Analytical and organoleptic profiles of lactic acid-fermented cucumber juice with addition of onion juice

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Summary

Analytical and organoleptic profiles of the fermented cucumber juice with the additions of 0.5, 1.0 and 2.0% of the onion juice has been studied using *Lactobacillus plantarum* as a starter culture. The juices obtained the highest score in harmonic taste, odour, flavour and acceptable pH (3.50–3.55) after 48 h of fermentation. Regarding the ratio of juices, that the cucumber juice with the addition of 0.5% of the onion one was evaluated as the most appropriate resulting in the highest score by sensorial analysis.

Keywords

fermentation; cucumber; onion; organic acids; nitrates; *Lactobacillus plantarum*; sensorial analysis

Modern food processing is also based on a range of preservation methods to ensure equal quality from the time of manufacture to the time of consumption. One of the oldest methods of food preservation is fermentation of raw materials of plant and animal origin to obtain foods with special taste and aroma. However, this preservation depends directly on the biological activity of microorganisms able to produce metabolites suppressing growth and survival of undesirable microflora in foodstuffs [1].

Fermented foods are defined as products that have been treated mainly by microorganisms and/or enzymes to obtain specific organoleptic profile. The microorganisms responsible for the fermentation may be the microflora either indigenously present on the substrate or it may be added as a starter culture [2]. Fermented foods contribute considerably to the human diet in many countries because fermentation is inexpensive, accesible and convenient preservation technology improving substantially the nutritional value and the organoleptic profile of food products [3].

Fermentation of vegetable juices is usually carried out by strains of *Lactobacillus plantarum*, *L. bavaricus*, *L. xylosus*, *L. bifidus* or *L. brevis* [4], which are able to considerably improve the original sensorial properties of raw material to the desirable organoleptic profile. Criteria to be used for assessment of strain suitability are as follows: total production of organic acids, quick change of the substrate pH, minimum loss of nutrients, decrease or even elimination of nitrates and nitrites, and minimal formation of biogenic amines [5]. During fermentation, pH of the juices decreases usually from 6–6.5 to 3.8–4.5 [6]. A rapid decrease of pH at the beginning of fermentation has decisive importance for the quality of the final product [7, 8].

In recent years, several authors have dealt with lactic acid fermentation of vegetables (garlic, carrot, cucumber, onion) [9-12] or vegetable juices (cabbage, red beet, carrot) [8, 13-15].

In this paper, lactic acid fermentation of cucumber juice with the addition of the onion one has been studied using *L. plantarum* as a starter culture and the organoleptic profiles of final products as well as chemical parameters were evaluated by sensorial analysis to find the optimum fermentation time as well as the most appropriate ratio of the juices.

MATERIAL AND METHODS

Chemicals

For analysis of total acidity and reducing saccharides, following chemicals were used: sodium hydroxide p.a. (Chemapol, Praha, Czech Repub-

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lic), potassium iodide p.a. (Mikrochem, Pezinok, Slovakia), phenolphtalein pure grade, sodium tiosulphate p.a., potassium sodium tartarate p.a., copper sulphate pentahydrate p.a., potassium hexacyanoferrate trihydrate p.a., zinc sulphate pentahydrate pure grade, soluble starch pure grade (Lachema, Brno, Czech Republic), sulphuric acid 97% (Merck, Darmstadt, Germany).

Chemicals for isotachophoretic determination

For isotachophoretic determination of organic acids (lactic acid, citric acid, acetic acid, ascorbic acid), nitrites and nitrates, the following chemicals were used: citric acid p.a., hydrochloric acid p.a., caproic acid p.a., poly(vinylpyrrolidone) p.a., potassium nitrate, sodium nitrite (Lachema), acetic acid p.a. (Mikrochem), lithium lactate 98%, ascorbic acid p.a. aminocaproic acid p.a., β-alanine p.a. (Merck), methyl hydroxyethyl cellulose p.a., 1,3-bis[tris(hydroxymethyl)methyl-amino]propane p.a. (Sigma-Aldrich, Steinheim, Germany).

Equipment

Measurement of pH was performed using a LABOR-pH-meter (CG-834, Schott, Mainz, Germany).

Organic acids (lactic, acetic, citric and ascorbic acids), nitrates and nitrites were determined by capillary isotachophoresis using an isotachophoretic analyser and the ZKI 01 column connection technique using the conductivity detector (Villa Labeco, Spišská Nová Ves, Slovakia).

