *Lactococcus lactis* strains were centrifugated (4000 rpm, 4 °C, 5 min), the LM17 broth after cultivation was discarded and cells were resuspended in sterile reparation solution of the same volume as discarded LM17 broth (0.1% $MnSO_4$, 0.5% $KH_2PO_4$, 0.1% pyruvic acid in sterile water, pH 7) and allowed to repair at 25 °C for 1 h. After it all samples were enumerated for colony forming units on LM17 agar (Oxoid).

## RESULTS AND DISCUSSION

The knowledge of microbial cell injury due to freezing and thawing is useful in the preservation of starter cultures (R a y, 1986). The knowledge of bacterial injury of starter cultures by different sublethal heat treatments used in dairy technology alone or in combination with nisin action may be used for prediction of changeable running of starters such as during the production and ripening of cheeses. In our work we tested the changes in viability of two nisin-producing strains of *Lactococcus lactis* subsp. *lactis*. These nisin-producing strains that can be comprised in the dairy starters used for cheese production may be useful for their protective activity against genera *Staphylococcus, Streptococcus, Listeria, Bacillus* and

I. Resistance of injured cells of *Lactococcus lactis* strains to nisin

| Strain | Injury | Growth of cells [%]* nisin concentration [IU/ml] 10 | 100 | 1000 |
|---|---|---|---|---|
| *L. lactis* ssp. *lactis* R5 | control+ | 98 | 93 | 9 |
| | heating | 88 | 50 | 1 |
| | freezing | 99 | 83 | 7 |
| *L. lactis* ssp. *lactis* 702/68 | controlx | 99 | 77 | 19 |
| | heating | 50 | 35 | 1 |
| | freezing | 98 | 69 | 20 |

* Growth of cells [%] = $100 \cdot \frac{A_{615}\ \text{nis x}}{A_{615}\ \text{nis o}}$

$A_{615}$ nis o.....$A_{615}$ in LM17 broth after 6 h of cultivation at 30 °C

$A_{615}$ nis x.....$A_{615}$ in LM17 broth with nisin concentration 10, 100, 1000 IU/ml after 6 h of cultivation at 30 °C

+unstressed cells of *Lactococcus lactis* subsp. *lactis* R5

x unstressed cells of *Lactococcus lactis* subsp. *lactis* 702/68

*Clostridium* (Hurst et al., 1993; Delves-Broughton, 1990).

Effect of injury by heating and freezing and thawing on the nisin resistance of two strains of *Lactococcus lactis* subsp. *lactis* is presented in Table I. Both *Lactococcus lactis* subsp. *lactis* strains with different levels of nisin resistance reduced resistance to nisin as compared to the respective unstressed controls. The cells of both *Lactococcus lactis* subsp. *lactis* strains injured by heating were more sensitive to nisin concentrations higher than 10 IU/ml, while the freezing caused increased sensitivity of both strains to nisin concentrations higher than 100 IU/ml.

The effect of freezing and thawing and heating alone or combined with nisin action on viability of two strains of *Lactococcus lactis* subsp. *lactis* is presented in Table II. Reduction in population varied greatly with the medium used for resuspendation of cells, as it can be concluded also from the results in Table III.

Freezing (–20 °C/24h) and rapid thawing and common effect of this physical treatment with nisin action decreased the viability of *Lactococcus lactis* subsp. *lactis*

II. Loss in viability of stressed *Lactococcus lactis* strains by freezing (–20 °C/24h) and heating (50 °C/10min)

| Strain | Control | | Treatment | | Treatment + nisin | Treatment + nisin |
|---|---|---|---|---|---|---|
| | water | LM17 broth | water | LM17 broth | water with nisin 100 [IU/ml] | LM17 both with nisin 100 [IU/ml] |
| | **Log CFU/ml after treatment by freezing** | | | | | |
| *L. lactis* subsp. *lactis* R5 | 5.9 | 5.6 | 4.2 | 5.2 | 0 | 4.5 |
| *L. lactis* subsp. *lactis* 702/68 | 5.3 | 5.7 | 4.3 | 4.9 | 0 | 4.8 |
| | **Log CFU/ml after treatment by heating** | | | | | |
| *L. lactis* subsp. *lactis* R5 | 6.0 | 5.6 | 3.8 | 4.1 | 0 | 3.4 |
| *L. lactis* subsp. *lactis* 702/68 | 5.2 | 5.5 | 3.3 | 4.7 | 0 | 3.2 |

II. Effect of reparation procedure on the viability of injured cells of *Lactococcus lactis* strain

| Strain | Injury | Nisin concentration [IU/ml] | Log CFU/ml after injury in water | reparation in water | injury in LM17 medium | reparation in LM17 medium |
|---|---|---|---|---|---|---|
| | | 0 | 3.8 | 3.9 | 4.0 | 4.1 |
| | heating | 10 | 0 | 0 | 3.8 | 4.1 |
| | (50 °C/10min) | 100 | 0 | 0 | 3.4 | 4.0 |
| *L. lactis* subsp. | | 1000 | 0 | 0 | 0 | 0 |
| *lactis* R5 | | 0 | 4.2 | 4.3 | 5.2 | 5.6 |
| | freezing | 10 | 0 | 0 | 4.5 | 4.7 |
| | (–20 °C/24h) | 100 | 0 | 0 | 4.5 | 4.6 |
| | | 1000 | 0 | 0 | 4.6 | 4.6 |
| | | 0 | 3.3 | 3.5 | 4.7 | 5.0 |
| | heating | 10 | 0 | 2.2 | 4.6 | 3.8 |
| | (50 °C/10min) | 100 | 0 | 0 | 3.2 | 3.5 |
| *L. lactis* subsp. | | 1000 | 0 | 0 | 0 | 0 |
| *lactis* 702/68 | | 0 | 4.3 | 4.4 | 4.9 | 5.1 |
| | freezing | 10 | 2.0 | 2.3 | 4.9 | 5.0 |
| | (–20 °C/24h) | 100 | 0 | 0 | 4.8 | 5.1 |
| | | 1000 | 0 | 0 | 5.0 | 5.0 |

strains only slightly. The sensitivity of both strains to nisin present in the environment (Table I) was more influenced by this treatment. Heat treatment (50 °C/10 min) alone or in combination with nisin action decreased the viability of lactococci to a higher extent. Combined heat treatment and 100 IU of nisin/ml decreased the number of cells to form colonies on LM17 agar by 2 log cycles. Heating also evidently increased the sensitivity of nisin-producing strains to added nisin. Already nisin concentration 10 IU/ml that did not influence the growth of both uninjured strains and by freezing injured tested strains decreased the growth rate of the same strains injured by heating. The similar results were published (Kalchayanand, 1992) in the past. Where Gram-positive nisin resistant *Lactococcus lactis* and *Pediococcus acidilactici* became more sensitive to their respectively produced bacteriocin-nisin and pediocin after injury.

As it was mentioned above from the results in Table III it is obvious that the character of environment where the injury is realized affected the viability of lactococci very strongly.

Both heating and freezing of cells in water in combination with 10 IU nisin/ml caused total loss of viability of cells. Heating in LM17 broth influenced the viability of cells more compared with freezing in the same enviroment. Combined treatment by heating 50 °C per 10 min and the highest nisin concentration 1000 IU/ml caused total loss of viability at both tested lactococci strains while the combined action of freezing and 1000 IU nisin/ml did not influence the viability evidently.

The reparation procedure did not affecte the viability of cells injured by heating and freezing in water where the celullar damage was probably greater and resulted in cell death. The reparation procedure seemed to have a low stimulative effect on the repairing of injured in LM17 broth cells with no regard to the type of injury (physical alone or combined physical and nisin action).

As it was published (Valdéz et al., 1985) LM17 broth (also used alone in our work) had only slightly lower effect on viability of *Lactococcus lactis* strains compared with LAPT recovery medium (Raibaud et al., 1961) containing: peptone, tryptone, glucose, yeast extract and Tween 80. The effectivenes of LAPT medium with recovery solution in combination with LM7 base will be compared in future.

The knowledge of the injury of lactic acid bacteria due to physical treatment alone or combined with bacteriocin action and knowledge of increased sensitivity of injured lactic acid bacteria to bacteriocins is useful in the field application of frozen and freeze-dried starter cultures and in prediction of non-standard course of technological operations in dairy industry.

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**Souhrn**

PLOCKOVÁ M., ŠVIRÁKOVÁ E., NEVAŘILOVÁ P.: **Účinek nisinu na poškozené buňky *Lactococcus lactis* subsp. *lactis***. Czech J. Food Sci., *16* (1998): 3–7.

V důsledku poškození buněk u dvou nisin-rezistentních a zároveň nisin-produkčních kmenů *Lactococcus lactis* subsp. *lactis*, buď záhřevem (50 °C/10 min), nebo mrazením (–20 °C/24 h) s rychlým rozmrazením, došlo ke zvýšení citlivosti těchto kmenů k nisinu.

Během samotného mrazení a rozmrazování došlo ve srovnání se záhřevem u obou kmenů *Lactococcus lactis* k méně výraznému snížení životaschopnosti buněk.

Kombinovaný účinek fyzikálního působení a působení nisinu snižoval životaschopnost buněk mnohem intenzivněji, než v případě samotného fyzikálního působení. Kombinace záhřevu nebo mrazení s působením nisinu vykazovala zvýšený antibakteriální účinek na kmeny *Lactococcus lactis* ve sterilní vodě než v LM17 bujónu.

Reparační procedura měla nízký stimulační účinek na reparaci poškozených buněk v LM17 bujónu na rozdíl od nulového účinku reparace poškozených buněk ve vodě.

nisin; poškozené buňky; *Lactococcus* sp.; záhřev; mrazení

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# Authenticity and Adulteration of Food – the Analytical Approach (FECS No. 220)

The latest event organised by the FECS, Division of Food Chemistry together with the Swiss Society of Food and Environmental Chemistry took place in Interlaken, September 24–26, 1997. 314 participants from 36 different countries attended the meeting held at the Congress Centre of Interlaken, amidst the famous peaks of the Swiss Alps. The well organised catering and the nice lecture theatre together with the large congress Hall, where the exhibition of analytical equipment and the poster sessions took place, guaranteed a stimulating atmosphere for interesting discussions. The congress was opened by Welcome addresses by Dr. Urs Klemm, Head of the Food Science Division of the Federal Office of Public Health, by Dr. Jean-Luc Luisier, President of the Swiss Society of Food and Environmental Chemistry and by Dr. Reto Battaglia, Chairman of FECS Division of Food Chemistry and Head of the Organising Committee.

The scientific programme was divided 1nto 8 sessions, each consisting of 1 maul and 3 short lectures followed by corresponding poster sessions during the long coffee or lunch breaks. For the posters there was an additional session with miscellaneous subjects. Each participant received the very comprehensive proceedings*) consisting of 3 volumes and a total of about 800 pages already upon registration. So the 32 lectures and 129 posters were very well documented. The following main topics were presented at the conference: Legal Aspects and the Role of Food Analysis; Authenticity of Meat, Adulteration of Meat Products; Adulteration of Milk and Dairy Products; Plant Products: Adulteration of Spices, Flavours and Aroma; Fruit Juice Adulteration; Carbohydrates and Adulteration; SNIF NMR and Authenticity; Chemotaxonomy and Authenticity.

A prize (free registration for Euro Food Chem X, to be held from 22nd to 24th September, 1999, 1n Budapest, Hungary) was awarded by the Scientific Committee for the three best poster presentations. The prizes were awarded to the following presentations: "Detection of Genetically Modified Food" (E. Köppel, E. Studer, J. Lüthy, P. Höbner, Switzerland); "Determination of Honey Authenticity and its Botanical Origin by Micellar Electrokinetic Chromatography (MEKC) and HPLC" (C. Corradini, G. Canali, A. Cavazza, E. Cogliandro, I. Nicoletti, Italy); "Authenticity of Boronia and Osmanthus Absolutes and Raspberry Flavours by Chiral GC/MS Analysis of Alpha and Beta Ionones" (N. Bouter, P. de Valois, F. P. Scanlan, The Netherlands).

The conference provided ample opportunities for the interchange of scientific information on specific topics of interest to participants. Considerable attention was also paid to the exhibition of analytical equipment. During the whole Conference, all participants profited from exceptionally nice weather, which cannot be taken for granted in this region, and had the possibility to attend either of the following two small tours: to Schynige Platte, a mountain 1967 m above sea level with an overwhelming panorama view especially of the famous Alpine peaks Eiger, Mönch and Jungfrau, or to the Open Air Museum Ballenberg, a park-like museum complete with houses, farmhouses and rural crafts from past cultural eras. Most of the participants also enjoyed the exquisite conference dinner in the comfortable dining room of the Congress Centre. Surely all the participants took only the best of impressions from this conference back home to their lab or office.

*Dr. O. Zoller (Berne)*

*) Copies of the Proceedings are available and may be ordered for CHF 50.– + shipping directly from Dr. J.-L. Luisier, Bibliotheque Ecole d'Ingénieurs du Valais, Route du Rawyl 47, CH-1950 Sion, Switzerland; or E-MAIL: jluc.luisier@eiv.vsnet.ch

# Production of Sterols by *Saccharomyces cerevisiae* in Fed-Batch Culture

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*Institute of Chemical Technology – Department of Fermentation Chemistry and Bioengineering, Prague, Czech Republic*

**Abstract**

There are many influences affecting sterol production and their composition in baker's yeasts. One of the most important factor is feeding strategy with respect to the growth rate of microbial population. Experiments were carried out in the fed-batch system and the feeding rate was according to the profile of $CO_2$ in off-gases during cultivation run. Each cultivation was divided into two parts – phase of growth and phase of retarded growth. It was not proved that sterols accumulate in cells kept at low growth rate. Also the higher ethanol concentration (50 g/l) in the culture had no stimulating effect for sterol biosynthesis. Nevertheless, ethanol had got the positive influence on the ergosterol content in total sterol fraction, that exceeded 95% (w/w). Changes in yeast sterols were observed in the course of storing of biomass at various conditions. The cultivation conditions leading to the different specific biomass growth rates were simulated (using PSI/C simulation software) by means of the mathematical physiological model. Simulations were verified by cultivations. Model predicted data for profiles $CO_2$ exploited for controlling of feeding were in a good agreement with real courses.

sterols; ergosterol; *Saccharomyces cerevisiae*; fed-batch cultivation; model simulations

Production of the yeast biomass is a wide spread biotechnology. The microbial biomass is a rich source of the many valuable substances as well.

Low level of growth rate is generally considered to be a positive factor to accumulate ergosterol in yeast membranes. Arnezeder and Hampel (1990) found linear correlation between growth rate and amount of ergosterol in yeast cells without respect to utilized substrate. Increased sterol formation was found at low growth rate at the end of exponential phase and in the stationary phase. That is supported by a presence of excess of carbon source. Sterol intermediates are esterificated by fatty acids as a cellular reserves at their overproduction. Běhalová et al. (1994) explained that yeast culture characterized by high content of ergosterol without a parallel accumulation of its ergosterol precursors is probably unattainable. Influence of physiological state of cells and medium composition was studied many times. The results show that nitrogen deficient medium and sporulation medium caused increasing in the total amount of sterol content (Běhalová et al., 1992). The role of oxygen is very important. Sterols are synthesised in the endoplasmatic reticulum and transported to the plasmatic membrane under aerobic conditions. In the absence of oxygen their synthesis is inhibited (Hunáková, Hapala, 1996).

