Lactic acid fermentation of some vegetable juices

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Summary
Suitability of various kinds of vegetables (cabbage, tomatoes, pumpkin and courgette) for the preparation of vegetable juices processed by lactic acid fermentation was tested. Based on the results obtained from the assessment of selected analytical and sensory parameters, juice from cabbage and courgette in particular was recommended for the preparation of this product type. The juices tested showed sufficiently low pH values (3.6) after 72 or 48 h of fermentation, they contained 1.4% and 1.5% of acids (expressed as lactic acid) respectively, had a pleasantly sour taste and a flavour typical of vegetable products processed by lactic acid fermentation.

Keywords
fermentation; vegetable juices; *Lactobacillus plantarum*

Fermentation of foodstuffs is a desirable process of biochemical modification of primary food products, with the major role in this respect played by microorganisms and their enzymes. Fermentation improves flavour and taste, extends the shelf-life, and increases the nutritional value of thus treated products [1].

There are 21 different kinds of vegetable products processed by lactic acid fermentation produced currently in Europe, including vegetables and vegetable juices [2]. The importance of this method of preserving food in the modern world is underlined by the wide range of uses in both developed and developing countries, because of its low price and significant sensory characteristics of thus preserved food [3-5].

The preparation of products with the required characteristics requires selection of suitable microorganism strains for processing of the different raw materials by lactic acid fermentation; selection criteria may include speed and total production of acids [6], representation of various organic acids [7], change in pH, loss of substances of nutritional relevance, reduction of concentrations of nitrates and nitrites, production of biogenic amines [6], substrate ability to accept the given starter culture, type of metabolism (homofermentative, heterofermentative), ability to produce desirable characteristics of the fermented product, etc. [5].

The worldwide apparent trend of increasing interest in products prepared from vegetables processed by lactic acid fermentation is due to consumers' increasing preference of natural, biological methods of preservation as well as to the new data suggesting favourable chemoprotective (health protecting) effects of such foods. On the one hand, these products preserve high proportions of protective substances contained in the original raw material; on the other hand, during the fermentation process lactic bacteria produce additional health-promoting components. The substances formed, antibiotics as well as other substances, while providing for taste and flavour, reduce the risk of civilisation diseases and contribute to the health-relevant significance of this group of foods [3].

The aim of the present work was to compare the course of fermentation of different vegetable juices inoculated with *Lactobacillus plantarum* CCM 7039 using defined analytical and sensory parameters, and to recommend the juice, which would the best raw material for the production of a product processed by lactic acid fermentation; in addition, optimum duration of fermentation was to be determined.

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MATERIALS AND METHODS

Preparation of sample vegetable juices

White cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba*, variety HOLT), tomatoes (*Lycopersicon esculentum*, variety Tornádo), pumpkin (*Cucurbita pepo* L., variety Kveta), and courgette (*Cucurbita pepo* L, Goldline F1 hybrid) purchased in retail were used for the preparation of vegetable juices. Fresh vegetable juices were obtained by extracting the juice from the raw materials using a kitchen juicer. The pH value of the tomato juice was adjusted by $NaHCO_3$ to 6.5. Subsequently, the juices were gauze-filtered, fortified by adding 2% D-glucose and 0.5% NaCl, and inoculated with a culture of the lactic bacteria *Lactobacillus plantarum* CCM 7039 (Czech Collection of Microorganisms, Faculty of Sciences, Masaryk University, Brno, Czech Republic) at 10^6 KTJ.ml^{-1} juice. Thus treated juices were poured into 250 cm^3 graduated flasks, which were stoppered with sterile stoppers and left to ferment in a thermostat for 168 hours at 21 °C. During the fermentation, samples were withdrawn at pre-specified intervals for analytical determinations and sensory assessment.

Chemical analyses

The following analytical parameters were studied during fermentation: pH, titration acidity, reducing saccharides according to Schoorl [8], and content of organic acids. Lactic, acetic, citric, and L-ascorbic acids were determined using the method of capillary isotachophoresis [9, 10]. Isotachophoretic measurements were done using an isotachophoretic analyser and the ZKI01 columns connection technique (Villa Labeco, Spišská Nová Ves, Slovak Republic), equipped with conductivity detector and two-line recorder TZ 4200 (Laboratórní přístroje, Prague, Czech Republic).