Cultures

Lactobacillus plantarum CCM 7039 applied as a starter culture originated from the Czech collection of microorganisms, Masaryk University, Faculty of Science, Brno, Czech Republic.

Preparation of vegetable juices

Cucumber (*Cucumis sativum*, variety Lavina F1) and yellow onions (*Allium cepa*, variety Raasford) were purchased in a local market in Bratislava, Slovakia. After peeling, they were pressed and filtered. The cucumber-onion juice mixtures were prepared by the addition of the onion juice into the cucumber one in the ratio of 0.5, 1.0 and 2.0%. Then, 0.5% D-glucose and 2% NaCl addition as well as inoculation by *L. plantarum* at a concentration of 10^6 $CFU.cm^{-3}$ were carried out. Fermentations were carried out in a thermostat at 21 °C ± 0.1 °C for 120 h. At 24-h time intervals, samples were refrigerated and analysed for pH, total acidity, reducing saccharides, organic acids, and sensorically assessed for colour, sediment, turbidity, appearance, odour, taste, acceptance of odour, acceptability of taste and flavour. Cucumber juice without the onion addition was fermented at the same conditions as a control sample.

The initial concentration of microorganisms was measured by the plate-count technique on LS-agar (Imuna, Šarišské Michaľany, Slovakia). The plates were incubated for 48 h at 37 °C ± 0.1 °C. Individual volumes of 150 ml of the juice were poured into 250-cm^3 flasks and closed with sterile rubber stoppers to ensure microaerobic conditions.

Analytical methods

Total acidity was determined by visual titration with a standard solution of NaOH (c_{NaOH} = 0.1 $mol.dm^{-3}$). Determination of reducing saccharides was performed according to Schoorl, lactic, acetic, citric and L-ascorbic acids, nitrates and nitrites were determined by capillary isotachophoresis [5, 8, 16]. Quantitative analysis was performed by calibration of standard solutions. Each sample was analysed in triplicate and each result of analysis represents an average value of 3 measurements of the same sample. Limits of determination for the acids were 2 $mg.dm^{-3}$ and for nitrites and nitrates 2.1 $mg.dm^{-3}$ and 1.9 $mg.dm^{-3}$, respectively. Relative standard deviation for the determination of lactic acid varied from 2.2% to 6.7%, and for the determination of nitrites and nitrates between 2.2% and 4.8%.

Statistical method

For processing of the analytical and sensory results, the multivariate statistic method - principal component analysis (PCA) was used. The results were arranged into a data matrix and analysed by the statistic programme SGWIN Statgraphic for Windows, version 1.4 (Statpoint, Herndon, Virginia, USA).

Sensory evaluation

The samples were evaluated by 10 assessors. Before the sensory evaluation, samples were defrosted and allowed to reach a laboratory temperature of 15–18 °C. Turbidity and appearance were evaluated by a 5-point intensity scale (1 - non-turbid, 5 - very strongly turbid and 1 - non-typical, 5 - typical). The colour was evaluated by a 6-point scale. For evaluation of odour and taste, 100 mm graphical non-structured abscissae with the description of extreme points were applied (minimal or maximal intensity of descriptors). For odour evaluation, the following descriptors were chosen: sweet, acid, cucumber, sharp, spicy, smell, sweet-acid, onion, cucumber-onion. For taste evaluation, the following descriptors were chosen: sweet, acid, cucumber, salt, spicy, bitter, sharp, harmonic, sweet-acid, onion and cucumber-onion. The evaluation of appearance of odour, appearance of taste and flavour was done by 100 mm graphical non-structured abscissae with the description of extreme points. Results of sensory evaluation represent average values from the evaluation of 10 assessors.

RESULTS AND DISCUSSION

A rapid decrease in pH in the beginning of fermentation is of great importance for the quality of the end product [7]. The rapid increase in acidity minimizes the influence of spoilage bacteria. In the slowly acidified medium, lactic acid fermentation can be suppressed by butyric bacteria activity [17]. pH of the raw material (cucumber-onion juices) was 5.4 and of the control sample (cucumber juice) 5.35. A gradual fall of pH was observed during fermentation. After 120 h of fermentation, pH was reduced to 3.0–3.2 (the control sample to 3.45) (Fig. 1). Simultaneously, there was also observed a decrease in reducing saccharides and citric acid concentrations, while total acidity, lactic and acetic acid concentrations increased considerably.

