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Contribution of lactic acid fermentation to improved nutritive quality vegetable juices enriched with brewer's yeast autolysate

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Abstract

Vegetables are rich sources of the biologically active compounds which have beneficial effects in prevention of some diseases and certain types of cancer. From the point of view of protection and health, the objective of this paper was to optimize food content in order to obtain a functional food. In order to improve the nutritive and protective properties of the product, the beetroot and carrot juices enriched with brewer's yeast autolysate were subjected to lactic-acid fermentation with *Lactobacillus acidophilus* NCDO1748.

Chemical compositions of produced fermented bioproducts showed that fermented carrot juice with brewer's yeast autolysate had higher contents of some minerals (Ca, P, Fe) and β-carotene than had beetroot juice with brewer's yeast autolysate. Higher mineral content in the carrot juice better affected production of lactic acid in that sample. Fermented beetroot juice with brewer's yeast autolysate had higher contents of betanin and vitamin C, which were in accordance with the contents of these components in raw beetroot that did not significantly vary during the processing of the material (pasteurization, fermentation). Thus the fermented bioproduct 3, which is a mixture of beetroot and carrot juices with brewer's yeast autolysate, represents the product with optimum proportions of pigments, vitamins and minerals.

Keywords: Fermented juice; Beetroot; Carrot; Brewer's yeast autolysate; Chemical composition

1. Introduction

Besides numerous natural nutrients present in food that positively affect human health, of particular interest are phytonutrients from vegetables (Kanner, Harel, & Granit, 2001). Many investigations have confirmed the antitumorous effects of vegetable extracts, as well as their utilization as colorants in food and pharmaceutical products (Kapaida, Tokuda, Konoshima, & Nishino, 1996; Norrish, Jackson, Sharpe, & Skeaff, 2000; Pedreno & Escribano, 2001; Slattery et al., 2000).

Thanks to the presence of carbohydrates, vegetable juices are suitable substrates for lactic-acid fermentation. Through fermentation, these juices are preserved and protective and nutritive properties of the products are improved (Campbell Platt, 1994). Lactic-acid bacteria synthesis vitamins (Crittenden, Martinez, & Playne, 2003) and antimicrobials (Cleveland, Montville, Nes, & Chikindas, 2001) and increase their contents in fermented products.

In this paper, through realization of one of the principles in production of functional food, vegetable juices have been enriched with brewer's yeast autolysate before lactic-acid fermentation. The addition of autolysate favourably affects the increase of the number of lactic-acid bacteria during fermentation (Aeschlimann & von Stocar, 1990; Rakin, Baras, & Vukasinovic, 2004), reduction of time of fermentation and enrichment of vegetable juices with amino acids, vitamins, minerals and antioxidants (Chae, Joo, & In, 2001; Dziezak, 1987). In this work, we used spent brewer's yeast from a brewery, which is important for economic optimization of this fermentation.

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2. Materials and methods

2.1. Substrate for fermentation

Beetroot and carrot were purchased from a local store in Belgrade. The juices of beetroot and carrot were prepared by slicing the fresh vegetables, followed by separation of the juice in a juice maker. Subsequently, the juices were pasteurized at 70 °C for 20 min. Brewer's yeast was collected from Belgrade Beer Industry (BIP-Belgrade). Brewer's yeast was first debittered with 4% NaCl, washed and then autolysed at 55 °C for 3 h, and finally poured into the pasteurized vegetable juices. The juices, enriched with brewer's yeast autolysate, were inoculated with 2% of 24-h old inoculum of isolated *Lactobacillus acidophilus* NCDO1748 that was prepared in MRS broth at of 37 °C and under anaerobic conditions. Initial number of bacteria in inoculated juices was between 10^5 and 10^7 CFU/ml, depending on the substrate. The temperature of fermentation was 370 °C, while the duration of fermentation was 8 h. By the lactic acid fermentation the following products were obtained:

Biofermented product 1 – beetroot juice and brewer's yeast autolysate in a 1:1 ratio, calculated on the dry matter.

Biofermented product 2 – carrot juice and brewer's yeast autolysate in a 1:1 ratio, calculated on the dry matter.

Biofermented product 3 – mixture of carrot and beetroot juices and brewer's yeast autolysate in 0.5:0.5:1 ratio, calculated on the dry matter.

