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# The choice of strains of *Lactobacillus* species for the lactic acid fermentation of vegetable juices

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**Abstract** The reasons for using lactic acid bacteria are to make food durable, to improve its taste and to maintain the nutritive, physiological and hygienic value of the fermentation products. Sixteen strains of the genus *Lactobacillus* were tested on samples of white fresh cabbage and of a sterilized cabbage and carrot juice mixture. After 7 days of lactic acid fermentation at 27 °C or 30 °C, reducing sugars, total acidity, pH value, lactic, citric and acetic acids, ammonia, nitrates and nitrites were measured in the samples. On the basis of the criteria mentioned above three strains were acceptable. These strains reduced the content of nitrates in the original samples.

**Key words** Vegetable juices · Lactic acid fermentation · *Lactobacillus* species

## Introduction

The lactic acid fermentation of vegetables, applied as a preservation method for the production of finished and half-finished products, is again being ranked as an important technology and is being further investigated because of the growing amount of raw materials processed in this way in the food industry. The main reasons for this interest are the nutritional, physiological and hygienic aspects of the process and their corresponding implementation and production costs. These aspects play an important role in the evaluation of the increased use of lactic acid fermented vegetable juices [1, 2].

The desirable properties of fermented vegetable juices can be achieved by choosing *Lactobacillus* strains suitable for the lactic acid fermentation of individual raw materials. The criteria used to gange a strain's suitability are as follows: the rate and total production of acids, change of pH, loss of nutritionally important substances, decrease of nitrate concentration and production of biogenic amines [3–5]. The factors mentioned above have become the subject of discussions on the specificity of *Lactobacillus* strains and their dependence for growth on the supply of nutrients (substrate), the availability (or lack) of air (oxygen), the chemical and physical environment, temperature and other factors [6-8].

The aim of this work was to select *Lactobacillus* strains suitable for the lactic acid fermentation of vegetable juices. The criteria used for strains selection were the rate of the pH decrease, organic acid production, nitrate and nitrite reduction and low content of biogenic amines.

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## Materials and methods

*Preparation of cabbage samples and their fermentation.* Fresh white cabbage (type Dobrovodská late) was purchased at a local and fruit vegetable market in Slovakia. After removing its outer leaves and the addition of 1.5% NaCl, the cabbage was crushed with a chopper. The stirred throughly homogenized cabbage (300 g batches) was with 100 ml of the inoculum containing ca. 10 microorganisms in 1 ml and placed in 500 ml flasks. The flasks were sealed with a ground-in cap with a vacuum valve through which air was evacuated using a vacuum pump. The samples prepared in this way were cultivated in a thermostat at 27 °C for 7 days.

*Preparation of cabbage and carrot juice samples and their fermentation.* The cleaned fresh white cabbage was homogenized with a chopper after the addition of 1% NaCl. The juice was obtained by pressing the crushed material. The cleaned and shredded carrot (type Delicia, Slovakia) was boiled in a pressure pot at ca. 110 °C with water in the ratio 1:1 for 10 min and then the juice was separated from the carrot pith. The cabbage and the carrot juice were mixed in the ratio 2:1, then 3% D-glucose was added and the salt concentration adjusted to 1.5%. The juice was placed in several flasks and sterilized in an autoclave for 10 min at 121 °C. After cooling, L-ascorbic acid (15–25 mg/100 ml) and vitamin $B_1$ (0.01 mg/100 ml) were added to the flasks and the mixture was inoculated with pure cultures of lactobacilli. The juice volume in

each flask was made up to 300 ml and the flasks were sealed with sterile rubber plugs. The samples were cultivated at 30 °C for 7 days.

*Cultivation of pure cultures.* All pure cultures were cultivated is liquid APT medium prepared for lactobacilli. In the experiments the following strains were used: *Lactobacillus plantarum* 178, *L. plantarum* 186, *L. plantarum* 189, *L. casei* 598, *L. lactis* 447, *L. plantarum* BIL, *L. pentosus*, *L. plantarum* 190, *L. plantarum* 195, *L. delbrueckii* 237, *L. delbrueckii* 238, *L. plantarum* 181, *L. plantarum* 187, *L. plantarum* 192, *L. plantarum* 976 (Food Research Institute, Modra, Slovakia) and L. acidophilus CCM 2913, *L. helveticus* CCM 3826, *L. plantarum* CCM 551 (Culture Collection of Microorganisms, Brno, Czech Republic).