In all juice mixtures, the highest decrease in pH was observed after 48 h fermentation. During this period, a negligible increase in pH (from 5.35 to 5.4) in the control sample was observed. The decrease in pH during lactic acid fermentation was due to the formation of organic acids, in particular lactic acid. The initial total acidity value for the raw material was low, between 1.29 $g.dm^{-3}$ and 1.89 $g.dm^{-3}$ (the value for the control sample was 0.98 $g.dm^{-3}$). After fermentation for 120 h, the total acidity values of juices increased in the range of 11.96–14.45 $g.dm^{-3}$, (the value for the control sample was 11.46 $g.dm^{-3}$).

Acid-fermented vegetables are important sources of vitamins and minerals [18]. The cucumber-onion juices contained at the end of fermentation 37.4–38.5% of the original concentration of L-ascorbic acid present in the raw material. Degradation of L-ascorbic acid can be caused by chemical (minor contents of oxygen or metals in the raw materials) or by enzymatic oxidation (by enzymes naturally present in the raw materials).

At the end of fermentation, the cucumber-onion juices contained 10–20.7% of the original concentration of reducing saccharides present in the raw material. The saccharides concentration was found to decrease during fermentation. The highest degradation of saccharides was found in the cucumber juice with a 0.5% addition of the onion juice (from 34.91 $g.dm^{-3}$ to 3.50 $g.dm^{3}$). Further, the decrease in the concentration of saccharides during fermentation was due to their bioconversion into lactic acid to a larger extent, and due to their utilization during multiplication and metabolism of *L. plantarum* to a lesser extent.

The organic acids, mainly lactic acid and acetic acid, produced by lactic acid bacteria are effective antimicrobial agents, and they reduce the pH in the foods to prevent the growth of hazardous food microorganisms [18]. Dependence of concentration of lactic acid on the time of fermentation of cucumber-onion juices and of the control sample (cucumber juice) are presented in Fig. 2. After 72 h of fermentation, a stimulating effect of the onion juice addition on lactic and acetic acids production (7.18–7.65 $g.dm^{-3}$ respectively 1.10–

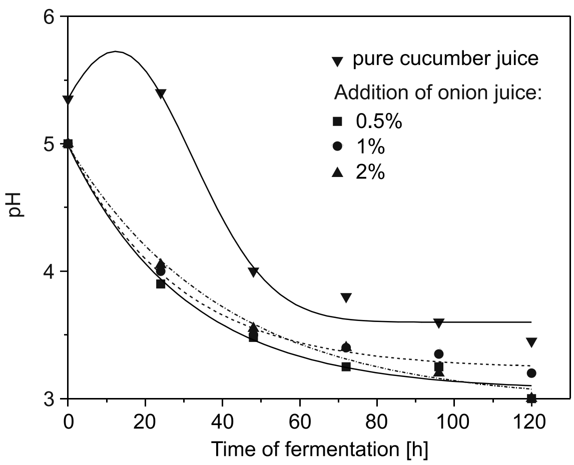

Fig. 1. Dependence of pH on fermentation time of the cucumber-onion juices mixtures.

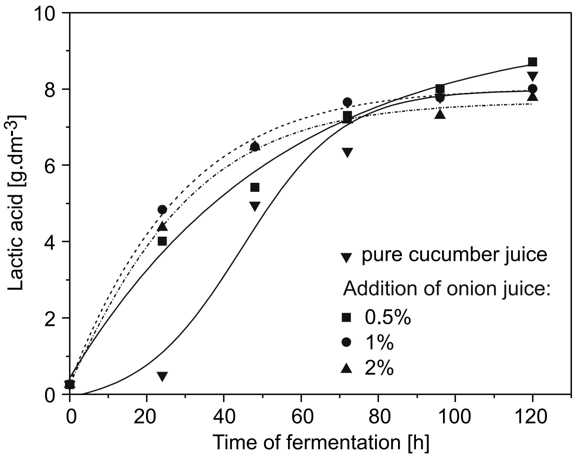

Fig. 2. Dependence of lactic acid concentration on fermentation time of the cucumber-onion juices mixtures.

1.85 g.dm^{-3}) was observed in comparison with the control sample (6.36 g.dm^{-3} and 0.35 g.dm^{-3} respectively). The highest accumulation of these acids was recorded during fermentation of cucumber juice with a 1% addition of the onion juice. However, in final stages of fermentation (96–120 h), the lower production of lactic acid and acetic acids in the juices with 1 and 2% of onion juice in comparison with the control juice was observed. It was also found that after 120 h of fermentation, the highest production of lactic and acetic acids were recorded in the cucumber juice with a 0.5% addition of the onion juice (8.71 g.dm^{-3} and 3.35 g.dm^{-3}, respectively). The production of lactic acid in these juices was higher than found by Roberts and Kidd after one week fermentation of onions (5 g.dm^{-3}) [12].