The membrane structure changes in yeast cells are intensively affected by presence of ethanol in the medium. Sterol deficient strains of yeast cells show higher sensitivity to the ethanol (Novotný et al., 1992b). Same authors were dealing with relation between lipidic composition of membranes and ethanol induced death of cells in another work. Their results do not seem to sustain their foregoing conclusion (Novotný et al., 1992a).

Modelling of the yeast growth has a importance for controlling of the bioprocesses. There are some models for yeast culture grown in various conditions (Keulers, 1993; Rychtera et al., 1996a). Various parameters (ethanol, $CO_2$, dissolved oxygen concentration) were tested for controlling of feeding strategy for fed-batch process (Rychtera et al., 1996b; Konopásková et al., 1994). Respiratory quotient and carbon dioxide production rate were found to be suitable for the controlling of continuous processes (Meyer et al., 1984).

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## MATERIALS AND METHODS

### Microorganism

*Saccharomyces cerevisiae* D7 strain was used for all cultivations. This strain which was improved to the higher production of sterols was acquired from collection of microorganisms on Department of Fermentation Chemistry and Bioengineering (Institute of Chemical Technology, Prague). The yeast strain was maintained on malt agar slants at 4 °C.

### Medium

The medium for inoculum contained per 1 litre: 20 g glucose, 5 g yeast extract, 5 g $(NH_4)_2SO_4$, 0.6 g $KH_2PO_4$, 1 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2$. Production medium contained per 1 litre: 250 g glucose, 31.25 g yeast extract, 3.75 g $KH_2PO_4$, 3.13 g $MgSO_4.7H_2O$, 2.5 g $CaCl_2.6H_2O$. C : N ratio of production medium was 10 : 1. For both media pH value were adjusted at 5.0.

### Inoculum

Yeast cells from agar slants were grown on 100 ml of inoculum medium on rotary shaker at 30 °C for 24 h. The cells were harvested by centrifugation and used as inoculum for cultivation.

### Bioreactor and equipment

Cultivations were carried out in laboratory bioreactor 7L-MBR-Bioreactor (Switzerland). The working volume was 5 l at start of cultivation. Temperature, pH, speed of agitation and excessive foam formation were controlled by IMCS 2000 unit. The MBR bioreactor was equipped by probe for measurement of dissolved oxygen (DOC) and analyzers of ethanol, $O_2$ and $CO_2$ in outlet gases. Dosing of medium was assured by programmable peristaltic pump (Verdeflex, Germany). Dosing frequency was controlled according to signal from $CO_2$ gas analyser (the amount of dose was always the same). $CO_2$ and $O_2$ concentration in off-gases was analysed by Infralyt 5 (VEB Junkalor Dessau, Germany) and Permolyt 2 (VEB Junkalor Dessau, Germany). Ethanol in outlet was measured by Metrex analyzer (ICT, Prague).

### Cultivation conditions

Twenty-two-hour cultivations were led in the fed-batch system at 30 °C and constant pH value 5.0. The culture was stirred (600 rpm) and aerated by 3 l/min.

### Storing of biomass

Centrifuged and washed wet biomass taken from stationary phase of growth was stored (in suspended form) in distilled water or in 50 g/l of ethanol solution at 4 °C and 20 °C for 14 days.

### Analytical methods

Biomass was determined immediately as a dry weight at 105 °C after centrifugation and twice washing with distilled water.

Ethanol and glucose in the medium and culture supernatants were determined by HPLC on a cation exchange column (Ostion LGKS 0800 $H^+$ form) with flow rate 1.0 ml/min $H_2SO_4$ solution ($c$=5 mmol/l) as a mobile phase and using a flow refractive index detector.

Sterol content in cells was measured after alkaline hydrolysis and extraction of sterol fraction by HPLC using a reverse phase C18 column with flow rate 1.0 ml/min of methanol solution 95% (v/v) as a mobile phase and UV detector (282 nm).

To estimate viability of cells methylene blue staining was used. Total, budding and living cells were counted in a Bürker counter in the culture. Morphology of cells was observed microscopically.

## RESULTS AND DISCUSSION

The main aims of our work were to investigate relation between growth rate (ethanol addition, respectively) and formation of sterols (ergosterol especially), verification of mathematical model with foregoing simula-tions and acceptability of carbon dioxide in off-gases as a control parameter for feeding strategy.

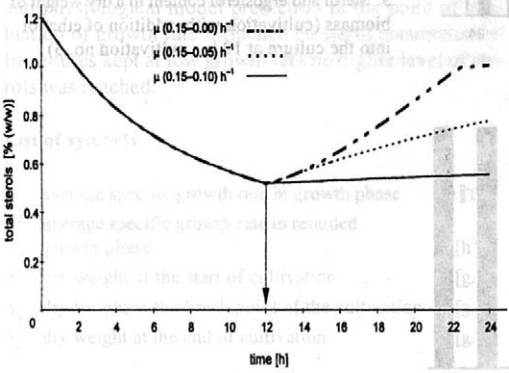
Five 24hour cultivations were carried out. To verify applied mathematical model cultivation two experiments were taken. These cultivations (no. 1 and 2) serve for assess-

I. Cultivation strategy

| Cultivation No. | Growth phase $\mu_1$ [$h^{-1}$] | Break point [h] | Retarded growth phase $\mu_2$ [$h^{-1}$] | Biomass [$g.l^{-1}$] $X_s$ | $X_{bp}$ | $X_e$ | Ethanol addition [$g\,l^{-1}$] |
|---|---|---|---|---|---|---|---|
| 1 | 0.10 | 17 | 0.00 | 1.1 | 15.7 | 15.4 | – |
| 2 | 0.15 | 15 | 0.01 | 1.7 | 13.1 | 16.8 | – |
| 3 | 0.10 | 12 | 0.07 | 1.3 | 6.4 | 12.0 | 50 |
| 4 | 0.15 | 12 | 0.00 | 1.2 | 13.8 | 13.2 | 50 |
| 5 | 0.15 | 12 | 0.00 | 1.3 | 9.8 | 9.7 | 50 |

ment of the influence of the growth rate on sterol accumulation in the yeast culture. The others (nos. 3–5) were intended on evaluating of yeasts behaviour at the presence of ethanol in the culture (Table I).

According to used mathematical model (Rychtera et al., 1996a) the production of sterols was predicated for different levels of the specific growth rates. Different values of specific growth rates were simulated by PSI/c simulation language (Fig. 1).




1. Prediction of sterol formation by yeast cell for different values of the specific growth rate based on the mathematical model

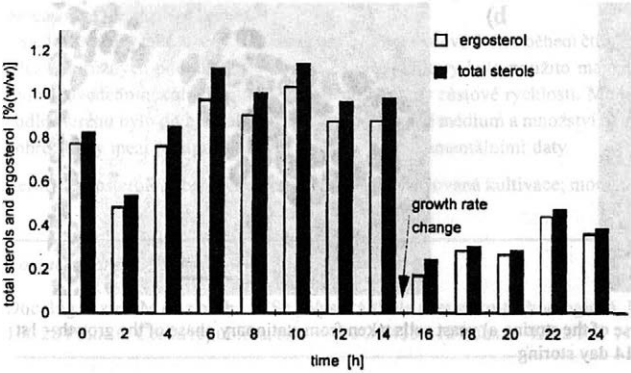
Each of cultivations was divided into the two parts – the first phase of growth and the second phase of retarded growth. In the phase of retarded growth higher production of sterols was expected. During this phase the growth rate was kept nearly at zero by two ways – the first was decreasing of feeding rate and the second was increasing the ethanol concentration in the culture on the level which inhibits the growth. Ethanol was added into the culture up to concentration of 50 g/l.

In the cultivation no. 2 (in comparison with the cultivation no. 1) the phase of retarded growth was prolonged in order to achieve higher sterol yield. This fact confirmed the hitherto knowledge about sterol formation at low growth rate (Arnezeder, Hampel, 1990). The growth rate was set on 0.15 $h^{-1}$ during the phase of growth and close to 0.00 $h^{-1}$ in the phase of retarded growth. Sudden decrease in the sterol content took place after break point at the 15 h of this cultivation (Fig. 2). Although, it was not so evident in the cultivation no. 1 any increasing in the amount of sterols formed. Acquired level of total sterol was inconsistent with mathematical model for these cultivations.

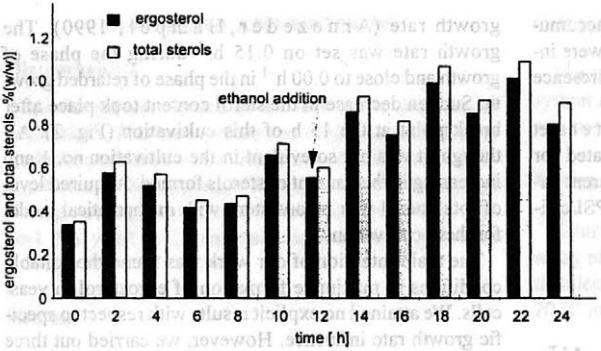
The main intention of our work was found the suitable conditions to maximize formation of ergosterol in yeast cells. We attained no explicit results with respect to specific growth rate influence. However, we carried out three cultivations with addition of ethanol into the culture (Table I) in order to estimate influence on sterols accumulation and composition of this fraction. Yeast culture grown at the presence of higher level of ethanol and in stationary phase (cultivation no. 5; at 12 h ethanol was added and feeding was stopped; no fresh glucose medium was fed into the bioreactor) gradually increased in the sterol content. During the growth phase amount of total sterols fluctuated from 0.35 % (w/w) to 0.71% (w/w). Level cell sterols increased to 1.09 % (w/w) at 22 h of cultivation no. 5 (Fig. 3). Under the same conditions (cultivation no. 4) there appears very pure ergosterol in the sterol fraction exceeded 95 % (w/w) almost whole second part of this cultivation (Fig. 4).

We tried to observe changes in yeast sterols in the course of storing of yeast suspension under the variable conditions. We could find no evident trend depending on duration or on the storing temperature (4 and 20 °C). Only ethanol addition affected purity of ergosterol as was mentioned above. The significant changes in morphology of cells were observed during the storing in the presence of ethanol (50 g/l). Morphological properties typical for yeast cells in the stationary phase were changed during the biomass storing. There was evident trend to loose oval shape and to agglutinate into the small clusters (Fig. 5).

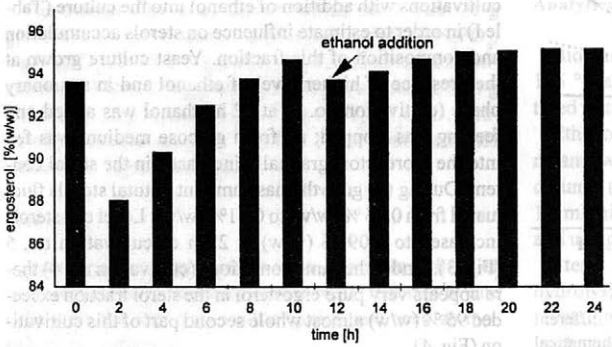
$CO_2$ in the off-gases can be a indicator of physiological state, especially biomass synthesis because its level is directly connected with growth of yeasts. In our cultivations $CO_2$ in outlet gas was used as the control parameter



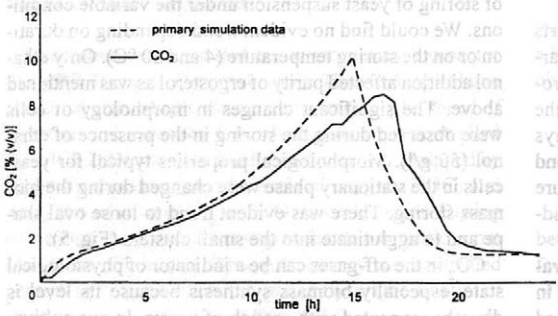

2. Sterol and ergosterol content in a dry weight of biomass (cultivation no. 2). At 15 h the feed rate was dramatically decreased



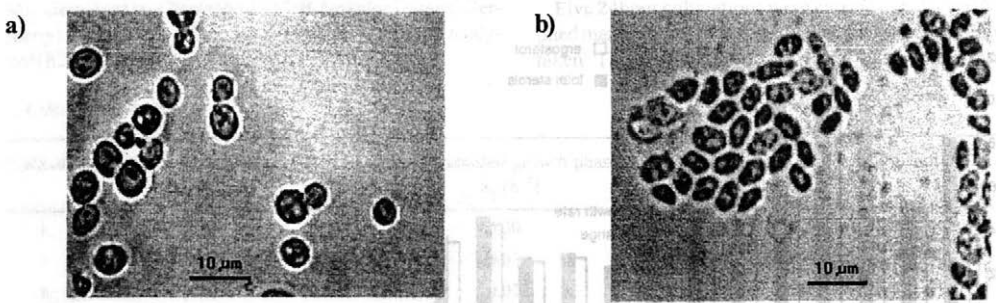

3. Sterol and ergosterol content in a dry weight of biomass (cultivation with addition of ethanol into the culture at 12 h – cultivation no. 5)




4. Ergosterol content in the sterol fraction in yeast biomass (cultivation no. 4)




5. Comparison of physiological model simulation and real course of carbon dioxide concentration in outlet gas (cultivation no. 2)




6. Changes in morphology of yeast cells in the course of the storing a) yeast cells taken from stationary phase of the growth – 1st day of the storing, b) yeast cells at the end of the 14 day storing

for dosing of the fresh medium into the bioreactor. That was found to be suitable for fed-batch cultivations after the comparison of real and simulated courses of $CO_2$ in off-gases curves (Fig. 6). In this case the mathematical model was in a good agreement with real data, but it should be corrected and precised for description of sterol and ergosterol content in the cells.

Results received from presented experiments are contradictory to earlier suggestions of some authors (Arnezeder, Hampel, 1990; Běhalová et al., 1994) and physiological model prediction in the point of evaluation of growth rate influence on sterol accumulation. In cultures kept at low growth rate no higher level of sterols was reached.

**List of symbols**

| | | |
|---|---|---|
| $\mu$ | average specific growth rate in growth phase | $[h^{-1}]$ |
| $\mu^2$ | average specific growth rate in retarded growth phase | $[h^{-1}]$ |
| $X_s$ | dry weight at the start of cultivation | $[g.l^{-1}]$ |
| $X_b$ | dry weight at the break point of the cultivation | $[g.l^{-1}]$ |
| $X_e$ | dry weight at the end of cultivation | $[g.l^{-1}]$ |

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**Souhrn**


VEDLICHOVÁ Z., DUBOVÁ R., ČERMÁK J., PATÁKOVÁ P., RYCHTERA M., MELZOCH K. (1998): **Produkce sterolů kvasinkou *Saccharomyces cerevisiae* v přítokované kultivaci.** Czech J. Food Sci., *16*: 9–19.