*Determination of reducing sugars according to Schoorl.* Reducing sugars were determined according to Davidek et al. [9].

*Determination of total acidity.* Total acidity was determined by visual titration with a 0.1 M solution of NaOH against phenolphthalein. The result was calculated for lactic acid [9].

*pH determination.* Measurement of pH was performed using a Pracistronic MV 88 apparatus.

*Determination of L-ascorbic acid content.* L-Ascorbic acid was determined by titration with a solution of 2,6-dichlorophenolindophenol [9].

*Isotachophoretic determination of organic acids.* The sample was homogenized and filtered before the isotachophoretic analysis. The measurement was carried out using a CS ZKI 01 isotachophoretic analyzer equipped with a conductivity detector. The electrolytic system used for identification and determination had the following composition: concentration of leading electrolyte $10^{-2}$ mol/dm$^3$ HCl; counter-ion, 6-aminocapronic acid, pH 4.5; additive 0.1% methylhydroxyethylcellulose; terminating electrolyte $5\times10^{-3}$ mol/dm$^3$ capronic acid; $5\times10^{-3}$ mol/dm$^3$ histidine, pH 4–5. The samples were analyzed at a driving current of 200 μA in the preparation column and 50 μA in the analytical column. Quantitative analysis was performed by calibration [10].

*Determination of ammonia by microdiffusion according to Conway.* The ammonia was expelled from the sample extract by potassium carbonate in a Conway vessel and then absorbed by boric acid [9].

*Determination of nitrates and nitrites by flow injection analysis.* Nitrates and nitrites were determined using N-(1-naphthyl)ethylenediamine dihydrochloride [9].

*Determination of biogenic amines.* The biogenic amines (histamine, putrescine, cadaverine) were studied and a high-performance liquid chromatography isocratic system along with precolumn derivatization of amines with a dansyl chloride were used for isolation [11].

## Results and discussion

The aim of the study was to investigate the suitability of various microorganisms as starting cultures during the lactic acid fermentation of vegetables. The selection of the microorganisms was based mainly on the production of lactic acid and the utilization of nitrates.

The inoculation by individual microorganisms was chosen in the range of $10^4$ to $10^6$ CFU/g of raw material. The average results from a set of experiments are summarized in Table 1, which shows acid production and its influence on pH, and the amount of individual organic acids (lactic, citric, acetic) produced by selected lactic acid bacterial cultures. Under the experimental conditions chosen it was assumed that the heterofermentative course of lactic acid fermentation and the production of concomitant substances resulted from the presence of raw material microflora. Different rates of decrease of nitrates were evident. From the point of view of the lactic acid fermentation the concentration growth of ammonia was interesting and despite its relationship to the applied determination method it was observed in all cases.

**Table 1** Parameters determined for fresh cabbage and after 7 days of fermentation by various strains of *Lactobacillus*