In general, the highest accumulation of lactic acid and a decrease in pH were observed between 0 and 24 h and the highest accumulation of acetic acid and a decrease in pH were observed between 48 and 72 h of fermentation. In case of the fermentation of the control sample (pure cucumber juice), fermentation process started slower and the highest production of lactic acid and acetic acid, as well as the decrease in pH were recorded between 24 and 48 h, or 96 and 120 h of fermentation.

Lactic acid fermentation of cucumber-onion juices resulted in a 7 times reduction in the citric acid concentration (from 3.23 g.dm^{-3} to 0.46 g.dm^{-3}) and only in a 2.8 times reduction in the concentration of this acid during fermentation of the pure cucumber juice. In all cases, the highest accumulation of citric acid was observed after 24 h of fermentation. Citric acid can be degraded by several species of the genus *Lactobacillus* to oxalacetic acid and acetic acid, and by decarboxylation of oxalacetic acid, pyruvic acid can be formed. Lactic acid and carbon dioxide are the end products of degradation of citric acid (reduction of pyruvic acid to lactic acid and carbon dioxide).

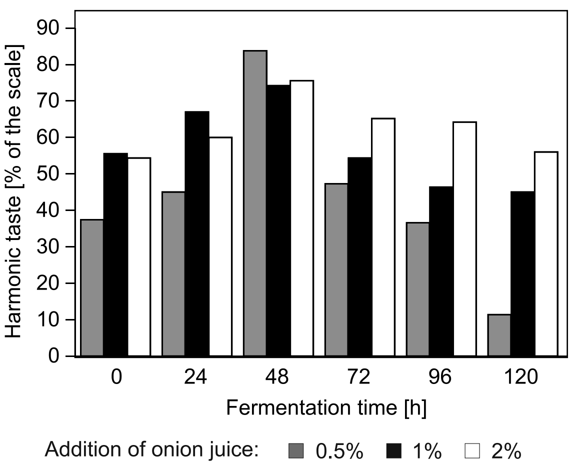

Fig. 3. Dependence of taste on fermentation time of the cucumber-onion juices.

At the start of fermentation process, the cucumber-onion juices contained from 707.89 mg.dm^{-3} to 785.40 mg.dm^{-3} of nitrates and the contents of nitrites varied between 25.38 mg.dm^{-3} and 33.31 mg.dm^{-3}. Horvath [19] and Andersson [20] described that fermentation of vegetable products decreased the nitrate contents to about ten percent of the original amount. During fermentation of cucumber-onion juices, contents of nitrates was reduced to 28.8–39.2% of their original concentration. The highest reduction in nitrates was recorded in cucumber juices with the addition of 0.5% of the onion juice. Contents of nitrites at the end of fermentation remained at the same level except for the cucumber juice with the addition of 2% of the onion juice (reduction to 31.2% of the original contents).

Results of sensory evaluation

Roberts and Kidd [12] performed lactic acid fermentation of sweet, white and yellow onions. Sensory evaluation of these products showed that yellow onions were favourable products with respect to colour, texture and flavour. Based on these results we applied yellow onion for preparation of lactic acid-fermented cucumber-onion juices. Sensory evaluation showed that all juices had the highest intensity of harmonic taste after 48 h of fermentation. In individual juices, following intensity of harmonic taste was reached (% of the scale): cucumber juice with a 0.5% addition of the onion juice 83.8%, cucumber juice with a 1% addition of the onion juice 74.2% and cucumber juice with a 2% addition of the onion juice 75.6%. In Fig. 3, dependence of harmonic taste intensity for cucumber-onion juices on fermentation time is presented. pH of juices after 48 h of fermentation varied between 3.5 and 3.55 and juices contained from 5.42 g.dm^{-3} to 6.48 g.dm^{-3} of lactic acid. Figs. 4 and 5 present graphical chart of the sensory profile of odour and taste of cucumber-onion juices after 48 h of fermentation. In this hour of fermentation, the juices distinguished mainly in the intensity of onion, cucumber-onion and sharp odour and taste. After 48 h of fermentation, all juices were strongly turbid and a typical appearance and presence of sediment were recorded. Cucumber juice with a 0.5% addition of the onion juice had in this hour of fermentation a yellow-green colour, and cucumber juices with 1% and 2% additions of the onion juice had a light green colour with a yellow hue.