Produkce a složení kvasničných sterolů závisí na použitém mikroorganismu, fyziologickém stavu kultury, složení media a dalších faktorech. V této práci jsme studovali vliv růstové rychlosti a přídavku etanolu ke kultuře na produkci sterolů. Bylo provedeno pět přítokovaných 24hodinových kultivací a dávkování média bylo řízeno podle koncentrace $CO_2$ ve výdechových plynech. Každá kultivace byla rozdělena do dvou fází, které se lišily hodnotami růstové rychlosti – fáze růstu a fáze zpomaleného růstu, kde byla očekávána zvýšená produkce sterolů. Akumulace sterolů v biomase kvasinek *Saccharomyces cerevisiae* nebyla za snížené růstové rychlosti prokázána. Přídavek etanolu ke kultuře se projevil pozitivně ve zvýšení podílu ergosterolu ve sterolové frakci, a to za současné nízké růstové rychlosti.

Sledovány byly také změny v množství a složení sterolové frakce během čtrnáctidenního skladování biomasy ve formě kvasničného mléka za různých podmínek. K popisu chování kultury bylo použito matematického strukturovaného fyziologického modelu. Bylo provedeno několik simulací pro různé hodnoty růstové rychlosti. Model byl využit k predikci průběhu koncentrace $CO_2$, podle kterého bylo do bioreaktoru dávkováno čerstvé médium a množství akumulovaných sterolů. V případě $CO_2$ bylo dosaženo dobré shody mezi předpovězenými průběhy a experimentálními daty.


steroly; ergosterol; *Saccharomyces cerevisiae*; přítokovaná kultivace; modely simulace


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**Agricultural Research Institue Kroměříž, Ltd.**
**Mendel University of Agricultural and Forestry Brno**
**Food Research Institute Prague**

**organized in July 7–11, 1998 at Mendel University of Agricultural and Forestry Brno**
**Conference with international participation**

# CEREALS FOR HUMAN HEALTH AND PREVENTIVE NUTRITION

The following topics are planned to be discussed:

- Chronic deseases and their relation to cereals
  a) Cereals as a source of dietary fibre and other important nutrients
  b) Human clinical tests with fibre and individual nutrients in preventing and treating chronic diseases
- Development of cereal varieties for various food uses, initial genetic resources, breeding objectives
- Optimum post-harvest treatments and storage of food cerals in order to preserve nutrient content and desired quality (for example to avoid fat rancidity in oats, etc.)
- Technological methods for processing grain and whole crops and products from it
- Functional foods – new directions of the use of grain, its components and whole crops
- Legislation concerning cereals used for health beneficial nutrition applied in the Czech Republic and abroad – food codex, nutrition claims, quality parameters and limits, etc.

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**Zemědělský výzkumný ústav Kroměříž, s.r.o.**
**Mendelova zemědělská a lesnická universita Brno**
**Výzkumný ústav potravinářský Praha**

**pořádají ve dnech 7.–11. července 1998 na Agronomické fakultě MZLU v Brně konferenci s mezinárodní účastí**

# OBILOVINY VE ZDRAVÉ A PREVENTIVNÍ VÝŽIVĚ LIDÍ

Plánované okruhy problematiky projednávané na konferenci:

- Civilizační choroby a jejich vztah k obilovinám
- Obiloviny jako zdroj dietetické vlákniny a dalších důležitých živin
- Klinické pokusy s vlákninou a jednotlivými živinami v prevenci výskytu civilizačních chorob
- Tvorba odrůd obilovin pro nejrůznější směry potravinářského využití, výchozí genetické zdroje, směry šlechtění
- Optimální posklizňové ošetření a uskladnění potravinářských obilovin s cílem zachování obsahu a požadované kvality živin (např. ochrana před žluknutím tuku u ovsa apod.)
- Technologie zpracování zrna, jeho produktů i celých rostlin obilovin
- Funkční potraviny – nové směry využití zrna obilovin a jeho dílčích složek
- Legislativa obilnin ve zdravé výživě u nás a v zahraničí – potravinový kodex, ukazatele kvality, nutriční omezení apod.

Předběžné přihlášky a dotazy zasílejte na adresu:

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# Malolacid Fermentation in the course of Wine Formation

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**Abstract**

The concentration changes of basic organic acids in wine were observed during the course of spontaneous and regulated malolactic fermentation. The main acids investigated were tartaric, malic and lactic acids while citric, succinic and acetic acids were also examined. An HPLC chromatograph HPP 5001 was used for all analyses. The course of malic acid degradation was observed during a period of six months. It is possible to confirm the high and rapid malic acid degradation observed after application of the pure culture of lactic bacteria to wine.

malic acid; lactic acid; spontaneous fermentation; controlled malolactic fermentation

## Significance of Malolactic Fermentation

Forty years ago, malolactic fermentation (MLF) was an unknown phenomenon in oenology. Unless MLF has proceeded completely during the maturation of wine, there is a risk that, owing to the temperature increase, MLF could start after bottling. This would cause undesirable sparkling of wine because of carbon dioxide formation. At the same time, sparkling amplifies the astringence and the acidity. These were the reasons why the MLF was considered to be a wine disease instead of a natural occurrence in wine production.

The oenologic laboratories of today are equipped with analytical apparatus and/or methods which enable determination of malic and lactic acids and, consequently, if MLF has or has not occurred. Liquid chromatography, HPLC, capillary isotachophoresis or enzymatic methods belong to the methods currently in use (C a r m e n e r e , 1993).

MLF begins after ethanol fermentation is finished. This is because lactic bacteria need nutrients and vitamins resulting from yeast autolysis (F a r k a š , 1983).

Lactic bacteria degrade not only malic acid but also citric acid (C a r m e n e r e , 1993):

Malic acid → lactic acid + carbon dioxide

Citric acid → diacetyl + ethylacetate + acetic acid

Formatiou of lactic acid from L-malic acid can proceed by three metabolic ways (R i b e r e a u - G a y o n , 1975):

1. L-malic acid —(malic dehydrogenase)→ oxalacetic acid —(oxalacetate decarboxylace)→ pyruvid acid —(dehydrogenase)→ lactic acid

2. L-malic acid —(malic enzyme)→ pyruvic acid —(dehydrogenase)→ lactic acid

3. L-malic acid —(malolactic enzyme)→ lactic acid

**MLF contributions**

- Natural de-acidification: The malic acid with a rough and hard flavor is transformed to a mild lactic acid which reduces the astringence and intensifies the colour of red wine.
- New aroma formation: Some primary aromas originating in the grapes and secondary aromes resulting from the fermentation can disappear while some new ones can appear (e.g. diacetyl with a butter flavour) (C a r m e n e r e , 1993).

• Biological stability: After MLF is finished, the liability of wine to bacterial and yeast attack is decreased.

MLF is almost inevitable in the case of astringent red wines because of need to reduce its acidity.

With white wines the situation is different. Preservation of a certain acidity level is necessary for maintaining the freshness and the primary (varietal) and secondary (fermenting) aromas. MLF can subsequently be prevented by adding the sulphur dioxide to wine after ethanol fermentation (Navarre, 1994).

In extremely cold years the grapes cultivated in northern wine regions can exhibit high acidity, especially high concentration of malic acid. In this case MLF is desirable even in white wines (Malík, 1996).

The process has to be prepared as follows: removal of muds, very moderate sulphuration and leaving the wine on the yeasts. A temperature between 17 and 20 °C is necessary for MLF initiation. If cellars are too cold between the end of October and November, the MLF will start not earlier than in the spring.

The white wines after MLF completion are fatter and more stable, but less fresh than the white wines without any MLF. In the first case, the wines are tolerant enough to maturation and ageing.

The MLF is a complex process which is not easy to control.

Convenient conditions are as follows: Temperature between 17 and 20 °C, pH = 3–3.6 (not lower). Even if the conditions are kept within these limits, the MLF initiation is not guaranteed.

The MLF can start several days after ethanol fermentation is finished but usually it occurs any time during the following two months. If the contents of the tanks are exposed to lower temperatures, the MLF is delayed until spring.

Once the MLF is finished, the sulfur dioxide is added into wine to prevent bacterial and yeast development and for protection of wine against the oxidation process (Carmenere, 1993).

Controlled MLF is employed all over the world. It consists of the inoculation of pure lactic bacteria cultures (*Leuconostoc oenos*) into the wine after ethanol fermentation is finished. The temperature is kept at 18 °C and the malic acid degradation starts immediately. The yeast sediments taken from other tanks where the MLF has already finished, can also be used for inoculation.

Spontaneous MLF is caused by lactic bacteria naturally present in wine (e.g. the strains of *Lactobacillus*, *Leuconostoc* and *Pediococcus*). L-malic acid can be also degraded by the yeast strains of *Schizosaccharomyces pombe*. Carbon dioxide and water is formed in aerobic conditions, ethanol and carbon dioxide in anaerobic conditions (Minárik, Navara, 1967). As mentioned above, the initiation is very hard to control and so the spontaneous MLF is an unpredictable process.

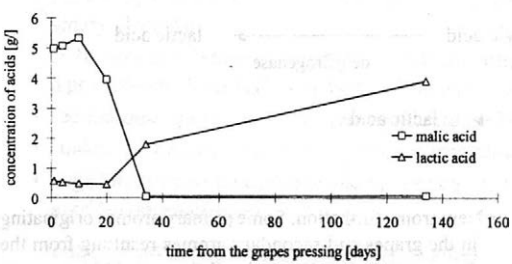
## MATERIAL AND METHODS

### Equipment

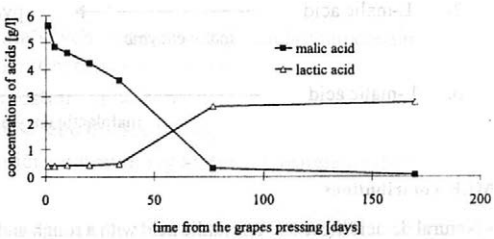
A liquid chromatograph HPP 5001 connected with a loop LCI 30 (Laboratorní přístroje, Prague) and a UV detector LCD 2082 (ECOM, Prague) were used. Measurements were carried out at a wavelength of 206 nm. Separation proceeded in a steel column 25 × 0.8 cm filled with ionex Ostion LGKS 0800 in an $H^+$ cycle (Tessek, Prague) at a temperature of 55 °C. The results were analysed using an Apex integrator (DataApex, Prague). The mobile phase was 0.005M sulphuric acid. Isocratic elution with a flow rate of 0.5 ml/min was employed. Samples were filtered through an acetate-cellulose filter of 0.2 μm pore size (Sartorius AG, Goetingen, Germany) (Kučerová, Čepička, 1996).

## RESULTS AND DISCUSSION

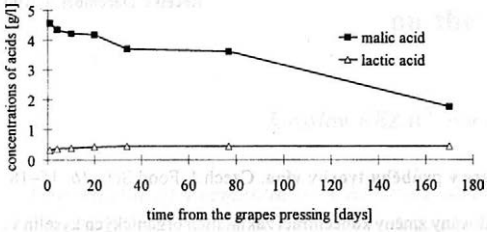
Wine samples from the Velké Pavlovice vine growing region, vintage 1995, were analysed. At various stages of wine production, the total acidity and the content of individual acids were investigated. White wines were produced using classical technology including spontaneous acid degradation while red wines were prepared using regulated degradation.



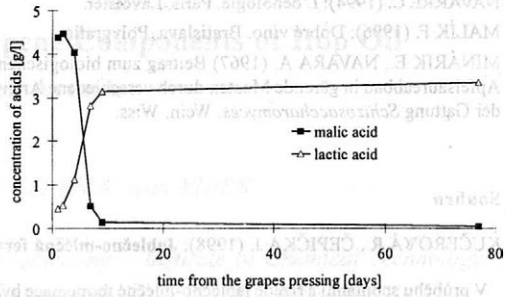

1. Lactic and malic acids changes – Spontaneous fermentation variety Welschriesling




2. Lactic and malic acids changes – Spontaneous wine fermentation variety Neuburger




3. Lactic and malic acids changes – Spontaneous wine fermentation variety Müller-Thurgau




4. Lactic and malic acids changes – Regulated wine fermentation

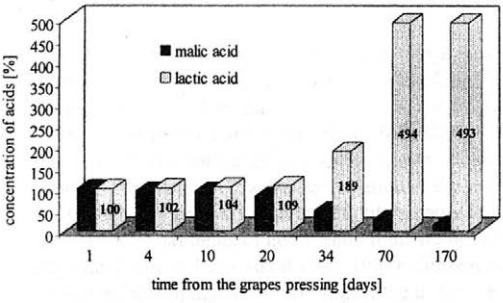
The following figures demonstrate concentration changes of malic and lactic acid over the period of 170 days from the grape pressing.

In the case of spontaneous fermentation three wine varieties were investigated – Müller-Thurgau, Neuburger and Welschriesling. During the fermentation of the Welsch Riesling variety almost all the malic acid was degraded to lactic acid after about 30 days.

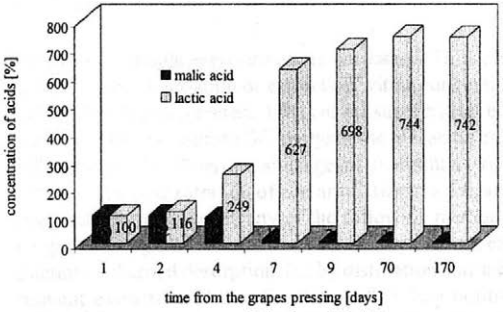
The Neuburger variety required more than twice as much time as the Welschriesling, with most of the malic acid being degraded within 70 days.

The slowest degradation was found for the Müller-Thurgau variety, as after 70 days no considerable changes in the above-mentioned acids were observed. During the next five months the malic acid was degraded to only half of its original amount.

All samples from regulated fermentation are represented in one figure owing to the similar course of concentration changes for the acids. In the course of regulated fermentation lactic bacteria were added to fermenting wine immediately after the violent stage and the total degradation of malic acid was observed after only seven days.




5. Course of malic acid degradation and lactic acid formation – Spontaneous fermentation




6. Course of malic acid degradation and lactic acid formation – Regulated fermentation

Differences in the time of malic acid degradation and lactic acid formation during the course of spontaneous and regulated fermentation were examined. In the case of **spontaneous fermentation** the first considerable change was observed on average after about 30 days. The decrease of malic acid was 49% and the increase of lactic acid was 189% of the original content. In the course of regulated fermentation the considerable changes were already observed in 7 days after grape pressing with the malic acid being degraded to 12 % of its original content and lactic acid increasing more than six times.

It is possible to confirm the rapid and high level of malic acid degradation observed after application of the pure culture of lactic bacteria to fermenting wine. To preserve lactic bacteria activity it is necessary that the addition be made before the drawing of wine from the yeast sediment owing to the vitamin and nutrient demand.

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**Souhrn**

KUČEROVÁ R., ČEPIČKA J. (1998): **Jablečno-mléčná fermentace v průběhu tvorby vína.** Czech J. Food Sci., *16*: 15–18.