Selection of sensory methods [11, 12]

In evaluating the appearance of samples, the most important parameters included colour, turbidity, sediment, and overall appearance. A 5-point intensity scale was used to assess turbidity and overall appearance. Colour was assessed by a descriptive method. In assessing taste and flavour, the overall perception was split into the different taste and flavour descriptors and 100mm graphical non-structured line segments with specified end-points were used. Also, pleasantness of taste and flavour were assessed using 100mm graphical non-structured line segments with specified end-points.

RESULTS AND DISCUSSION

Cabbage, tomatoes and carrots are the vegetables most frequently used to prepare vegetable juices processed by lactic acid fermentation as they contain large amounts of fermentable saccharides [13]. Prompted by this fact, we tested the suitability of cabbage, tomatoes, courgette, and pumpkin for the preparation of vegetable juices processed by lactic acid fermentation. The juices tested contained between 24.4 g.dm^{-3} (tomato juice) and 49.5 g.dm^{-3} (pumpkin juice) reducing saccharides. Since at least 4% saccharides are needed to provide for an optimal course of lactic acid fermentation [14], the juices obtained were supplemented with 2% D-glucose before the fermentation. Tomato juice has a pH value (4.3), which is unsuitable for the growth of lactic bacteria [13], and the pH value of this kind of juice had therefore to be adjusted with $NaHCO_3$ to 6.5.

Lactobacillus plantarum CCM 7039 used to ferment the juices was selected based on the results of preliminary analyses [14] to test the suitability of various microorganisms (*Lactobacillus plantarum* 92H, *Lactobacillus plantarum* CCM 7039, mixed starter culture of *Lactobacillus plantarum* 92H and *Saccharomyces cerevisiae* C11-3) for the preparation of cabbage juices processed by lactic acid fermentation.

During lactic acid fermentation, the pH value of the juices dropped from between 6–6.5 to 3.8–4.5 [3, 5, 15]. At the end of fermentation (at 168 h), the pH value of the juices prepared by us ranged between 3.35 (pumpkin juice) and 3.8 (tomato juice), with the initial pH of the input raw material being between 6.15 (cabbage juice) and 6.5 (tomato juice). Fig. 1 illustrates the changes in

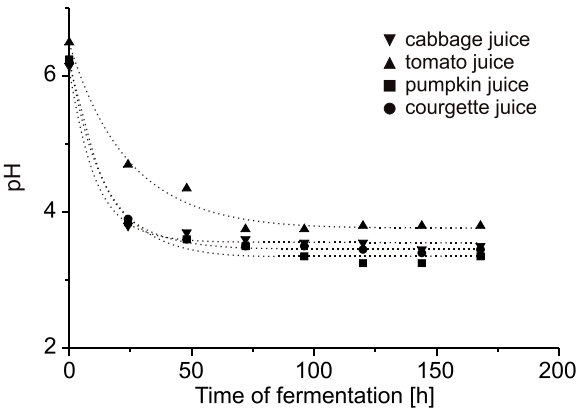

Fig. 1. Changes in pH during fermentation of vegetable juices.

pH occurring during the fermentation of the different juices. The largest drop in pH was recorded for pumpkin juice (from 6.25 to 3.25). A rapid pH reduction in the early stages of fermentation is important to obtain a high-quality final product [16]. In a slowly acidifying environment, lactic acid fermentation is suppressed by butyric acid producing bacteria [17].

At the end of the fermentation, different juices contained between 0.4% and 54% (cabbage juice) of the initial content of reducing saccharides. The largest reduction of the content of reducing saccharides was noted for courgette juice, which retained as little as 22% of the initial amounts within 48 hours after the start of fermentation.

Since L-ascorbic acid is an indicator of the sparingness of the technological process, the concentration of this acid was measured in also the end products. About 20 to 70% of the initial content of L-ascorbic acid remains preserved in the end-products, depending on the used method of processing [3]. At the end of fermentation, tested juices showed between 43% (tomato juice) and 56% (cabbage juice) of the original content of L-ascorbic acid.

Formation of lactic acid is considered to be the key factor of the preserving effect in lactic acid fermentation [18]. Fig. 2 shows production of lactic acid during the fermentation of juices. At the end of the fermentation, the juices contained between 15.19 g.dm^{-3} (pumpkin juice) and 18.10 g.dm^{-3} (cabbage juice) of lactic acid. All tested juices thus proved to be a suitable substrate for the production by lactic acid fermenting bacteria.