| Sample | Lactobacilli (CFU/g) | Reducing sugars (g/kg) | Total acidity (g/kg) | pH | Ammonia (mg/kg) | Nitrates (mg/kg) | Acids (g/kg) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lactic acid | Citric acid | Acetic acid |
| Fresh cabbage | | 47.21 | 3.10 | 6.20 | 56.12 | 339.20 | 0.40 | 4.82 | 0.11 |
| *Lactobacillus casei* 589 | $1.2\times10^6$ | 21.40 | 18.90 | 3.60 | 201.21 | 172.01 | 14.81 | 1.91 | 0.72 |
| *L. lactis* 447 | $1.5\times10^6$ | 27.90 | 14.31 | 3.70 | 262.24 | 195.80 | 11.02 | 2.05 | 1.41 |
| *L. helveticus* CCM 3826 | $2.3\times10^6$ | 27.30 | 17.92 | 3.60 | 264.33 | 188.51 | 14.20 | 2.43 | 0.60 |
| Fresh cabbage | | 41.03 | 2.41 | 6.31 | 60.01 | 314.13 | 0.13 | 4.90 | 0.22 |
| *L. plantarum* BIL | $4.8\times10^6$ | 27.32 | 15.60 | 3.81 | 205.25 | 258.02 | 13.51 | 2.06 | 0.73 |
| *L. pentosus* | $1.7\times10^6$ | 21.71 | 18.60 | 3.80 | 192.16 | 237.01 | 14.70 | 0.92 | 1.12 |
| *L. acidophilus* CCM 2913 | $3.5\times10^5$ | 23.60 | 17.22 | 3.60 | 162.11 | 245.60 | 14.80 | 1.04 | 0.91 |
| Fresh cabbage | | 43.84 | 2.12 | 6.31 | 40.32 | 363.70 | 0.20 | 4.71 | 0.14 |
| *L. plantarum* 190 | $1.2\times10^6$ | 16.11 | 13.63 | 3.80 | 250.81 | 24.41 | 10.21 | 2.52 | 4.61 |
| *L. plantarum* 195 | $3.2\times10^5$ | 17.90 | 15.90 | 3.60 | 272.64 | 25.51 | 13.31 | 1.81 | 3.13 |
| *L. delbrueckii* 237 | $1.9\times10^6$ | 17.80 | 13.81 | 3.80 | 247.32 | 28.30 | 11.03 | 2.40 | 2.61 |
| *L. delbrueckii* 238 | $2.5\times10^6$ | 14.42 | 15.63 | 3.71 | 245.24 | 104.41 | 13.43 | 2.22 | 1.62 |
| Fresh cabbage | | 38.83 | 2.41 | 6.02 | 45.87 | 259.51 | 0.62 | 4.06 | 0.09 |
| *L. plantarum* 181 | $3.0\times10^5$ | 11.40 | 13.81 | 3.41 | 198.11 | 83.50 | 15.40 | 2.41 | 0.51 |
| *L. plantarum* 187 | $1.6\times10^6$ | 11.53 | 13.02 | 3.40 | 213.52 | 86.53 | 16.51 | 1.91 | 0.54 |
| *L. plantarum* 192 | $1.9\times10^6$ | 12.43 | 12.51 | 3.70 | 277.13 | 24.50 | 11.24 | 2.04 | 5.22 |
| *L. plantarum* 976 | $4.0\times10^5$ | 9.41 | 16.62 | 3.30 | 153.19 | 81.03 | 16.60 | 2.72 | 0.42 |
| *L. plantarum* CCM 551 | $2.6\times10^5$ | 8.90 | 17.11 | 3.30 | 186.32 | 86.04 | 16.01 | 0.71 | 0.60 |

**Table 2** Parameters determined for cabbage-carrot juices and after 7 days of fermentation by various strains of *Lactobacillus*

| Sample | Lactobacilli (CFU/g) | Reducing sugars (g/kg) | Total acidity (g/kg) | pH | Nitrates (mg/kg) | Acids (g/kg) | | | Ascorbic (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lactic acid | Citric acid | Acetic acid | |
| Fresh sample | | 47.11 | 1.80 | 6.02 | 162.01 | 1.13 | 4.50 | 0.12 | 30.80 |
| *L. plantarum* 178 | $1.1 \times 10^4$ | 33.71 | 12.50 | 3.80 | 142.41 | 18.06 | 2.51 | 0.42 | 20.02 |
| *L. plantarum* 186 | $2.4 \times 10^4$ | 33.30 | 14.11 | 3.72 | 161.02 | 19.01 | 1.90 | 0.43 | 17.81 |
| *L. plantarum* 189 | $2.5 \times 10^4$ | 34.06 | 12.60 | 3.81 | 63.51 | 16.12 | 2.13 | 0.41 | 16.65 |
| Fresh sample | | 61.62 | 2.61 | 6.02 | 415.03 | 0.44 | 6.02 | 0.22 | 24.12 |
| *L. casei* 589 | $9.7 \times 10^5$ | 44.22 | 15.22 | 3.53 | 216.51 | 18.33 | 1.81 | 0.34 | 17.16 |
| *L. lactis* 447 | $1.3 \times 10^6$ | 46.51 | 15.01 | 4.11 | 367.34 | 16.11 | 1.63 | 0.52 | 11.33 |
| *L. helveticus* CCM 3826 | $1.4 \times 10^6$ | 45.16 | 15.83 | 3.74 | 413.37 | 16.92 | 1.60 | 0.37 | 17.54 |
| Fresh sample | | 64.52 | 1.73 | 5.5 | 230.06 | 0.39 | 5.71 | 0.22 | 24.50 |
| *L. plantarum* BIL | $2.5 \times 10^6$ | 51.38 | 12.74 | 1.02 | 77.90 | 19.07 | 2.64 | 0.54 | 18.31 |
| *L. pentosum* | $9.0 \times 10^5$ | 39.91 | 13.15 | 3.90 | 77.14 | 19.82 | 1.38 | 0.63 | 16.73 |
| *L. acidophilus* CCM 2913 | $1.8 \times 10^6$ | 49.96 | 14.32 | 3.91 | 90.22 | 18.90 | 2.51 | 0.51 | 17.97 |
| Fresh sample | | 52.21 | 1.91 | 5.40 | 299.23 | 0.32 | 4.81 | 0.26 | 22.85 |
| *L. plantarum* 190 | $6.3 \times 10^5$ | 32.15 | 10.82 | 3.60 | 19.71 | 10.04 | 1.82 | 2.36 | 7.44 |
| *L. delbrueckii* 237 | $1.0 \times 10^5$ | 34.32 | 11.16 | 3.61 | 9.22 | 9.94 | 1.26 | 2.35 | 9.73 |
| Fresh sample | | 50.11 | 2.33 | 6.04 | 385.15 | 0.35 | 4.84 | 0.12 | 28.91 |
| *L. plantarum* 181 | $1.6 \times 10^5$ | 35.77 | 11.11 | 3.22 | 158.31 | 16.06 | 1.65 | 0.37 | 15.33 |
| *L. plantarum* 187 | $8.3 \times 10^5$ | 31.03 | 10.12 | 3.22 | 156.44 | 19.93 | 2.05 | 0.34 | 14.87 |
| *L. plantarum* 192 | $1.0 \times 10^6$ | 31.04 | 9.80 | 3.63 | 5.91 | 9.71 | 1.42 | 2.05 | 15.81 |
| *L. plantarum* 976 | $2.1 \times 10^5$ | 36.61 | 12.11 | 3.11 | 149.20 | 11.22 | 2.06 | 2.61 | 15.60 |
| *L. plantarum* CCM 551 | $1.4 \times 10^5$ | 35.91 | 12.32 | 3.12 | 158.42 | 18.01 | 0.74 | 0.70 | 16.22 |