V průběhu spontánní a řízené jablečno-mléčné fermentace byly sledovány změny koncentrací základních organických kyselin ve víně. Byly zkoumány především kyseliny vinná, jablečná, mléčná, ale také citronová, jantarová a octová. Analýzy byly prováděny metodou kapalinové chromatografie na přístroji HPP 5001. Průběh odbourávání kyseliny jablečné byl pozorován po dobu šesti měsíců. Závěrem lze shrnout velmi účinnou a rychlou degradaci kyseliny jablečné pozorovanou po aplikaci čistých kultur mléčných bakterií do vína těsně po skončení alkoholové fermentace.

kyselina jablečná; kyselina mléčná; spontánní fermentace; řízená jablečno-mléčná fermentace

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# The Influence of the Main Terpene Components of Hop Oil on the Beer Aroma

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**Abstract**

The influence of hop essential oil on the aroma of beer was the subject of the present work. The aim was to correlate the achieved set of analytical data with the sensory profile of hop aroma. Sixteen samples of Czech, American, British and German aromatic and bitter hop varieties were analyzed. The laboratory and pilot scale brews were prepared from some of the analyzed hops. Total hop oil content was measured using steam distillation and extraction. The hop essential oil constituents were identified by a GC-MS system. Essential oil constituents were also isolated from beer using solid phase extraction and then identified by a GC-MS system. The beer sensory profiles were assessed with emphasis on hop aroma evaluation. Either α-humulene or myrcene content had the highest content in all hop varieties. A number of substances were identified, including terpenes, esters and ketones. The main hop oil constituents α-humulene, myrcene and β-caryophyllene were identified in beers as well as some oxidized sesquiterpenes and many other substances whose hop origin was not known. Using correlation analysis it was found that the essential oil composition of hop correlates with that of beer, on the basis of selected indicators. No correlation between the chosen sensory and analytical data of beer was found by either cluster analysis or principle components analysis.

hop essential oil; GC-MS analysis; hop flavour of beer; multivariate analysis

The chemistry of the hop bitter flavour in beer has been well understood for some time. Knowledge of the hop essential oil compounds that are responsible for various aspects of hop aroma is still incomplete. Both the composition of the hop essential oil and its flavour are influenced by the hop variety. However, correlation of the analytical and sensory analysis data is difficult because many of the major constituents of hop essential oil are not normally found in beer (P e p p a r d et al., 1989). Consequently, it is more advantageous to investigate the influence of the varietal factor on hop flavour in beer by reference to the spectrum of hop-derived components found in late-hopped beers, rather than to the composition of the essential oils in hop (P e a c o c k et al., 1981).

GC with Flame Ionisation Detection (FID) has found universal use in the analysis of hop aromatic compounds. Subsequently, GC with Flame Photometric Detection (FPD) revealed that hop oil can contain more than 20–30 sulphur compounds. Modern studies, however, have found GC coupled to Mass Spectrometry (MS) to be an indispensable tool, not only for the most sensitive quantification based on monitoring selected fragment ions but also for providing a vast amount of structural information about minor hop components (M o i r, 1994).

A sample of hop oil can be introduced directly to GC, or dissolved in a suitable solvent. For hops and hop products some sample pretreatment is necessary. This may involve steam distillation or extraction with a conventional solvent (S h a r p e et al., 1981) or the supercritical extraction with $CO_2$. Before GC analysis, the oil can be usefully split into fractions on a silica gel or aluminium oxide column. The concentration of hop aromatics in worts and beers can be achieved by any of the following methods: (i) gas flushing into adsorbents, followed by solvent extraction or thermal desorption, (ii) by distillation and subsequent extraction of the distillate with a low boiling point solvent such as Freon 11, (iii) by simultaneous distillation and extraction in the familiar Likens-Nickerson apparatus (H a l e y et al., 1983; M o i r, 1994). Solid phase extractions has become fashionable analytical tool in recent years. Flavour compounds are extracted from beer by passage through one of several proprietary $C_{18}$ cartridges commercially available followed by desorption by washing with a suitable solvent. The analysis of hop aromatics in boiled wort is much easier than in beer where they are usually swamped by fermentation volatiles. Analytical methods for trace organic compounds were improved and have led to an increased knowledge of the hop-derived compounds in beer (M o i r, 1994).

It appears that some of the hoppy flavour substances have very high flavour potencies, and produce flavour impacts at concentrations that are too low for detection by

conventional methods. Due to the fact that the knowledge and/or the ability to quantitatively measure the compounds responsible for certain aspects of hoppy aroma in beer is lacking, the sensory analysis plays an important role (Siebert, 1994).

A number of terms to describe flavour have been associated with hop aromas. They include "floral", "grapefruit", "citrus", "spicy", "fruity", "European hop", "Noble hop", "resinous", "piney", "herbal", "hop room aroma" and "cheesy". Most of these terms are thought to be fairly specific for flavours that originate in hops, while several are more general. Different beers have different amounts and proportions of these flavour characteristics, depending on the hop cultivar and processing procedures used to produce them. Depending on the purpose of sensory evaluation, different approaches are employed. In many cases a single term for "hoppy" is used as a part of an overall beer flavour profile. In some cases the intensity as well as the quality of the hoppy flavour may be evaluated. Often the nature of the hoppy flavour may be further characterized; perhaps as "dry hop", "kettle hop" or "hop oil", which are terms from the beer flavour wheel (Siebert, 1994).

For a better understanding of the problem associated with beer flavour, brewing scientists in recent years have made increasing use of multivariate statistical techniques. One of the objectives of applying multivariate statistics has been to correlate flavour descriptives or hedonic judgements with physicochemical measurements. However, such techniques do not prove any causal relationships (Peppard et al., 1989). A number of different multivariate methods have been applied in the past few years. Two of the most commonly used techniques are the principal components analysis and the factor analysis. Applied separately to matrices of sensory and instrumental data, these methods often enable discrimination between different beers and, furthermore can provide an explanation of the basis of such discrimination in terms of the individual variables used (Peppard et al., 1989). Because the olfactory sensitivity and consistency of sensory subjects are different for a given aroma note, their sensory ratings need to be weighted. The performance of subjects may be the evaluated by the analysis of variance, principal components analysis or multivariate analysis of variance. Based on these analyses, the scientist sometimes decides to eliminate a subject's data. This in itself is a form of weighting, as each retained subject is given a weight of 1, while each eliminated subject is given a weight of 0 (Yang et al., 1994).

## MATERIAL AND METHODS

### Hop Oil Isolation from Hop Sample

The amount of 100 g of natural hop was placed in a distillation flask together with 2 l of distilled water. The mixture was refluxed for 3 hrs. The separated hop oil was trapped into a collimator. The hop oil was poured into a separating funnel after steam distillation and extracted with 30 ml of diethyl ether. The upper organic phase was collected into an Erlenmayer flask and desiccated using anhydrous sodium sulphate and filtered. The solvent was removed from the sample by evaporation for 30 min at a temperature of 35 °C. After weighting, the sample was analyzed by gas chromatography.

### Hop Oil Concentration from Beer Sample

Hop essential oil was isolated from beer using solid phase extraction. An octadecyl-silicagel column Separcol C18 was employed as a solid sorbent. The column was conditioned using 3 ml of methanol and 3 ml of distilled water. The amount of 100 ml of distilled water was mixed with 100 ml of beer and the mixture was allowed to flow through the column. Isolated compounds were eluted by n-hexane, which was subsequently partially evaporated. Fresh samples were analyzed using a GC-MS system under the same conditions used for hop analysis.

### Chromatografic Conditions

GC-MS analyzer FISONS MD 800

Column: SPB-1, length: 50 m
inner diameter: 0.32 mm
stationary phase: phenylmethylsilicone
film thickness: 1 μm

Temperature program: from 50 °C, then 4 °C/min gradient to the final temperature of 280 °C

Carrier gas: helium

Mass detector: electron impact ionization
electron energy: 70 eV

### Pilot Scale Brewing

Pilot scale brews were performed for evaluation of the correlation between hop and beer. The brewing conditions were the same for all samples. Beers were brewed on a pilot scale (50 l) with proportions of hops added at the beginning of wort boiling (75% of the weight of hops) and 5 min before kettle knock-out (25% of the weight of hops), with a total boil time of 90 min. The quantities of hops added were designed to achieve a target of 12 g α-bitter acid in 100 l of wort. The raw materials used (i.e. malt, water and a yeast strain) were the same. The only difference was the type of the hop used. A list of the hops used is shown in the Table I.

### Sensory Analysis of Beers

The sensory of beer profiles were assessed with emphasis on hop aroma evaluation. Beers were tasted after five weeks of storage in a lager cellar. The panel consisted of nine staff and graduate students from the Department of Fermentation Chemistry. The evaluation form was de-

I. List of analyzed hop samples

| Sample No. | Harvest year | Variety | Descriptor |
|---|---|---|---|
| 1 | 1995 | Zlatan | Saaz aroma variety |
| 2 | 1995 | Sifem | Saaz aroma variety |
| 3 | 1995 | Osvald's clone 72 | Saaz aroma variety |
| 4 | 1995 | Sládek | New Saaz aroma hybrid variety |
| 5 | 1995 | Bor | New Saaz bitter hybrid variety |
| 6 | 1995 | Sm. 3231 | Saaz bitter hybrid |
| 7 | 1995 | Willamette | American aroma variety |
| 8 | 1995 | Nugget | American bitter variety |
| 9 | 1995 | Galena | American bitter variety |
| 10 | 1996 | Osvald's clone 72 | Saaz aroma variety |
| 11 | 1996 | Sládek | New Saaz aroma hybrid variety |
| 12 | 1996 | Bor | New Saaz bitter hybrid variety |
| 13 | 1996 | Premiant | New Saaz bitter hybrid variety |
| 14 | 1996 | Brewer's Gold | English bitter variety |
| 15 | 1996 | Northern Brewer | English bitter variety |
| 16 | 1996 | Hallertau Magnum | German bitter variety |

signed specifically to focus the taster's attention on the various aspects of hop aroma. There were 16 special descriptors for hop aroma evaluation. Intensity, character and flavour of hop aroma were assessed. Temperature of the tasted beers was 8–10 °C for usual evaluation and 20–22 °C for hop aroma evaluation.

### Statistical Evaluation

The analytical and sensory analysis data were evaluated by means of the correlation analysis, cluster analysis and principal components analysis on the basis of selected oil constituents in hop and beer.

## RESULTS AND DISCUSSION

### Sensory Profile of Beers

The hop aroma intensity, flavour and character as well as the overall sensory profile were analyzed. The results of the sensory analysis are shown in Table II. The sensory evaluation of the individual samples was different, especially in the case of hop aroma character. The aroma evaluation was probably influenced by the different bitterness and carbon dioxide contents of samples. In general, the beer sensory profiles were good, with strange odours and tastes having low intensity. Beer quality was well-balanced in all samples.

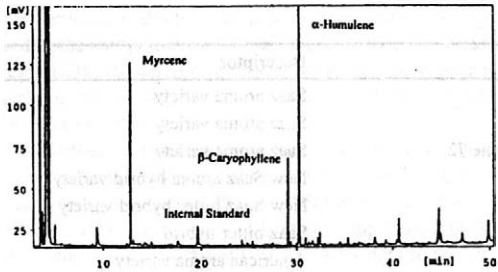
The hop aroma character was assessed mostly as "fresh", "fruity", "floral" or "redolent" but in some cases as "pungent" or "insipid". It seems to be a correspondence of some varieties between two years, e.g. Sládek variety was evaluated as "fresh" or "aromatic" in all beer samples. Sládek variety had the best evaluation in the first sample series in 1996, Osvald's clone 72 had the best evaluation in the second sample series in 1997. The hop aroma of Saaz aroma varieties were assessed to be better than that others. On the other hand, new Saaz bitter va-rieties (Bor and Premiant) were assessed to be worse than the Saaz aroma varieties. The hop aroma of American va-rieties Galena and Nugget was evaluated as "pungent".

II. Sensory analysis of beers of the first and second series

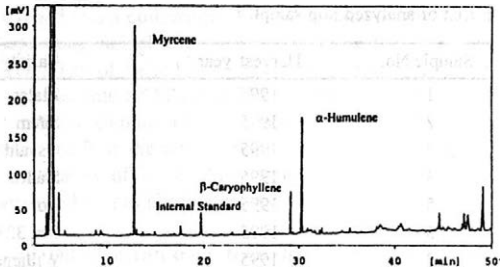
| Hop variety | Hop aroma | | |
|---|---|---|---|
| | intensity | flavour | character |
| **First series** | | | |
| Sládek | 3.00 | 2.44 | fresh |
| Bor | 2.44 | 2.78 | insipid, inexpressive |
| Sm 3231 | 2.78 | 3.00 | pungent |
| Zlatan | 2.22 | 3.11 | fruity |
| Sifem | 2.89 | 3.11 | fruity |
| Osvald's clone 72 | 2.67 | 2.89 | fresh, fruity |
| Willamette | 2.56 | 3.00 | insipid, inexpressive |
| Nugget | 2.78 | 3.22 | pungent |
| Galena | 2.44 | 3.00 | pungent |
| **Second series** | | | |
| Bor | 3.00 | 3.86 | sweet, floral |
| Northern Brewer | 2.71 | 2.86 | sweet, pungent |
| Premiant | 2.86 | 3.71 | pungent |
| Hallertau Magnum | 2.57 | 3.14 | insipid, inexpressive |
| Brewer's Gold | 3.00 | 3.43 | fruity, fresh |
| Sládek | 2.57 | 3.29 | fresh |
| Osvald's clone 72 | 2.86 | 2.57 | fresh, aromatic |

### Analytical Data of Beers

Hop essential oil components were measured in beers using solid phase extraction and GC-MS. The main terpe-




1. GC-MS chromatogram of Premiant beer




2. GC-MS chromatogram of Brewer's Gold beer

ne compounds, i.e. myrcene, b-caryophyllene, b-farnesene and a-humulene, were identified in all beer samples. The GC-MS chromatograms of hop oils in two beer extracts are shown in Figs. 1 (Premiant variety) and 2 (Brewer's Gold variety). The major component was α-humulene in the Premiant sample and, on the contrary, myrcene in the Brewer's Gold sample. Similar differences were found in the essential oil composition of original hops. The main terpenes content in some pilot scale beer are shown in Table III.

