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Dynamics of production of organic acids during lactic fermentation of vegetable juice

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Abstract

Strains of *Lactobacillus* were tested on prepared samples of cabbage and carrot juice mixture. The determination of phosphoric, citric, lactic, succinic and acetic acid was carried out after 40, 64, 136, 184 and 232 h of fermentation by capillary isotachophoresis. *L. plantarum* (190/86) produced the best sensoric values of fermented vegetable juices.

1. Introduction

The popularity of vegetable juices produced by lactic fermentation is growing because they represent a new type of drink and because of their high nutritive value, with a high content of vitamins and minerals [1]. They are sold either non-fermented or fermented. Because of the variety of materials used in their production, no generally used technology exists; the production method depends not only on the kind of material used, but also on the desired properties of the products [2–4]. Vegetable juices produced by lactic fermentation do not have salt or spices added, so they are suitable for dietetic use [5,6].

Enzymatic treatment of mashes can be done together with lactic fermentation, according to some workers [5,7,8]. It is stated that the best results were achieved by using *Lactobacillus plantarum* as a starting culture. From the nutritive point of view, the content of lactic acid in lactic-fermented juices is of interest [9,10].

The detection of individual acids in various food products is now carried out mostly by chromatographic methods, gas chromatography [11] and high-performance liquid chromatography [12] being the most widely used. However, the complete chromatographic separation of organic acids is not easily achieved, and the methods are time consuming and involve complex sample preparation. Organic acids in aqueous alcohols have been determined using zone electrophoresis [13]. Capillary isotachophoresis has been found suitable for the identification and determination of organic acids in foods [14–16].

In this work, we prepared a mixture of cabbage and carrot juice, fermented it and measured the organic acids produced with the application of selected microorganisms by capillary isotachophoresis.

2. Experimental

2.1. Preparation of cabbage and carrot juice samples and their fermentation

Cabbage and carrot juice were mixed in the

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proportions of 2:1, 3% of D-glucose was added and the salt concentration was adjusted to 1.5%. The juice mixture was placed in flasks and sterilized for 10 min at 121°C. L-Ascorbic acid and thiamine dichloride were added and pure cultures of lactic bacteria were inoculated. The samples were cultivated at 30°C.

2.2. Chemicals

The chemicals were of analytical-reagent grade. Standards of organic acids (here considered to include used phosphoric acid) and HCl were obtained from Lachema (Brno, Czech Republic), methylhydroxyethylcellulose (MHEC) and ε-aminocaproic acid from Serva (Heidelberg, Germany) and histidine from Sigma (St. Louis, MO, USA). Doubly distilled water was passed through a deionizer before use.

2.3. Equipment

A ZKI 01 isotachophoretic analyser (Labeco, Spišská Nová Ves, Slovak Republic) with standard columns and a conductivity detector and a TZ 4200 double-line recorder (Laboratorní Přístroje, Prague, Czech Republic) were used. An M-120 pH meter (Mikrotechnika, Prague, Czech Republic) was employed.

2.4. Electrolyte system for separation

For the identification and determination of organic acids the electrolyte system applied had the following composition: concentration of leading electrolye, 0.01 *M* HCl; counter ion, ε-aminocaproic acid, pH 4.5; additive, methylhydroxyethylcellulose (0.1%); and terminating electrolyte, $5 \cdot 10^{-5}$ *M* caproic acid–$5 \cdot 10^{-3}$ *M* histidine (pH 4–5).