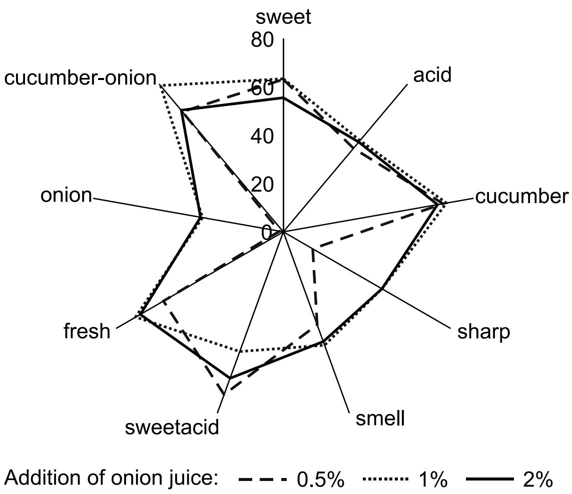

Fig. 4. Graphic chart of the sensory profile of odour for the cucumber-onion juices.

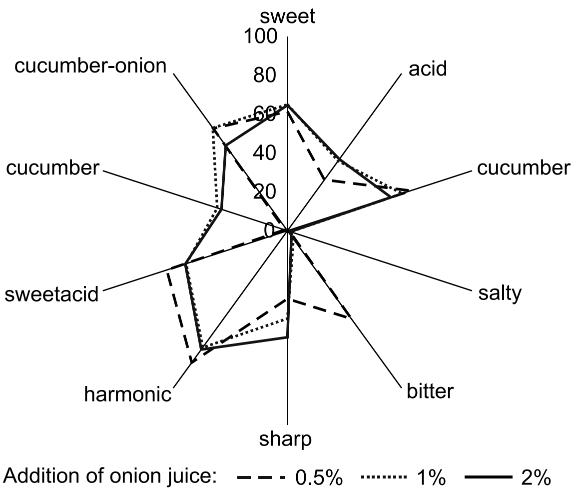

Fig. 5. Graphic chart of the sensory profile of taste for the cucumber-onion juices.

The highest intensity of the appearance of odour, appearance of taste and flavour were found after 48 h of fermentation (presented in Tab. 1). The highest intensity of these sensory parameters was recorded in the cucumber juice with a 0.5% addition of the onion juice. Cucumber juices with 1% and 2% additions of the onion juice had nearly identical intensities of appearance of odour and taste. The cucumber juice with a 1% addition of the onion juice had by about 10% lower intensity of flavour than cucumber juice with a 2% addition of the onion juice and by about 20% lower intensity of flavour than the cucumber juice with a 0.5% addition of the onion juice.

Statistical evaluation

Principal component analysis (PCA) reduces the number of original variables into a lower number of unobservable variables (principal components) that are linear combinations of the original ones [21]. By this, statistical data interpretation is simplified, relationship between different variables may be investigated, and the most important factor of variability in the data can be detected [22]. There are few studies when PCA was applied to evaluation of lactic acid-fermented food products analysis. Panda et al. [23] have evaluated sweet potato preserved by lactic acid fermentation using PCA. In previous studies, we used this method for evaluation of various lactic acid-fermented vegetable juices [8, 21]. In this paper, we applied this method for evaluation of the results of analytical and sensory parameters determined for lactic acid-fermented cucumber-onion juices.

PCA reduced the original analytical parameters to one principal component (PC1) that accounted for 81.4% % for the total variance of data. The highest loading values were found for pH and for the contents of lactic acid. It was found that to maintain sufficient amount of information encompassed in the original analytical data, determination of these two parameters is sufficient (PC1 accounted for 99.2 % of total variance).

Using PCA, the 9 original variables (odour descriptors) were reduced to two independent principal components PC1 and PC2, which had eigenvalues larger than 1. PC1 accounted for 71.6%, whereas PC2 accounted for 16.5% of total variations. When PC1 and PC2 were combined together, they accounted for 88.1% of total variations. Higher loading values were found for acid, cucumber, sweet and fresh odour (on the axis PC1) and for onion odour (on the axis PC2; Fig. 6). When

Tab. 1. The intensity appearance of odour, taste and flavour after 48 h of fermentation.