III. The ratios of the main terpenes content in beers of the first and second series

| Beer sample | M/H | M/C | F/M | F/H |
|---|---|---|---|---|
| **First series** | | | | |
| Sládek | 0.56 | 1.94 | 0.51 | 0.29 |
| Bor | 0.30 | 1.20 | 1.09 | 0.33 |
| Osvald's clone 72 | 0.25 | 0.46 | 3.48 | 0.25 |
| Galena | 1.34 | 3.64 | 0.24 | 0.33 |
| Zlatan | 0.25 | 0.40 | 1.67 | 0.61 |
| Sifem | 0.26 | 0.88 | 1.41 | 0.36 |
| Sm. 3231 | 0.54 | 1.72 | 0.71 | 0.38 |
| Willamette | 0.47 | 1.54 | 1.28 | 0.60 |
| Nugget | 0.20 | 0.69 | 1.39 | 0.27 |
| **Second series** | | | | |
| Osvald's clone 72 | 0.46 | 1.78 | 0.85 | 0.39 |
| Sládek | 0.68 | 1.65 | 0.00 | 0.00 |
| Bor | 0.65 | 1.79 | 0.00 | 0.00 |
| Premiant | 0.84 | 2.22 | 0.00 | 0.00 |
| Brewer's Gold | 1.11 | 1.68 | 0.00 | 0.00 |
| Northern Brewer | 0.59 | 1.27 | 0.00 | 0.00 |
| Hallertauer Magnum | 0.48 | 1.20 | 0.00 | 0.00 |

M/H – myrcene/α-humulene F/M – β-farnesene/myrcene
M/C – myrcene/β-caryophyllene F/H – β-farnesene/α-humulene

### Analytical Data of Hops

Hop oil composition was measured by steam distillation and GC-MS analysis. The results of these analyses are shown in Table IV. The major component in the first series was α-humulene in all Saaz aroma varieties. Myrcene was the major component in the new Saaz bitter varieties and in the American variety Galena. The Willamette variety had the most similar composition to Saaz aroma varieties. A lot of differences were found between Czech Saaz varieties and American varieties due to the fact that some esters were not present in the Saaz varieties. The analyses in the second series showed a lower content of myrcene in Osvald's clone 72 compared to bitter Saaz and imported varieties. Unfortunately, most of the hop oil substances were not detected in beers because of low concentrations, so it was not possible to use these substances as indicators in multivariate analysis.

### Statistical Evaluation

The aim of this work was to use some multivariate analysis methods to determine the relationship between hops and the corresponding beers brewed from these hops.

Correlation analysis was used for the evaluation of hop and beer essential oil composition. Hop samples and beer samples were used as variables. The correlation coefficient value corresponds to the similarity between hop and beer. The critical value of the coefficient was 0.4683 for a significance level of $P = 0.05$ and 0.5898 for a significance level of $P = 0.01$ in the first series of samples.

The results of the correlation analysis are shown in Table V, which shows the correlation coefficients for hops and beers. There exists mostly a positive correlation between hop and beer samples. Correlation coefficient value, for example, between Sládek hop and Sládek beer was 0.86, between Galena hop and Galena beer the value was 0.92. In the case of Bor, Zlatan and Willamette, positive correlations were found at the significance level of 0.05 which means the probability of 95%. One exception was the case of the Nugget variety, where no positive correlation was found between hop and beer.

The results of the second series proved a similar correlation between hops and beers, as it is shown in Table V.

The selected hop constituents and their ratios did not comprise all the differences between the samples, the refore it is not possible to conclude that the results are unambiguous and complete. In the future it will be neces-

IV. Hop oil constituents content in hop of first series

| Hop sample | Compound peak area / Internal standard peak area | | | |
|---|---|---|---|---|
| | myrcene | β-caryophyllene | β-farnesene | α-humulene |
| **First series** | | | | |
| Sládek | 29.94 | 11.17 | 3.05 | 27.39 |
| Bor | 34.11 | 8.82 | 0.49 | 28.85 |
| Zlatan | 11.87 | 10.01 | 16.66 | 24.39 |
| Sířem | 9.65 | 8.87 | 15.32 | 20.71 |
| Sm 3231 | 39.51 | 11.34 | 1.96 | 19.89 |
| Willamette | 10.48 | 12.38 | 7.32 | 22.01 |
| Osvald's clone | 8.90 | 11.91 | 13.84 | 21.56 |
| Galena | 21.59 | 10.04 | 0.20 | 19.77 |
| Nugget | 22.41 | 14.26 | 1.48 | 27.39 |
| **Second series** | | | | |
| Osvald's clone 72 | 17.81 | 10.02 | 15.17 | 38.65 |
| Sládek | 29.88 | 18.06 | 0.02 | 44.06 |
| Bor | 27.33 | 15.30 | 0.04 | 42.01 |
| Premiant | 31.68 | 14.30 | 0.08 | 37.85 |
| Brewer's Gold | 26.30 | 15.68 | 0.00 | 23.79 |
| Northern Brewer | 23.24 | 18.24 | 0.00 | 39.63 |
| Hallertau Magnum | 19.60 | 16.40 | 0.00 | 40.63 |

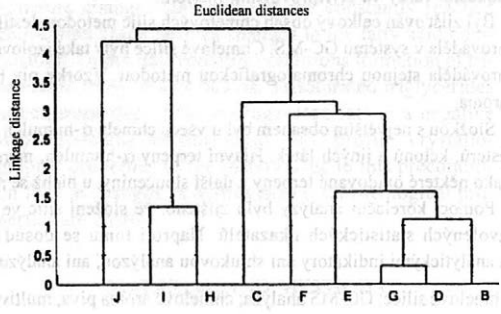
sary to determine more essential oil compounds and hop-derived compounds which affect the hop aroma of beer.

This analysis also proved a strong similarity in some groups of hop varieties. For example, the correlation coefficient between Saaz aroma varieties Zlatan and Sířem was 0.99, between the Osvald's clone 72 and Sířem 0.96. High positive correlation was also found between beers which were brewed from varieties of the same group.

V. Correlation analysis between hop and beer samples of the second series

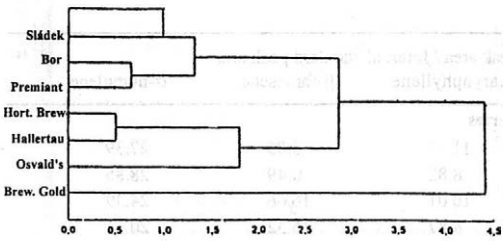
| Sample of hop and beer | Correlation coefficients hop vs. beer |
|---|---|
| **First series** | |
| Sládek | 0.86 |
| Osvald's clone 72 | 0.80 |
| Sířem | 0.85 |
| Zlatan | 0.49 |
| Bor | 0.50 |
| Sm. 3231 | 0.82 |
| Galena | 0.92 |
| Nugget | 0.28 |
| Willamette | 0.54 |
| **Second series** | |
| Sládek | 0.98 |
| Bor | 0.95 |
| Premiant | 0.86 |
| Northern Brewer | 0.97 |
| Hallertauer Magnum | 0.99 |
| Brewer's Gold | 0.70 |
| Osvald's clone 72 | 0.85 |

The correlation between sensory and analytical data was examined in the next part of this work. Three aroma descriptors were chosen for this evaluation being hop aroma intensity, flavour and character. Analytical data were obtained for ratios of selected essential oils of β-farnesene, β-caryophyllene, myrcene, α-humulene and 2-undecanone. PCA and cluster analysis were used for this purpose. Unfortunately no correlation between sensory and analytical data has been found. The dendrogram of the cluster analysis of sensory and analytical data is shown in Fig. 3. There are two main clusters; all sensory data (J, I, H indicators) and all analytical data (C, F, E, G, D, B indicators). The principal component analysis of the same indicators confirmed these results.




B, C, D, E, F, G – Analytical indicators (M/H, M/C, C/H, F/C, F/M, F/H, respectively)
H, I, J – Sensory indicators (intensity, flavour and character, respectively)

3. The cluster analysis of analytical and sensory indicators of the first series




4. The cluster analysis of beer samples of the second series

Correlation between individual beer and hop samples was also examined by the cluster analysis of the essential oil composition of hops and sensory profile of beers which were brewed from them. Brewer's Gold hop differed from the others because it is presented as the first main cluster and the others as the second one. Fig. 4 also shows the similarity of the new Saaz hybrid varieties Sládek, Bor and Premiant because they appeared in one cluster.

## Conclusion

The statistical evaluation of results proved a positive correlation between hop variety and the beer, which was brewed from this hop almost in all samples. Essential oil composition of hop correlated with that of beer, on the basis of selected indicators.

The correlation between chosen sensory and analytical data has not yet been proved by either cluster or principal component analysis.

The cluster analysis showed similarities and differences between beer samples and hop samples on the basis of selected indicators of oil composition and sensory profile.

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**Abstrakt**

ŘEZÁČ J., ČEPIČKA J., VIDEN I. (1998): **Vliv hlavních terpenických složek chmelových silic na aroma piva.** Czech J. Food Sci., *16*: 19–24.

Cílem práce bylo sledování vlivu hlavních terpenických složek chmelových silic na aroma piva. Snahou bylo zejména objasnit korelaci mezi vybranými analytickými a senzorickými ukazateli. K tomuto účelu bylo použito 16 odrůd českých, amerických, anglických a německých chmelů jak aromatických, tak hořkých. Tyto vzorky byly analyzovány a byly z nich připraveny pokusné várky ve čtvrtprovozním měřítku.

Byl zjišťován celkový obsah chmelových silic metodou destilace s vodní parou a extrakce. Identifikace jednotlivých složek se prováděla v systému GC-MS. Chmelové silice byly také izolovány z piv technikou extrakce na pevné fázi. Identifikace složek se prováděla stejnou chromatografickou metodou. Vzorky piv byly podrobeny senzorické analýze se zaměřením na chmelové aroma.

Složkou s největším obsahem byl u všech chmelů α-humulen nebo myrcen. Identifikováno bylo také množství dalších terpenů, esterů, ketonů a jiných látek. Hlavní terpeny α-humulen, myrcen, β-karyofylen a β-farnesen byly nalezeny v pivech podobně jako některé oxidované terpeny a další sloučeniny, u nichž se nedá prokázat chmelový původ.

Pomocí korelační analýzy bylo zjištěno, že složení silic ve chmelu pozitivně koreluje s jejich složením v pivu na základě zvolených statistických ukazatelů. Naproti tomu se dosud nepodařilo prokázat korelace mezi vybranými senzorickými a analytickými indikátory ani shlukovou analýzou, ani analýzou hlavních komponent.

chmelové silice; GC-MS analýza; chmelové aroma piva; multivariační analýza

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# Effect of the Recycled Ni-catalyst on the Quality of the Hydrogenated Rapeseed Oil

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**Abstract**

A change of standard properties of Ni-catalyst occurs in the process of recycling. It is especially activity and selectivity and at the same time hydrogenated fat property changes occur. Property changes of the hydrogenated rapeseed oil saturated into a constant value of iodine number about 75 under the standard laboratory conditions with the use of fresh and used in operation Pricat 9910 catalyst are discussed. In dependence on the loss of catalyst activity a change in quality of the hydrogenated oil occurs: increase of melting point, changes of SFC profile in temperature range 10–40 °C. Rheological properties of these fats are affected in a negative way.

activity; canola oil; hydrogenation; Ni-catalyst; rheology; selectivity; SFC

Standard properties of the modern chemical and food products are one of the main production requirements. In the case of the fat products where partially hydrogenated vegetable oil represents the main physical property determinant it is necessary to attain standard properties of this hydrogenated product. At the moment SFC profile represents the commonest characteristic of partially hydrogenated oils and fats besides melting point. The attainment of the limited SFC values at chosen temperatures is usually required (Unichema Int., 1991). One of preconditions for the attainment of hydrogenated fat standard properties is a good-quality hydrogenation catalyst, standard refined oil, suitable reactor (Edvardsson, Irandoust, 1994) and control of the double bond saturation process. Double bond saturation is usually controlled by the refractive index changes (Cole et al., 1994). The variability of Ni-catalyst properties at recycling remains to be a variable parameter. Vice versa it is possible to answer the question how many times it is possible to recycle catalyst so that the changes of hydrogenated fat properties were still acceptable.

The Ni-catalyst properties are not constant in the course of rapeseed oil hydrogenation. The property changes already occur in the course of hydrogenation of the first batch. The catalyst properties are being further changed at recycling. Due to interaction of triglyceride accompanying components – residues of sulphur substances and chlorophyll pigments (Cho-Ah-Ying, Man, 1989), phospholipides, alkaline soaps, water (Irandoust, Edvardsson, 1993), free fatty acids (Filip, Zajíc, 1987) – with the Ni/NiO crystallite system changes of the catalyst surface characteristics take place. Consequently changes of the catalyst function properties of which activity and selectivity are usually characteristic take place. The catalyst activity decreases in proportion to the great of these changes. Linoleic selectivity values change (Albright, 1965). Linoleic selectivity changes are usually accompanied by triglyceride selectivity changes (Coenen, 1976). In these cases the undesirable increase of stearic acid takes place. In extreme cases the stearic acid is located to triglyceride trisaturated structures.

Especially at the Ni-catalyst recycling the triglyceride structure changes take place at the constantly reached conversion grade besides changes in the fatty acid composition. Under unfavourable conditions formation of trisaturated triglycerides occurs. Trisaturated triglycerides affect properties of the hydrogenated fats in a negative way: especially melting point, SFC profile, crystallographic properties of the system. Due to this precondition for the changes of hydrogenated fat rheological properties are created.

## MATERIAL AND METHODS

### Catalyst and Oil

Fresh and recycled Pricat 9910 catalyst was worked with. Recycled catalyst were used for semirefined canola oil hydrogenation. This catalyst is destined for partial cata-

lytic hydrogenation of vegetable oils. Recycled catalyst with different activity decrease from the hydrogenation plant were chosen for evaluation in an accidental way. Numeric indication at "recycled" ones indicates exlusively tha fact that the catalyst in question in a different one, and that, at the same time, catalyst "recycled x" and "recycled x + 1" do not have any mutually direct technological bond. The refined canola oil was used for testing of the catalyst properties.

**Catalyst Activity and Selectivity**

The catalyst activity was established from the change of refined oil iodine number with 0.025 % Ni in a laboratory reactor (Zajíc, Filip, 1984) under the defined condition (180 °C, constant proportions of hydrogen agitation) for one hour of the reaction. On the assumption of behaviour according to the kinetics of the first order reaction the activity was expressed from the relation as $k = \ln (IN^0/IN)/\tau$, where $IN^0$ and IN are the values of iodine number established by titration methods at the beginning of the reaction and after one hour. The activity was further calculated by optimization of 4–5 iodine number values calculated from the fatty acid composition at the initial stage of the reaction when the iodine number values are in the interval 115–75 (Filip, Štětina, 1995). Linolic and linolenic selectivity was calculated by a standard methods from the model of the first order consecutive reactions. Problems in these reactions systems were discussed earlier (Filip, Štětina, 1995).

The SFC values were established by a pulsed NMR method, trans isomers by an IR spectroscopy method, rheological parameters on the Haake Viscotester VT 500 apparatus with cone and plate (Filip et al., 1992).

## RESULTS AND DISCUSSION

The properties of the four recycled Pricat 9910 Ni-catalysts with the fresh one are compared in Table I. The recycled catalysts differ in the Ni content which is 1–3% lower in comparison with the fresh one. Concentration differences are not great. Striking decrease on the recycled catalyst activities can be seen from the results. The activities are 2–4 times lower compared to the fresh catalyst.

I. Activity of recycled Pricat 9910 Ni-catalyst

| Catalyst | Ni (% w/w) | $IN_{60}$ | $k_{IN}^{EXP}.10^3$ ($min^{-1}$) |
|---|---|---|---|
| Fresh | 20.1 | 52.3 | 13.3 |
| Recycled 2 | 16.8 | 94.6 | 3.2 |
| Recycled 4 | 19.0 | 79.8 | 6.0 |
| Recycled 5 | 16.9 | 84.9 | 5.0 |
| Recycled 6 | 16.8 | 90.1 | 4.0 |

The catalyst set was chosen in such a way so that the differences of the recycled catalyst activities were significant. It is possible to show the differences of the activity values in Table II expressed from the two experimental points (starting and final iodine number) and from four to five iodine number values calculated from the fatty acidcompositions where experimental errors and deviations from ideal behaviour of the reaction system occur besides the different calculation methodology (Filip, Štětina, 1995).