There are controversies in the literature as to the effects of acetic acid concentrations upon the quality of fermented products. Absence of acetic acid in products is known to give the products unilaterally sour taste, with a flat and atypical aroma. On the other hand, acetic acid has a favourable effect on preservation [19]. In our experiments, the fermented juices contained at the end of the fermentation between 0.23 g.dm^{-3} (courgette juice) and 1.64 g.dm^{-3} (cabbage juice) of acetic acid. Production of this acid during fermentation of the different juices is shown in Fig. 3. Comparable concentrations of acetic acid formed in courgette and pumpkin juice. In these juices, citric acid was degraded during the fermentation, with values at the end of the process below the detection limit of the method used (1.6 g.dm^{-3}).

YOON, WOODAMS and HANG [20] reported pH reduction to 3.5 and titration acidity expressed in terms of lactic acid of 1.67% during 72-h fermentation of tomato juice by *Lactobacillus plantarum* C3. At 72 h of fermentation, tomato juice fermented by *Lactobacillus plantarum* CCM 7039 showed a pH value of 3.75 and titration acidity of 1.51% (titration acidity expressed as lactic acid).

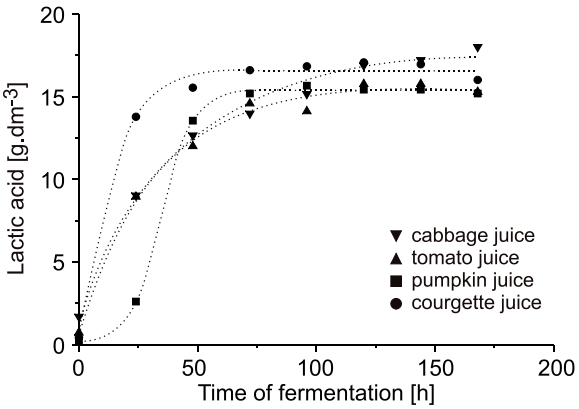

Fig. 2. Lactic acid production during fermentation of vegetable juices.

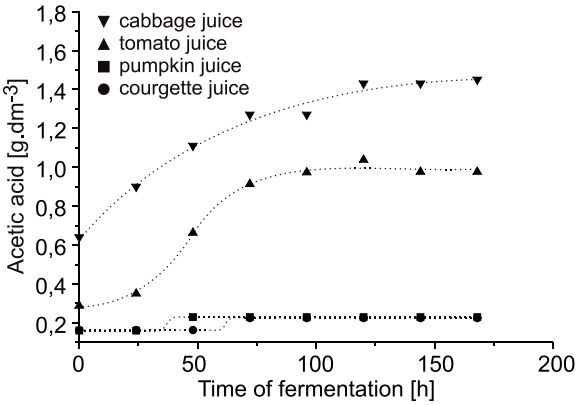

Fig. 3. Acetic acid production during fermentation of vegetable juices..

KUCHTA et al. [21] used *Lactobacillus plantarum* uh1Y to prepare pumpkin salad processed by lactic acid fermentation. After a 4 day fermentation, pumpkin contained 8.1 g.dm^{-3} of lactic and 2.3 g.dm^{-3} of acetic acid, pH of the medium was 2.8, and the medium contained 11.4 g.dm^{-3} of lactic and 1.4 g.dm^{-3} of acetic acid. After 4 days of fermentation, pumpkin juice fermented in our experiments had pH of 3.35, and its lactic acid and acetic acid concentration was 15.66 g.dm^{-3} and 0.23 g.dm^{-3}, respectively.

The sensory parameters assessed included: colour, turbidity, sediment, appearance, taste and flavour of juice, pleasantness of taste and flavour, and overall tastefulness of selected juices. Since the goal of the sensory assessment was to choose

Tab. 1. pH, titration acidity, lactic acid and acetic acid concentrations at 48 h or 72 h of fermentation.

Juice	pH	Titration acidity [%]	Lactic acid [g.dm^{-3}]	Acetic acid [g.dm^{-3}]
courgette (48 h)	3.60	1.49	15.54	0.16
pumpkin (72 h)	3.60	1.43	13.54	0.22
tomato (72 h)	3.75	1.51	14.58	0.91
cabbage (72 h)	3.60	1.42	13.97	1.27

Tab. 2. Flavour and taste pleasantness and overall tastefulness of the juices.