This work was also concerned with the study of biogenic amines. In the first stage, the analysis of samples of sauerkraut obtained from a trade network and the determination of putrescine, cadaverine and histamine were accomplished. The concentrations of these substances were ranging as follows: from 12 to 45.5 mg/kg for cadaverine, from 56 to 90 mg/kg for histamine and from 157 to 169 mg/kg for putrescine. In the sample exposed to a spontaneous fermentation under laboratory conditions the concentration of putrescine was 145 mg/kg. In the samples fermented by starter cultures the concentration of putrescine ranged from 100 to 120 mg/kg, in accordance with the published data. The values of histamine were between 10 and 25 mg/kg, and of cadaverine were between 35 and 50 mg/kg. The results obtained suggest that by applying a suitable starter culture the concentration of putrescine can be substantially reduced, owing to a rapid decrease in pH (lactic acid production). Naturally, this positive role is attributed to an increase in the starting concentration of microorganisms. The efficient production of histamine can be attained by completing the fermentation within the time interval in which the total acid converted to lactic acid is 1.2%.

The subsequent experiments were implemented on the sterile medium, the composition of which was appropriate for the cultivation of *Lactobacillus*. To ensure the comparability of experiments, the sterilized juice obtained from the cabbage and carrot juice mixture was fortilied by applying thiaminedichloride and ascorbic acid. The inoculum of individual microorganisms was added in amounts comparable to those used in the experiments with the cabbage. Again, from a suitable group of microorganisms, i.e. those that gave the best results during the lactic acid fermentation of cabbage, a variety of different additions of microorganisms was chosen. The results obtained from the study of the fermented cabbage and carrot juice samples are shown in Table 2. On the basis of both experiments and by comparing the results, the strains *L. plantarum* 192, *L. plantarum* 190 and *L. delbrueckii* 237 were selected. The application of these strains enabled us to obtain comparable and long-lasting results. In all cases the reduction of nitrate content was measured, but the lowest concentrations of nitrates in juices were found using the three chosen strains. The dynamics of this process were investigated as part of our study [12]. As well as determining the nitrate content, we observed the content of nitrites at the beginning and the end of the fermentation process. The initial and final nitrite concentrations were only trace amounts, indicating that the method used was not of an adequate sensitivity.

It is apparent that the concept of our work, based on repeated experiments with individual strains from collections and from our own group of microorganisms, and on the subsequent choice of strains suitable for the lactic acid fermentation of the chosen vegetable species, is correct. It is obvious that the application of particular strains in controlled fermentation, which has a great effect on the finished product, is possible provided that there are sufficient amounts of nutrients and growth factors.

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