An interesting discovery results from the mutual comparison of the catalyst activities with the calculated values of linoleic selectivity. The catalyst activity decrease is followed by the proportional decrease of linoleic selectivity. This is a rather surprising discovery. It should be added to that that the fresh Pricat 9910 Ni-catalyst represents a modern catalyst type which is destined to the direct use and, as it was assertained, the starting value of linoleic selectivity is high (relatively in the described arrangement). Linolenic selectivity within 1.5–2 represents standard values.

The catalyst activity and selectivity represent values calculated from the kinetic data and they indicate the catalyst properties in an indirect way on the assumption of work under the comparable, i.e. equal conditions. Another and also transferred picture of properties and state of the catalyst are properties of the reaction product. As far as carrying out of such a comparison it is necessary to choose another constant parameter besides standard conditions of the reaction control. The constant conversion grade can be considered to be advantageous. It is usually substituted by iodine number in fat chemistry. In this case the rapeseed oil was hydrogenated to the constant IN value about 75 corresponding to 33.2% conversion of double bond saturation. The oil hydrogenated with a fresh catalyst has the melting point 37 °C and the respective SFC values are 43% and 7% at the temperatures 10 and 30 °C. The stearic acid and trans-isomer increase is comparatively low (Table III).

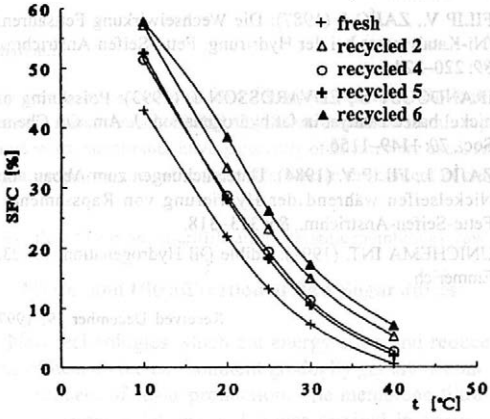
Recycling of the used catalyst has a negative effect on the hydrogenated fat properties. Dramatic increase of the melting point as much as by 11 °C, analogically of the solidification point as much as by 9 °C takes place. The SFC profile shows a considerable shift to the higher values (Fig. 1), at the temperature 10 °C as much as by 15%

II. Activity and selectivity (calculated values) of recycled Pricat 9910 Ni-catalyst

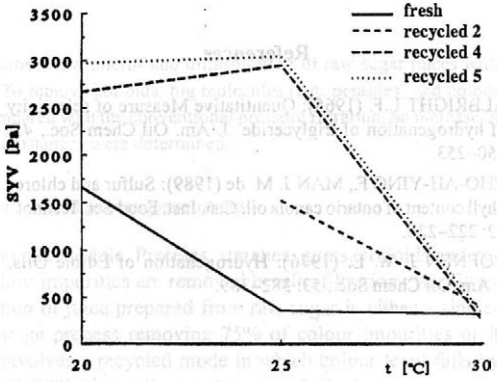
| Catalyst | Ni (% w/w) | $k_{IN}^{calc}.10^3$ ($min^{-1}$) | $S_{Li}$ (1) | $S_{Ln}$ (1) |
|---|---|---|---|---|
| Fresh | 2.0. | 19.1 | 15.6 | 2.0 |
| Recycled 2 | 16.8 | 6.0 | 8.0 | 1.9 |
| Recycled 4 | 19.0 | 10.6 | 9.7 | 2.0 |
| Recycled 5 | 16.9 | 10.8 | 9.9 | 1.5 |
| Recycled 6 | 16.8 | 7.7 | 8.0 | 1.9 |

III. Properties of hydrogenated canola oil (IN about75)

| Catalyst | IN | Melting point (°C) | Solidification point (°C) | $SFC_{10}$ (%) | $SFC_{30}$ (%) | $c_{St}$ (%) | $c_{Ti}$ (%) |
|---|---|---|---|---|---|---|---|
| Fresh | 75.9 | 37.4 | 20.9 | 42.9 | 7.2 | 8.5 | 37.2 |
| Recycled 2 | 75.4 | 46.6 | 27.3 | 57.2 | 15.0 | 14.4 | 45.7 |
| Recycled 4 | 74.5 | 42.0 | 24.4 | 51.4 | 11.3 | 14.1 | 39.0 |
| Recycled 5 | 75.2 | 43.0 | 23.5 | 52.5 | 10.5 | 13.6 | 40.1 |
| Recycled 6 | 74.9 | 47.8 | 27.6 | 50.1 | 15.2 | 14.5 | 39.7 |



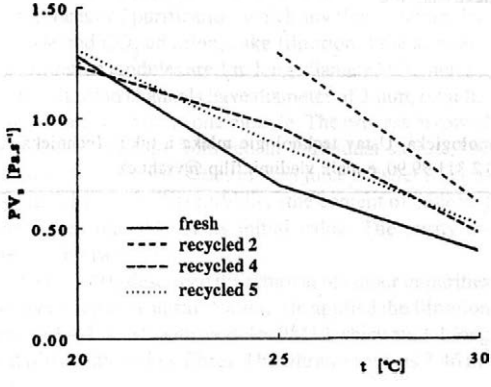

1. SFC profile of hydrogenated fats (IN about 75)



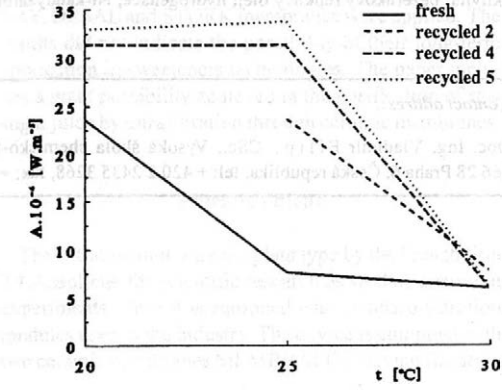

2. The dependence of static yield value on the temperature

and at the temperature 30 °C as much as by 7%. This is in correspondence with the increase of stearic acid and trans-isomer concentration. Hence considerable deterioration of triglyceride selectivity takes place. Triglyceride selectivity was not directly measured but the stated SFC values indicate this tendency.

Rheological parameters of the hydrogenated fats with the use of a recycled catalyst – a static yield value and plastic viscosity (Bingham) in dependence on the temperature (Figs. 2 and 3) indicate considerable change of flow properties compared to the comparative fat which was hydrogenated with a fresh catalyst. The presence of tri-




3. The dependence of plastic viscosity (Bingham) on the temperature




4. The dependence of area of flow curve hysteresis loop on the SFC

glycerides with a higher melting point and higher SFC values results in relative independence of both values at the temperatures 20–25 °C after which rapid decrease of both values at the temperature 30 °C follows. The fat hydrogenated with a fresh catalyst displays a completely different rheological characteristic.

This difference can be also well shown by the dependence of the area of flow curve hysteresis loop on the SFC (Fig. 4) and hence on the temperature. The area of the hysteresis loop has the physical dimension $kg/m.s^3$ which corresponds to the unit $W/m^3$. It is a work related to the time and space unit which is necessary for the destruction of the system under the condition of an experiment.

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**Souhrn**

FILIP V., DRDA A., NĚMEC Z. (1998): **Vliv opakovaného použití Ni-katalyzátoru na kvalitu ztuženého řepkového oleje**. Czech J. Food Sci., *16*: 25–28.

Opakovaným používáním dochází ke změně standardních vlastností Ni-katalyzátoru. Jedná se zejména o aktivitu a selektivitu a současně dochází ke změnám vlastností ztužených tuků.

Jsou diskutovány změny vlastností hydrogenovaného řepkového oleje ztuženého do konstantní hodnoty jodového čísla cca 75 za standardních laboratorních podmínek s použitím čerstvého a upotřebeného katalyzátoru Pricat 9910 z provozního zařízení. V závislosti na poklesu aktivity katalyzátoru dochází ke změně kvality ztuženého oleje: nárůstu bodu tání, změnám SFC profilu v rozmezí teplot 10–40 °C, nepříznivě jsou ovlivněny reologické vlastnosti těchto tuků.

aktivita; bezerukový řepkový olej; hydrogenace; Ni-katalyzátor; selektivita; SFC

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# Cross-flow Micro- and Ultrafiltration Applied on Ceramic Membranes in Impure Sugar Solutions*

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**Abstract**

The results of experiments on a filtration unit T.I.A. Bollene for cross-flow micro- and ultrafiltration of raw sugar juices with ceramic membranes MEMBRALOX are contained in this report. To remove colloids, big molecules (e.g. peptides) and colour substances, membranes having porosity of 20 nm were used. As compared with the conventional pressure filtration, an increase in permeate purity and subsequent decrease in the content of colour substances were determined.

cross-flow filtration; microfiltration; ceramic membranes; raw sugar juice; impure sugar solutions

## Micro- and Ultrafiltration of Raw Sugar Juices

New technologies which cut energy costs and reduce bad effects on the environment gradually get into a complex process of sugar production. The membrane filtration of sugar solutions and juices applied in various phases of the production process has great possibilities. Quite unconventional technologies are designed to apply the filtration. Their applications are limited by their costs compared with the standards of practice. Changes in the equipment and processes of the sugar industry may only be expected if such a barrier is overcome.

Vern et al. (1995) mentioned membrane technologies in his vision of future sugar refinement. By microfiltration of raw juice through FILMTEC SELECTFLO CMF synthetic membranes such purity of raw juice was achieved that direct crystallization was possible without the complex process of purification which involves calcium hydroxide and $CO_2$ addition, cake filtration, lime kiln, etc. The filtration modules are 1 m long, diameter 150 mm. Internal filtration channels have diameter of 3 mm, total filtration area is 5.5 $m^2$ in one module. The process involved cross-flow filtration followed in individual stages. The content of colour impurities in final juice is lower (approximately by 80%). The turbidity (the content of colloids) fell to less than 1% of its initial value. The purity increased by two points.

Mak (1991) described the removal of colour impurities from raw sugar by ultrafiltration. He applied the filtration unit ALFA-LAVAL with modules PM10 which are 1.1 long and filled with hollow fibres. The filtration area is 2.46 $m^2$ in one module. Proteins, starches, gums, colloids and colour impurities are removed by ultrafiltration. The filtration of juice prepared from raw sugar is either a single-stage process removing 75% of colour impurities or it involves a recycled mode in which colour level falls by 60–90% (depending on the type of juice).

Lancreon et al. (1993) analyzed the application of microfiltration (the size of pores was 0.1–10 μm) and ultrafiltration (2–200 nm) in sugar beet and sugarcane refinements. The APPLEXION system is described with ceramic membranes CARBOSEP (carbon base) and KERASEP (aluminium oxide or titanium base). In experimental ultrafiltration of sugar beet raw juice, a permanent output of 200 l/h.$m^2$ was achieved with the described filter.

Konkock et al. (1997) described the nanofiltration experiments with various membranes to examine possible long-term efficiency in purification of thin juice. The TORAY, DESAL and STORK membranes were applied. The results did not indicate the possibility of their long-term application in sweeteners technologies. The paper includes a great possibility achieved in the purification of raw sugar juice by ultrafiltration through ceramic membranes.

## Filtration Plant

The filtration unit is a pilot plant type by the French firm T.I.A.Bollene for scientific research as well as operation experiments, since it is equipped with standard filtration modules used in the industry. The device is equipped with two ceramic membranes MEMBRALOX having filtration

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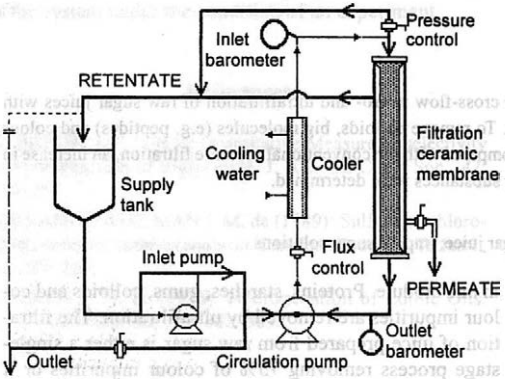
area of 2 x 0.2 $m^2$, porosity 20–100 nm for ultrafiltration and 0.2–5 μm for microfiltration. The limits within which experiments may be done are: temperatures up to 85 °C, pressures up to 0.6 MPa with pH in a large range of 0.5–13.5. The pilot plant is designed for the following research applications in food technologies: milk and whey thickening, clarifying and thickening of fruit and sugar juices, protein suspensions, filtration in wine-growing and brewing industries, water treatment (removing of microorganisms, sterilization), waste water purification (separation of oil etc.).




1. Filter unit scheme

## Filtration Tests

### Properties of Raw Juice Used

Raw juice taken from an extractor in a sugar refinery (sugar beet campaign 1996 and 1997) and raw juice concentrate were measured. Raw juice concentrate was measured particularly to create the possibility of performing the newly designed technological process not only during a season but the whole year. Even a filtration area may be reduced in an all-year-round operation. This may possibly reduce the increased capital expenditure on membrane filtration.

During the sugar beet campaign of 1996 the concentrate was prepared by the thickening of raw juice on a film-type evaporator. The evaporator is a pilot plant by the English firm ARMFIELD. The evaporation forms a climbing film. The whole process is very quick. It may be performed as pressure decreases. Such conditions are suitable particularly for the treatment of thermolabile extracts such as raw juice from sugar beet. The initial dry substance content was 70%. It then fell to 14.9% after dilution of the total volume of 28 l. Other properties of the original raw juice and the concentrate are summarized in Table I.