Juice	Pleasantness of flavour	Pleasantness of taste	Overall tastefulness
	[% of scale]		
cabbage	83.5	89.3	91.5
courgette	83	84.5	85
pumpkin	71	73	75
tomato	81	80	80

the product, which might be the most acceptable for the consumer, the key parameter of assessment was harmonic taste representing an optimal consonance of all taste descriptors. The highest intensities of harmonic taste were reached after 72 h (cabbage and tomato juice) or 48 h of fermentation (courgette and pumpkin juice). By their harmonic taste, the juices tested ranked as follows: cabbage juice (93.2% of the scale), courgette juice (92% of the scale), tomato juice (79% of the scale), and pumpkin juice (78% of the scale).

After 72 h of fermentation, cabbage juice was slightly turbid and its colour was creamy-orange-brown with a green tint. Courgette juice was moderately to strongly turbid after 48 h, and its colour was bright green with a creamy tint. Tomato juice was moderately to strongly turbid after 72 h of fermentation, and its colour was bright red. Pumpkin juice was slightly turbid after 48 h of fermentation, and its colour was bright yellow with a creamy tint. There was sediment in all the juices.

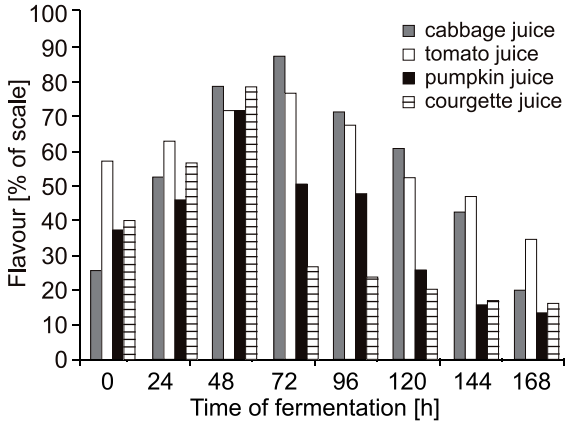

Fig. 4. Relationship between overall tastefulness of vegetable juices and fermentation time.

Lower than 6.3 pH values of vegetable juices processed by lactic acid fermentation are not desirable from the sensory point of view [22]. The pH values measured at maximal intensity of harmonic taste for the juices tested in our experiments did not exceed the above limit (Table 1).

The time of reaching the highest intensities of pleasantness of taste, flavour and overall tastefulness of the different juices (Fig. 4) coincided with the time of reaching the highest intensities of harmonic taste (after 72 h of fermentation for cabbage and tomato juice, after 48 h for courgette and pumpkin juice; Table 2). The lowest intensity of the above descriptors was found for pumpkin juice; this juice moreover presented a rather flat flavour. Consequently, such kind of fermented juice requires addition of spices to enhance the flavour.

CONCLUSION

Different types of vegetables (cabbage, tomatoes, pumpkin and courgette) were tested for suitability to be used for the preparation of vegetable juices processed by lactic acid fermentation. From the point of view of the production of lactic acid and adequate pH reduction (from between

6.15–6.5 to between 3.35–3.8) during the fermentation process, all tested vegetable juices have proven to be suitable substrates for lactic acid fermentation. After being fermented for 158 hours, the juices contained between 15.19 $g.dm^{-3}$ and 18.10 $g.dm^{-3}$ of lactic acid. As compared to the cabbage juice, much less acetic acid formed in pumpkin and courgette juice.

The key parameters in assessing sensory acceptability of the end product included harmonic taste, pleasantness of flavour and taste, and overall tastefulness. The highest intensities of the parameters mentioned were reached at 48 hours (pumpkin and courgette juice) or 72 hours of fermentation (cabbage and tomato juice). At these intervals, the pH values of the juices were sufficiently low (3.6 to 3.75) to provide for a good preservation effect.

The cabbage, courgette and tomato juices were of pleasantly sour taste and of a flavour typical of vegetable products processed by lactic acid fermentation. Compared with other juices, the flavour and sour taste of the pumpkin juice was less pronounced.

Based on the results of our studies, it is mainly cabbage and courgette juice that can be recommended to be used for preparation of vegetable-based products processed by lactic acid fermentation.

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