2.5. Measurement procedure

Prior to isotachophoretic measurement of organic acids, samples of the fermented mixture of cabbage and carrot juice were filtered and diluted 1:25 with water. The samples were injected into the column using the four valves of the instrument. The samples of juices were analysed at a driving current of 250 μA in the preseparation column and 40 μA in the analytical column. Quantitative analysis was performed by calibration. Based on the presumed presence of the individual organic acids, standard solutions of

Table I
Organic acids in mixture of cabbage and carrot juice

Microorganism	Fermentation time (h)	Organic acid ($g\ l^{-1}$)				
		Phosphoric	Citric	Lactic	Succinic	Acetic
–	Day zero	1.03	0.78	0.25	0.38	–
L. plantarum (189/86)	40	0.62	2.69	8.23	0.23	0.37
	64	–	1.39	12.90	0.71	0.36
	136	–	3.24	11.78	0.70	0.13
	184	–	1.75	11.96	0.36	0.55
	232	–	1.11	12.43	0.31	0.28
L. plantarum (190/86)	40	–	1.06	6.96	1.61	1.71
	64	–	0.80	8.48	1.49	1.76
	136	–	2.54	8.56	–	0.85
	184	–	1.23	11.28	0.89	2.08
	232	–	2.01	7.23	0.86	1.23
L. delbrückii (237/86)	40	0.59	2.22	0.59	–	–
	64	–	1.33	8.31	0.73	0.94
	136	–	1.16	7.94	0.52	0.23
	184	–	0.36	12.36	0.77	3.11
	232	–	2.01	7.23	0.86	1.23

lactic, acetic, citric, phosphoric and succinic acid of concentration 0.01 *M* were prepared.

3. Results and discussion

Samples of cabbage and carrot juice mixture were analysed on day zero before fermentation by capillary isotachophoresis and the concentrations of phosphoric, citric, lactic, succinic and acetic acid was determined. Various strains of microorganisms were applied to the prepared juice and the results of fermentation was observed after 7 days. Not only the concentrations of the individual organic acids, but also results of the determination of pH, titration acidity, decrease in reducing sugar and utilization of nitrates served for the selection of the microorganisms. On the basis of these results, we used only *Lactobacillus plantarum* (189/86), *L. plantarum* (190/86) and *L. delbrückii* (237/86) for further experiments.

Samples fermented with the selected microorganisms were analysed at set time intervals. The time intervals chosen for sampling and the results of isotachophoretic analyses were used to determine the time of maximum production of lactic acid and also of the production of acetic acid by the tested microorganism and for demonstrating its suitability for the production of fermented juices.

Isotachophoretic analyses for the determination of phosphoric, citric, lactic, succinic and acetic acid were carried out after 40, 64, 136, 184 and 232 h of fermentation. Table I gives the concentrations of organic acids in the mixture of cabbage and carrot juice on day zero and after fermentation with *L. plantarum* (189/86), *L. plantarum* (190/86) and *L. delbrückii* (237/86). The individual data are average values calculated from three measurements. Fig. 1 shows the analysis of cabbage and carrot juice mixture fermented with *L. plantarum* (189/86).

From the results for lactic acid production in Table I, it is obvious that the greatest increase, from the starting value of 0.25 g l^{-1} to 12.90 g l^{-1}, occurred in the sample with *L. plantarum* (189/86) after 64 h of fermentation. After 136 h there was a decrease and then a slight increase. Production of acetic by this microorganism increased to 0.53 g l^{-1} after 184 h of fermentation and subsequently decreased.