I. Properties of original raw juice in the campaign of 1997 and diluted concentrate of raw juice in the campaign of 1996

| | Original raw juice (1997) | Diluted concentrate (1996) |
|---|---|---|
| Dry substance (%) | 15.9 | 14.9 |
| Purity (%) | 88.7 | 85.8 |
| Colour substances ($cm^2/kg$) | 7.25 | 2.55 |
| pH | 6.22 | 6.12 |
| Acetic acid (% dry subst.) | – | 0.45 |
| Lactic acid (% dry subst.) | 0.08 | 0.30 |

### Methodology

The filtration kinetics (i.e. the permeate flux velocity dependence on time, temperature and operating pressure) was particularly observed. The permeate and retentate samples were measured in terms of colour, the content of dry substance (= saccharization), the content of sucrose (= polarization), conductivity, the contents of invert sugar (i.e. the contents of glucose and fructose produced in saccharose inversion) and lactic and acetic acids. The variables were measured as follows:

- Refractometric permeate dry substance: measured on the automatic ABBEMAT refractometer by the firm KARNCHEN (Germany).
- Refractometric retentate dry substance: measured on the ZEISS refractometer (Germany). An automatic refractometer was not possible to use due to particle sedimentation.
- Permeate polarization: measured after clearing up (dosing 2 g of alkaline lead (II) acetate in 100 g of raw juice).
- Retentate polarization: determined on the same apparatus as the permeate (clearing up with 40 ml of Herles reagents I, II in the 13g sample, half-an-hour downtime and then completion of the volume of 100 ml) after filtration.
- Permeate colour: determined as an absorption coefficient measured with a spectropho-tometer (wavelength 560 nm, dilution 1:1 with destilled water and then filtration with kieselguhr through a pressure filter).
- Conductivity: determined on the conductometry base with the universal apparatus THERM 2290-3 having an attached electrode FYA641-LF by the firm AHLBORN (Germany).
- Lactic and acetic acids: determined on the isotachophoretic analyzer IONOSEP 900.1 by the firm RECMAN (Czech Republic).
- Invert sugar: measured with the chromatographic analyser HPLC 4001, LP Prague (Czech Republic).
- Permeate flux: determined by measuring the permeate volume taken in 10–60s intervals, which was converted to output DE ($l/m^2.h$) with temperature 20 °C as follows:

$$DE = (P \cdot KT) / (S \cdot p)$$

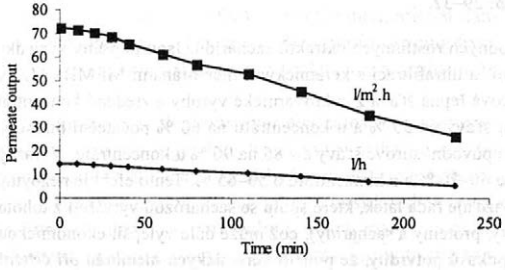
where: $P$ – permeate flux (l/h)
$S$ – membrane filtration area (m²)
$p$ – membrane pressure difference (MPa)
$KT$ – temperature coefficient

## Methods of Measurement

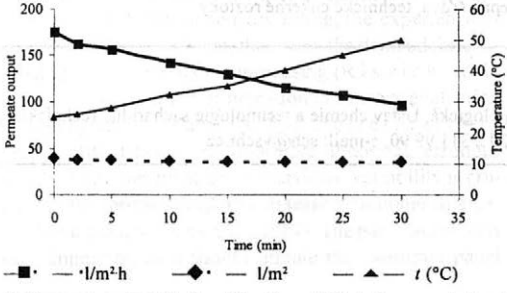
Filtration proceeded in the mode of retentate recycling with constant membrane pressure difference (0.1 MPa) and changing working temperature 22–24 °C (original raw juice) and 25–50 °C (diluted concentrate of raw juice). The recycling retentate was not cooled by filtration of diluted concentrate. The raw juice was filtered on membranes with porosity of 20 nm. Permeate and retentate samples were regularly taken in 10–20min intervals for 230 min (original raw juice) and in 2–5min intervals for 30 min (diluted concentrate). Permeate flux was measured in the same intervals for the determination of filtration kinetics (Figs. 2 and 3).

### Permeate

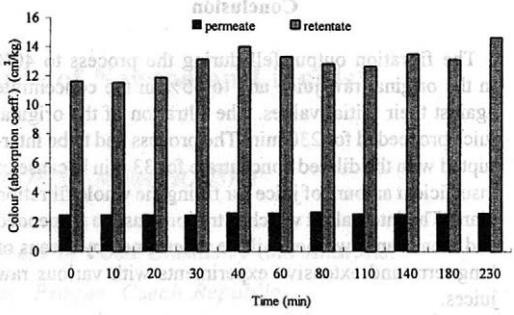
The values of dry substance and sucrose content, purity and colour (Figs. 4 and 5) were measured during filtration. Conductivity and the contents of lactic and acetic acids (Fig. 6) were measured in the first and the last samples of diluted concentrate (permeate "0" and "30") to compare these values with those of the retentate.



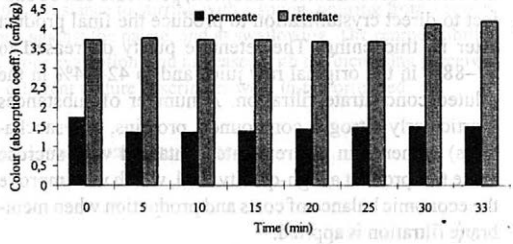

2. Permeate output during filtration of original raw juice




3. Permeate output during filtration of diluted concentrate of raw juice



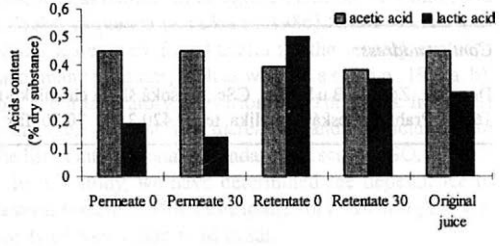

4. Permeate and retentate colour during filtration of original raw juice




5. Permeate and retentate colour during filtration of diluted concentrate of raw juice

### Retentate

Prior to the actual determinations of dry substance and sucrose content, colour and purity, the retentate samples had to be filtered with increased pressure to become suitable for the following measurements (Figs. 4 and 5). A thin layer of sample was applied to the Abbey refractometer which was used to remove the negative effects of particle sedimentation on the surface of the prism in the refraction measurements occurring with very impure juices. Furthermore, the contents of lactic and acetic acids at the beginning and the end of filtration of diluted concentrate were determined (Fig. 6 – retentate "0" and "30").




6. Lactic and acetic acids content during filtration of diluted concentrate of raw juice

## Conclusion

The filtration output fell during the process to 40% in the original raw juice and to 55% in the concentrate against their initial values. The filtration of the original juice proceeded for 230 min. The process had to be interrupted with the diluted concentrate for 33 min because of insufficient amount of juice for filling the whole filtration plant. The intervals at which filtration must be suspended and membranes washed will be determined by means of long-term and extensive experiments with various raw juices.

An interesting result was achieved in increasing the juice purity from the initial value 89% to the final value 91–92% of the original raw juice and from 86% to 96% of the diluted concentrate. In addition, permeate can be subject to direct crystallization to produce the final product after its thickening. The retentate purity decreased to 87–88% in the original raw juice and to 42–44% in the diluted concentrate filtration. A number of substances (particularly nitrogen compounds, proteins, polysaccharides) gathered in the retentate contained with sucrose make this product a high-quality feed, which will improve the economic balance of costs and production when membrane filtration is applied.

Another significant result is the decrease in the content of colour impurities in the original raw juice by 60–70% and 50–60% in the concentrate. The turbidity (colloids) fell to less than 1% of its initial value. These effects are necessary for permeate further treatment to white sugar. An interesting effect with lactic acid (Fig. 6), the content of whichincreased in retentate, but decreased in permeate during the diluted concentrate filtration, will be further studied in various raw juices.

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**Souhrn**

BUBNÍK Z., HINKOVÁ A., KADLEC P. (1998): **Mikrofiltrace a ultrafiltrace technických cukerných roztoků na keramických membránách s příčným tokem.** Czech J. Food Sci., *16*: 29–32.

Práce je součástí výzkumu zabývajícím se metodami čištění vodných rostlinných extraktů sacharidů. Jsou popsány výsledky filtračních experimentů na filtrační jednotce na cross-flow mikro- a ultrafiltraci s keramickými membránami MEMBRALOX s filtrační plochou 0,2 $m^2$ a porozitou 20 nm. Byla filtrována surová řepná šťáva z cukrovarnické výroby a zředěný koncentrát surové šťávy. Výkon filtrace v průběhu procesu klesl u původní šťávy na 55 % a u koncentrátu na 60 % počáteční hodnoty. Významné bylo zvýšení čistoty šťávy z počátečních 89 na 96 % u původní surové šťávy a z 86 na 96 % u koncentrátu. Výrazný je též pokles obsahu barevných látek u původní čerstvé šťávy až o 60–70 % a u koncentrátu o 50–65 %. Tento efekt je nezbytný pro další zpracování permeátu na bílý cukr. V retentátu se shromažďuje řada látek, které spolu se sacharózou vytvářejí z tohoto produktu velmi kvalitní krmivo (bohaté zejména na dusíkaté látky, proteiny a sacharidy), což může dále vylepšit ekonomickou bilanci pro aplikace membránové filtrace. Výsledky filtračních pokusů potvrdily, že použití keramických membrán při čištění cukerné surové šťávy může po stránce technologické nahradit dosavadní tradiční chemické epurační postupy v cukrovarnictví. Limitní podmínkou pro zavedení membrán do technologie cukru jsou zatím vysoké investiční náklady a nedostatečná kapacita provozu.

cross-flow filtrace; mikrofiltrace; keramické membrány; surová řepná šťáva; technické cukerné roztoky

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# Development of a Texture Profile of Newtonian Liquids*

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**Abstract**

The viscosity of Newtonian liquids was determined by sensory rating resistance to stirring with a spoon, pouring from a spoon, slurping under conditions of the ISO test, compressing by the tongue against the palate, and at swallowing. The reproducibility, expressed by the standard deviation, was comparable with that of flavour evaluation, and increased with the increasing perceived viscosity. Relative values of the standard deviations obtained with different texture descriptors were in-tercorrelated.

beverages; liquid foods; Newtonian fluids; sensory analysis; statistical evaluation; texture; viscosity

The viscosity of beverages and other liquid foods belongs to the most important properties that can be determined either using the instrumental analysis or the sensory analysis. Therefore, the determination of sensorically perceived viscosity was included in characteristics of a texture profile (ISO, 1994). After the international standard, the viscosity is determined by sample slurping from a spoon, and evaluating the viscosity on a eight-point rating scale.

In our opinion, the consumer evaluates the sample already before the ingestion, by mixing in the vessel, by manipulation with a spoon, and after ingesting it (sometimes, even by slurping, which is not considered as polite in Europe), also by mouthfeeling, which mainly consists of assessing the resistance to compression with the tongue against the palate and other parts of the oral cavity, and then, at swallowing. Therefore, we have developed a sensory profile, evaluating better and in more complex way, in our opinion, the real experience of a consumer.

For the validation of sensory rating, the experience of assessors is very important, therefore, the demand for qualified sensory personnel is increasing (Risvick et al., 1989). The calculation of precision of sensory evaluation is thus of great importance (Albi, Gutiérrez, 1991). Less qualified assessors often give abnormal, erronous results. The determination of individual variability is crucial for the lowest acceptable assessors' number in a panel (Neumann, Eckert, 1981). The panel selection is very important, as it should imitate the consumer panel (Lundahl, McDaniel, 1988). The concentration dependence of the accuracy should be determined (Pangborn et al., 1969) as it affects consumers' responses. The performance of assessors influences the number of assessors in the panel, necessary to obtain results of required quality (Basker, 1980). The experience is a factor which is very important for the reproducibility of results (McBride, Finlay, 1989). Panelists should be viewed as random effects in the sensory analysis, influencing the reproducibility (Lundahl, McDaniel, 1988). There may be substantial differences even between experienced assessors, of course (Neumann, Eckert, 1981).

The use of appropriate rating scales will contribute, naturally, to the reliability of assessment (Cloninger et al., 1976). Various types of rating scales were compared, and their suitability for evaluation of hot chocolate drinks evaluated (Pangborn et al., 1989). Category scales have some disadvantages as they are affected by several factors, such as number of categories, number of stimuli, and stimulus sequence (Riskey, 1986). Unstructured graphical scales were found useful for the sensory evaluation of many products, such as wine (Castino, 1983a, b), and in our laboratory, for various food products. In the last years, their application is increasing, and it is included into the list of internationally standardized scales (ISO, 1978).

In this study, we have determined the dependence of several texture profile descriptors for evaluating the viscosity of Newtonian food fluids.

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## MATERIAL AND METHODS

The total of 16 Newtonian fluids were analyzed (Table I) both by instrumental analysis and by sensory analysis. Fluid foods were purchased on the market, the sugar solution was prepared by dissolving 50 g of sucrose in 50 ml of water. Honey was diluted with tap water in the mass ratio of 90 : 10, if indicated.

Fluid foods were subjected to the measurement of their rheological properties (Houška et al., 1998). Low-viscosity fluid foods were measured by using a special cup. The measured time of pouring was calculated as the kinematic viscosity and as the dynamic viscosity. The viscosity of fluids having higher consistency ($0.1–10^3$ $s^{-1}$) was measured by Haake RVS rheometer using the system of coaxial cylinders. The viscosity was determined as a slope of shear stress-shear rate linear relationship. Samples were thermostated at 20.0 ± 0.1 °C for about 10 min after filling the rheometer before mesurement (thermostats Laude MGW and UH4 MLW, Germany).

For the sensory analysis, a group of assessors was selected, trained and monitored after the international standard procedure (ISO, 1989); they had the experience in texture profiling for at least 6 months (two sessions a week or more). The analysis was performed under standard conditions (ISO, 1985a, b) in a standard computerized test room (ISO, 1988), kept at 21–23 °C, and provided with 6 standard test booths. Samples were presented in random order at 20–21 °C. The sensory profile was based on the standard procedure (ISO, 1985a, b, 1994), using unstructured graphical scales. The first descriptor used consisted of the evaluation of effort necessary for stirring fluid food with a teaspoon in a 250 ml beaker, containing 50 ml of sample. The second descriptor consisted of evaluation of pouring the sample from a soup spoon into a beaker. The third method was based on evaluation of the effort necessary for slurping the sample from a table spoon into the oral cavity, as described in the standard procedure (ISO, 1994). The fourth descriptor concerned the effort perceived when compressing the fluid between the tongue and the palate and other parts of the oral cavity 2–5 s after introduction into the mouth. The fifth descriptor was the evaluation of the effort for swallowing the draught. The assessor had to swalow the sample within 10 s after the ingestion. Results of the fourth and fifth tasks were influenced by the amount of saliva, diluting the draught. The keeping of exact time helped in standardizing the procedure.

## RESULTS AND DISCUSSION

The list of 16 samples of Newtonian fluid foods analyzed is given in Table I with their kinematic and dynamic viscosities. Unstructured graphical scales were used for sensory viscosity assessment. Unstructured graphical scales may be considered as interval scales (McBride, Johnson, 1987; McBride, 1985), parametric methods could be used for statistical evaluation in this case.