Addition of onion juice to cucumber juice	Acceptance of odour	Acceptance of taste	Acceptance of flavour
	[% of the scale]		
0.5% addition	95.2	94.2	94.2
1% addition	82.0	75.8	75.2
2% addition	85.4	74.8	85.4

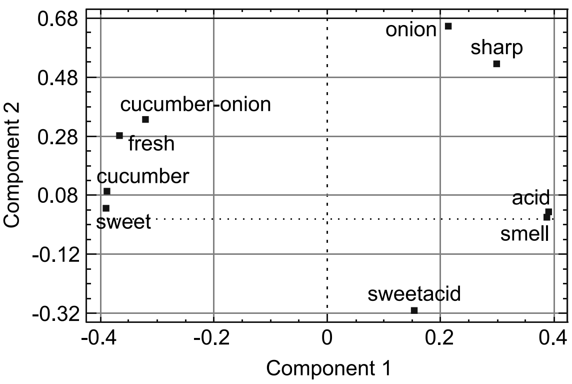

Fig. 6. Graphic representation of principal components (PC1 and PC2) of odour descriptors.

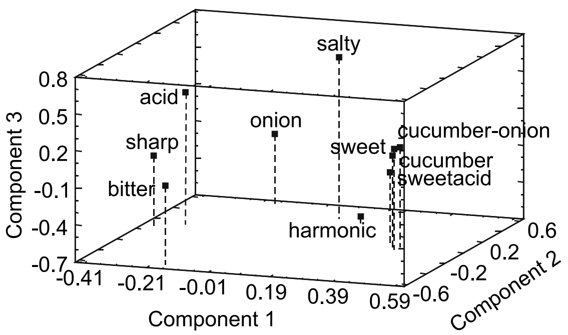

Fig. 7. Graphic representation of principal components (PC1, PC2 and PC3) of taste descriptors.

the variables for which PC1 was the most explanatory were retained, redistribution of data was reached. In this case, only one PC that accounted for 97% of total variance, was extracted.

PCA reduced the original 10 taste descriptors to three principal components (PC1, PC2 and PC3). PC1 accounted for 57.7%, PC2 22.7%, whereas PC3 accounted for 10.5% of the total variations. When combined, PC1, PC2 and PC3 together accounted for 90.9% of total variations. Higher loading values were found for cucumber, cucumber-onion, sharp and sweet taste (on the axis PC1) for onion and bitter taste (on the axis PC2) and for harmonic taste (on the axis PC3; Fig. 7). When the variables for which PC1 was the most explanatory were retained, redistribution of data was reached. In this case, only one PC that accounted for 98.4 % from total variance, was extracted. PCA showed that for taste evaluation of this type of juices, cucumber, cucumber-onion, sharp and sweet taste are the most important.

CONCLUSION

In this work, we studied cucumber juices with a 0.5%, 1% and 2% additions of the onion juices fermented by *L. plantarum* CCM 7039. In the initial stages of fermentation, the presence of onion in the juices positively influenced lactic and acetic acid production. However, in further course of fermentation, slight inhibition effects of onion in the fermented juices was observed, especially at elevated onion/cucumber ratio.

After 48 h of fermentation, the juices had the highest intensity of harmonic taste, acceptance of odour, acceptance of taste and flavour and the pH of juices (3.50–3.55) was sufficiently low to prevent the growth undesirable food microorganisms. In the initial stages of fermentation (to 48 h), the presence of onion in the juices positively influenced lactic acid production by lactic acid bacteria. The cucumber juice with an addition of 0.5% of the onion juices was selected by assessors as the most suitable for preparation of this type of juices.

It was also found that *L. plantarum* CCM 7039 reduced the concentration of nitrates in the cucumber and also in the cucumber-onion juices. We suggest that this strain of lactic acid bacteria is used for preparation of fermented juices from such vegetable species in which high contents of these compounds are present.

For evaluation of results, we applied PCA to reduce the dimensionality of variables (analytical and sensory parameters). PCA selected that for evaluation of cucumber-onion juices, pH, lactic acid, odour descriptors: acid, cucumber, sweet and fresh and taste descriptors: cucumber, cucumber-onion, sharp and sweet are the most important variables.

Acknowledgement
This work was supported by the Slovak Grant Agency for Science VEGA (Grant No. 1/3546/06) and National programme of Research and Development (Grant No. 749/s/2003)

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Received 9 January 2007; 1st revised 29 May 2007; 2nd revised 22 August 2007; accepted 27 August 2007.