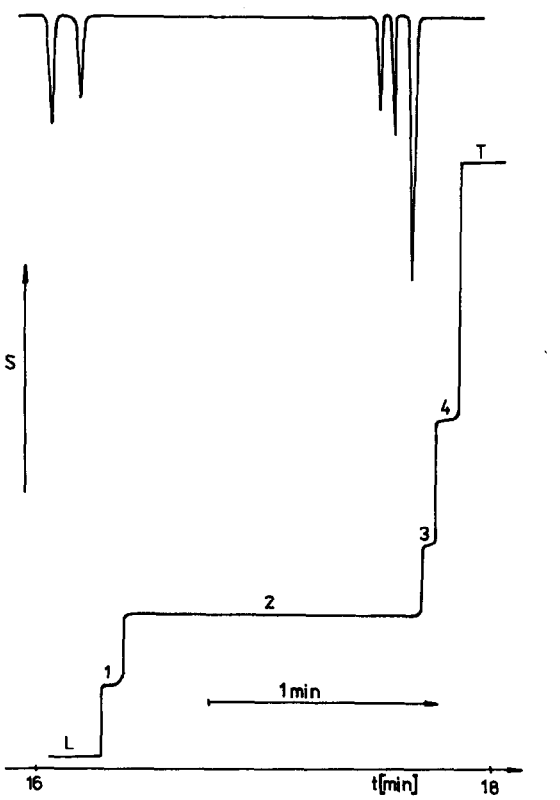

Fig. 1. Isotachophoreogram of organic acids in a sample cabbage and carrot juice mixture fermented with *L. plantarum* (189/86) for 64 h. *S* = Response of conductivity detector; L = leading electrolyte; T = terminating electrolyte; 1 = citric acid; 2 = lactic acid; 3 = succinic acid; 4 = acetic acid.

With *L. plantarum* (190/86), lactic acid production reached the maximum value of 11.28 g l^{-1} after 184 h of fermentation, then decreased. The acetic acid content also reached its maximum value of 2.08 g l^{-1} after 184 h.

L. delbrückii (237/86) gave a maximum production lactic acid of 12.37 g l^{-1} and of acetic acid of 3.11 g l^{-1} both after 184 h of fermentation.

The content of citric acid varied in the individual samples. The maximum content of 3.24

g l^{-1} occurred in the sample fermented with *L. plantarum* (189/86) for 136 h.

From the results, it is clear that *L. plantarum* (189/86) was the most suitable microorganism for lactic acid production because it produced this acid the most rapidly. However, *L. plantarum* (190/86) produced the best sensoric values of fermented vegetable juices because the concentration of acetic acid produced gave a very good taste.

For the determination of organic acids in individual fermented samples, the standard deviations, s_x [12], were as follows: phosphoric acid, 0.014–0.018; citric acid, 0.025–0.053; lactic acid, 0.016–0.042; succinic acid, 0.032–0.030; and acetic acid, 0.017–0.070 g l^{-1}. The relative standard deviations, s_r, were 0.35–1.87%.

Fig. 2 shows the changes in lactic acid content produced by fermentation with the individual microorganisms.

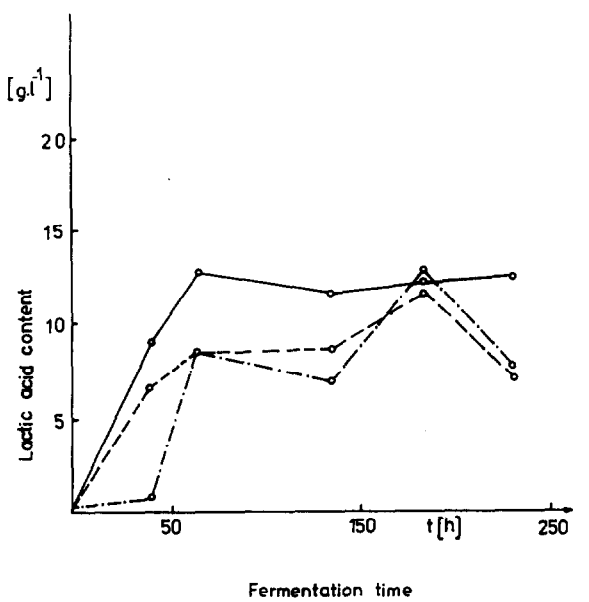

Fig. 2. Changes in lactic acid content during fermentation of cabbage and carrot juice mixture by individual microorganisms. ———, *L. plantarum* (189/86); – – – –, *L. plantarum* (190/86); – · – · –, *L. delbrückii* (237/86).

In conclusion, the application of isotachophoresis for the determination of organic acids is very advantageous. From the results of organic acid determination, a rapid choice of suitable microorganisms for further experiments is possible. Capillary isotachophoresis was selected as a suitable method for the determination of organic acids on the basis of its useful properties (simple sample preparation, rapid determination and good reproducibility).

4. References

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