Five descriptors of the texture profile were used, which are perceived by consumers. Stirring the sample with a spoon is often applied in course of consumption of fluid foods, and gives preliminary idea of the viscosity. Pouring the liquid from a spoon is also used in food consumption, and gives related results to the preceding procedure. The next descriptor is slurping, which is recommended by the international standard. Slurping is not

I. Analyzed samples of Newtonian fluids and their viscosities

| Sample code | Sample description | Viscosity μ (Pa.s) | Viscosity γ ($m^2.s^{-1}$) |
|---|---|---|---|
| A | potable water | 0.00089 | 0.00000089 |
| B | apple beverage | 0.001566 | 0.00000149 |
| C | orange beverage (50%, Limona, Uherské Hradiště,, PN 01/94) | 0.001936 | 0.000001859 |
| D | orange beverage (100%, Limona, Mnichovo Hradiště, PN 01/94) | 0.002362 | 0.000002258 |
| E | milk (reduced fat content, 15 g/l, Pelhřimov, PN-MP 02-06-91) | 0.002524 | 0.00000247 |
| F | full-fat milk (fat 35 g/l, Pelhřimov, PN-MP 02-06-91) | 0.002885 | 0.00000282 |
| G | Ovocit raspberry sirup (Limona, Mnichovo Hradiště, PN 56 8429) | 0.121 | 0.00009375 |
| H | fruit sirup (Limona, Mnichovo Hradiště, ČSN 56 8409) | 0.116 | 0.00009235 |
| I | honey (mixed origin) | 15.30 | 0.01057 |
| J | lime honey | 38.754 | 0.02708 |
| K | dark forest honey | 35.950 | 0.02508 |
| L | rape honey | 39.370 | 0.02722 |
| M | locust-tree honey | – | – |
| A | sugar solution | 0.447 | 0.00003349 |
| P | dark honey + 10% water | 3.509 | 0.002576 |
| Q | rape honey + 10% water | 2.738 | 0.002019 |

II. Sensory evaluation of viscosity: stirring with a table spoon (data expressed in p. c. of the graphical scale)

| Sample code | Number of responses | Lowest value | Highest value | Mean value (%) | Standard deviation (%) | Relative value (%) |
|---|---|---|---|---|---|---|
| | | | Stirring with a table spoon | | | |
| A | 20 | 0 | 43 | 20 | 18 | 90 |
| B | 18 | 5 | 47 | 19 | 13 | 68 |
| C | 14 | 7 | 42 | 28 | 11 | 39 |
| D | 18 | 8 | 54 | 23 | 13 | 57 |
| E | 19 | 3 | 57 | 22 | 17 | 77 |
| F | 19 | 3 | 51 | 23 | 13 | 57 |
| G | 13 | 23 | 78 | 58 | 18 | 31 |
| H | 11 | 37 | 80 | 53 | 17 | 32 |
| I | 14 | 73 | 98 | 82 | 7 | 9 |
| J | 18 | 65 | 98 | 82 | 8 | 10 |
| K | 18 | 64 | 93 | 78 | 13 | 17 |
| L | 17 | 73 | 96 | 85 | 8 | 9 |
| M | 17 | 72 | 94 | 81 | 21 | 26 |
| N | 18 | 43 | 72 | 60 | 8 | 13 |
| P | 18 | 37 | 72 | 58 | 8 | 14 |
| Q | 18 | 22 | 84 | 52 | 17 | 33 |
| | | | Pouring the fluid from a spoon | | | |
| A | 20 | 1 | 48 | 14 | 18 | 129 |
| B | 18 | 2 | 43 | 15 | 13 | 87 |
| C | 14 | 0 | 32 | 17 | 11 | 65 |
| D | 18 | 1 | 45 | 19 | 13 | 59 |
| E | 19 | 5 | 54 | 23 | 13 | 57 |
| F | 19 | 2 | 49 | 22 | 13 | 59 |
| G | 13 | 17 | 68 | 47 | 14 | 30 |
| H | 11 | 23 | 71 | 47 | 13 | 28 |
| I | 14 | 42 | 89 | 70 | 11 | 16 |
| J | 18 | 61 | 90 | 74 | 8 | 11 |
| K | 18 | 15 | 93 | 67 | 17 | 25 |
| L | 17 | 38 | 94 | 81 | 12 | 15 |
| M | 17 | 9 | 94 | 81 | 12 | 15 |
| N | 18 | 32 | 67 | 49 | 13 | 27 |
| P | 18 | 20 | 78 | 49 | 17 | 35 |
| Q | 18 | 5 | 72 | 44 | 17 | 39 |
| | | | Slurping from a spoon | | | |
| A | 20 | 1 | 28 | 10 | 9 | 90 |
| B | 18 | 0 | 30 | 15 | 8 | 62 |
| C | 14 | 5 | 35 | 17 | 7 | 41 |
| D | 18 | 4 | 36 | 20 | 8 | 40 |
| E | 18 | 2 | 57 | 17 | 13 | 76 |
| F | 19 | 3 | 55 | 19 | 13 | 68 |
| G | 13 | 21 | 94 | 50 | 22 | 44 |
| H | 11 | 20 | 68 | 47 | 13 | 28 |
| I | 14 | 25 | 94 | 84 | 19 | 23 |
| J | 18 | 66 | 100 | 88 | 8 | 9 |
| K | 18 | 36 | 100 | 80 | 17 | 21 |
| L | 17 | 31 | 100 | 89 | 16 | 18 |
| M | 17 | 65 | 100 | 92 | 8 | 9 |
| N | 18 | 47 | 82 | 59 | 8 | 14 |
| P | 18 | 20 | 93 | 59 | 17 | 29 |
| Q | 18 | 23 | 96 | 59 | 21 | 36 |

common in most European countries, and it is not used, generally, in consumption of very viscous fluids, such as honey, but for the evaluation of viscosity it is probably more accurate than the common way of fluid ingestion. The compression of the draught between the tongue and the palate gives an idea of the elasticity and of viscosity changes caused by saliva. It could express the mouth-feeling during the ingestion. The last descriptor, the viscosity perceived on swallowing, is affected by mixing with saliva (particularly, in the case of honey) and by temperature changes, but it is important in the process of consumption as it is the last experience with the sample that could affect the final opinion of the consumer. The whole procedure comprising the above five descriptors does not exceed 15–20 s. The assessors did not experience any problems in performing the complete test.

Results of the sensory analysis are shown in Tables II–IV, and average results at three main viscosity levels are summarized in Table V. The most difficult sample was, surprisingly, potable water, which was evaluated with far higher error than more viscous fluids. Absolute values of standard deviations were similar to those obtained in fla-

III. Sensory evaluation of viscosity: compression of fluid between tongue and palate

| Sample code | Number of responses | Lowest value | Highest value | Mean value (%) | Standard deviation (%) | Relative value (%) |
|---|---|---|---|---|---|---|
| A | 20 | 0 | 44 | 11 | 18 | 164 |
| B | 18 | 1 | 32 | 13 | 8 | 62 |
| C | 14 | 5 | 39 | 18 | 11 | 61 |
| D | 18 | 3 | 42 | 19 | 13 | 68 |
| E | 18 | 0 | 60 | 19 | 17 | 89 |
| F | 19 | 2 | 51 | 17 | 17 | 100 |
| G | 13 | 11 | 85 | 46 | 22 | 48 |
| H | 11 | 18 | 74 | 41 | 17 | 41 |
| I | 14 | 32 | 93 | 66 | 19 | 29 |
| J | 18 | 37 | 93 | 70 | 17 | 24 |
| K | 18 | 16 | 83 | 63 | 17 | 27 |
| L | 17 | 37 | 85 | 67 | 16 | 24 |
| M | 17 | 37 | 89 | 72 | 12 | 17 |
| N | 18 | 28 | 72 | 50 | 13 | 26 |
| P | 18 | 18 | 62 | 40 | 13 | 32 |
| Q | 18 | 17 | 62 | 42 | 13 | 31 |

IV. Sensory evaluation of viscosity: at swallowing (data expressed in p. c. of the graphical scale)

| Sample code | Number of responses | Lowest value | Highest value | Mean value (%) | Standard deviation (%) | Relative value (%) |
|---|---|---|---|---|---|---|
| A | 20 | 0 | 31 | 10 | 18 | 180 |
| B | 18 | 1 | 26 | 13 | 8 | 62 |
| C | 14 | 1 | 32 | 13 | 11 | 85 |
| D | 18 | 1 | 32 | 14 | 8 | 57 |
| E | 18 | 2 | 53 | 16 | 13 | 81 |
| F | 19 | 2 | 42 | 17 | 13 | 76 |
| G | 13 | 0 | 68 | 38 | 18 | 47 |
| H | 11 | 17 | 61 | 37 | 13 | 35 |
| I | 14 | 0 | 95 | 65 | 22 | 34 |
| J | 18 | 27 | 85 | 63 | 17 | 27 |
| K | 18 | 14 | 89 | 55 | 25 | 45 |
| L | 17 | 34 | 93 | 66 | 16 | 24 |
| M | 17 | 37 | 96 | 71 | 12 | 17 |
| A | 18 | 20 | 71 | 42 | 13 | 31 |
| P | 18 | 9 | 56 | 37 | 13 | 35 |
| Q | 18 | 15 | 83 | 37 | 17 | 46 |

V. Average standard deviations of sample viscosities at different levels

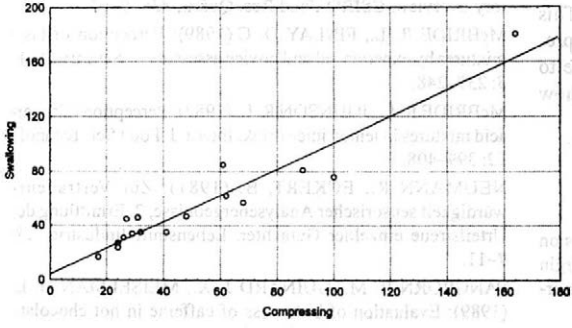
| Samples | Viscosity range (Pa.s) | Descriptor tested | Absolute value (%) | Relative value (%) |
|---|---|---|---|---|
| A–F | $(0.9–2.5).10^{-3}$ | stirring | 14 | 65 |
| | | pouring | 14 | 76 |
| | | slurping | 10 | 63 |
| | | compressing | 14 | 91 |
| | | swallowing | 12 | 90 |
| G–H, A–Q | 0.1–3.5 | stirring | 14 | 25 |
| | | pouring | 15 | 32 |
| | | slurping | 16 | 30 |
| | | compressing | 16 | 36 |
| | | swallowing | 25 | 39 |
| I–M | 15–40 | stirring | 11 | 14 |
| | | pouring | 12 | 16 |
| | | slurping | 14 | 16 |
| | | compressing | 16 | 24 |
| | | swallowing | 17 | 29 |

VI. Correlations between standard deviations obtained by different procedures of the profile ($n = 16$; $P = 0.95$; significant values are printed bold)

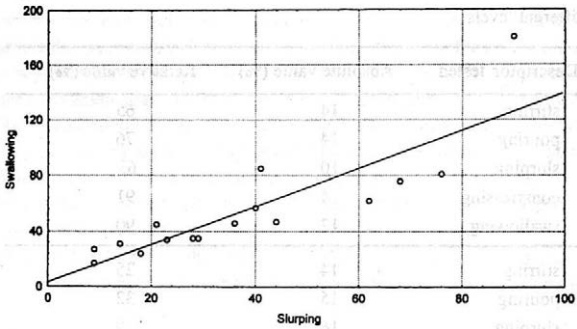
| Descriptor | Pouring | Slurping | Compressing | Swallowing |
|---|---|---|---|---|
| A. Correlation coefficients in the case of absolute values of standard deviations | | | | |
| Stirring | 0.35 | 0.02 | 0.10 | –0.10 |
| Pouring | | 0.39 | 0.03 | 0.25 |
| Slurping | | | **0.54** | **0.61** |
| Compressing | | | | 0.67 |
| B. Correlation coefficients in the case of relative values of standard deviations | | | | |
| Stirring | **0.89** | **0.93** | **0.89** | **0.82** |
| Pouring | | **0.88** | **0.90** | **0.92** |
| Slurping | | | **0.92** | **0.86** |
| Compressing | | | | **0.95** |

vour evaluation in our earlier studies (Pokorný, Kalinová, 1994; Pokorný, Valentová, 1994). The precision of about 15% would correspond to a one-point difference on a seven-point category scale, i.e. the value obtained in the case of flavour evaluation (Pilková et al., 1992).

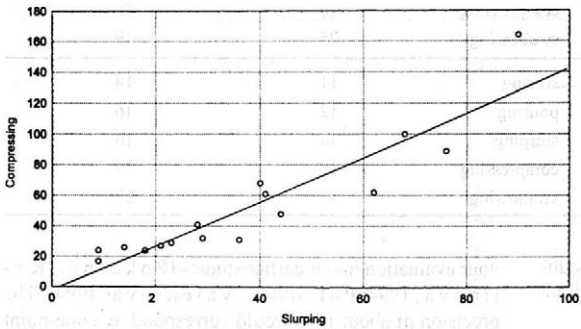
It may be seen from Tables II–V that absolute values of standard deviations are about the same, irrespective of the viscosity and of the position of rating marks on the unstructured scale. The necessary consequence is that relative values of standard deviations decrease with increasing viscosity. The number of panelists necessary for the analysis may be calculated from the standard deviations. If 12–18 assessors are taken for the analysis of each sample, the standard deviation of the mean value would then be equivalent to about 4% of the scale, which is acceptable even for detection of small differences in the sensory viscosity. Ratings varied from 0 to 100 mm of the scale in the evaluation of samples of different viscosities, which means that assessors used the whole scale.




1. Regression between relative standard deviations obtained at the viscosity assessment at compression between tongue and palate and at swallowing




2. Regression between relative standard deviations obtained at the viscosity assessment at slurping and at swallowing




3. Regression between relative standard deviations obtained at the viscosity assessment at slurping and compressing with the tongue against the palate

The comparison of standard deviations, obtained by different procedures of the profile, is interesting (Table VI). The precisions of stirring and pouring are in no relation with one another and with the procedures perceived in the mouth. On the contrary, significant relationships were obtained between the three characteristics tested in the oral cavity, even when the respective correlation coefficients were not high.

In the case of relative values of standard deviations, all relationships between the characteristics were significant, and their correlation coefficients relatively high. The regression between the values obtained by compressing and swallowing is given in Fig. 1, but relative standard deviations between the results obtained by slurping and swallowing are nearly acceptable as well (Fig. 2). Satisfactory relationships were obtained even between viscosities assessed by stirring and by slurping (Fig. 3), contrary to standard deviations expressed in absolute values. This is important for the evaluation of samples, which are present either in small quantities or which are not safe to taste and swallow. Other combinations could also show satisfactory relationships.

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**Souhrn**

VALENTOVÁ H., POKORNÝ J., HOUŠKA M. (1998): **Vypracování texturního profilu pro tekuté newtonské potraviny.** Czech J. Food Sci., *16*: 33–39

Pro newtonské kapaliny byl vypracován senzorický profil textury skládající se z pěti ukazatelů. Nejprve byla viskozita hodnocena při míchání lžičkou, pak při stékání ze lžíce, dále byl vzorek srkán ze lžíce, stlačován jazykem k patru a konečně polykán. V každém z uvedených bodů hodnocení byla viskozita stanovena s použitím nestrukturované grafické stupnice. Byla zkoumána řada 16 vzorků, jejichž viskozita se pohybovala od $0{,}9.10^{-3}$ (pitná voda) do 40 Pa.s (med). Hodnoty se pohybovaly po celé délce stupnice. Vzorky posuzovalo 11–21 hodnotitelů. Na všech hladinách viskozity byla směrodatná odchylka stanovení zhruba stejná (v průměru 13 % stupnice). Relativní odchylky proto značně závisely na viskozitě a s rostoucí viskozitou klesaly. Mezi relativními směrodatnými odchylkami zjištěnými různými způsoby byly zjištěny statisticky významné korelace o vysoké hodnotě korelačního koeficientu.

nápoje; tekutiny; newtonovské kapaliny; viskozita; senzorická analýza; statistické hodnocení; textura

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