2.2. Analysis

The progress of fermentation was monitored by determining total number of cells on a MRS agar plate, using the standard method of decimal dilution (Collins & Lynne, 1985). Production of lactic acid was determined by potentiometer (Herbert, Phipps, & Strange, 1971).

The samples were analyzed for moisture, ash, crude protein, lipid and carbohydrate using methods of the Association of Official Analytical Chemists (AOAC, 1990). The content of betanin was determined spectrophotometrically, following the method of Nillson (1970). The vitamins were determined by a reverse-phase HPLC technique (AOAC, 1995). Minerals (calcium, magnesium, iron and phosphorus) were determined using atomic-absorption spectrophotometry (AOAC, 1975). Sodium and potassium were determined using the flame photometric method (AOAC, 1975).

Lactic acid fermentation was carried out thrice, with variation below 5%, and all analyses were done in triplicate.

3. Results and discussion

The content of lactic acid at the end of fermentation, initial number of lactic-acid bacteria and the number of lactic-acid bacteria at the end of fermentation are shown in Table 1. Upon completion of fermentation, production of lactic acid was more intensive in fermented bioproducts with carrot juice. In addition to the increase of nutritive value, produced lactic acid contributes to better taste and structure of fermented bioproducts. A recent study of a group of the authors has shown that the lactic-acid fermentation is improved by addition of the carrot juice, due to high contents of minerals (Bergquist, Sandberg, Carlsson, & Andlid, 2005). The increase of the number of lactic-acid bacteria in tested products was best in the beetroot juice, fermented in the bioproduct 1. The addition of brewer's yeast autolysate stimulated the increase of the number of lactic-acid bacteria and production of lactic acid in comparison with fermentation of the beetroot juice without brewer's yeast autolysate, lasting for 48–72 h (Yoon, Woodams, & Hang, 2005). These products contain satisfactory number of active cells of lactic-acid bacteria, being in mind that minimum therapeutic daily dose usually ranges from 10^8 to 10^9 of viable cells (Rasic & Kurmann, 1983).

Table 1
Content of lactic acid and number of bacterial cells in products before and after fermentation with *Lactobacillus acidophilus* NCDO1748

Substrate	Lactic acid (g/l)	Initial number of bacterial cells (CFU/ml)	Number of bacterial cells after fermentation (CFU/ml)
Substrate 1	3.42 ± 0.04	$1.0 \times 10^7 \pm 0.2 \times 10^7$	$7.0 \times 10^8 \pm 1.2 \times 10^8$
Substrate 2	4.61 ± 0.03	$6.5 \times 10^6 \pm 0.4 \times 10^6$	$9.2 \times 10^7 \pm 0.6 \times 10^7$
Substrate 3	4.80 ± 0.03	$7.0 \times 10^5 \pm 0.6 \times 10^5$	$2.95 \times 10^7 \pm 0.8 \times 10^7$

The values given above are means of three determinations ±SD.

The basic chemical analysis of fermented bioproducts is given in Table 2. 30–40% of the total dry matter consists of proteins and 40% carbohydrates. The results show that there is a certain difference between the fermented bioproducts. The fermented carrot juice contains more carbohydrates and ash than does the beetroot juice, while the content of proteins is slightly higher in the fermented bioproduct based on beetroot juice (15%).

The contents of minerals in fermented bioproducts are given in Table 3. The results show that the carrot juice

Table 2
The chemical compositions of the fermented bioproducts based on vegetable juice and brewer's yeast autolysate

Chemical characteristics	Fermented bioproduct 1	Fermented bioproduct 2	Fermented bioproduct 3
Moisture (g/100 ml)	90.06 ± 0.08	89.97 ± 0.09	90.02 ± 0.08
Proteins (g/100 ml)	4.05 ± 0.03	3.42 ± 0.04	3.88 ± 0.06
Carbohydrates (g/100 ml)	4.13 ± 0.07	4.25 ± 0.05	4.18 ± 0.05
Lipids (g/100 ml)	1.32 ± 0.03	1.45 ± 0.02	1.39 ± 0.04
Ash (g/100 ml)	0.33 ± 0.02	0.47 ± 0.02	0.43 ± 0.01

Fermented bioproduct 1 – fermented beetroot juice and brewer's yeast autolysate in the ratio 1:1.
Fermented bioproduct 2 – fermented carrot juice and brewer's yeast autolysate in the ratio 1:1.
Fermented bioproduct 3 – fermented mixture of carrot and beetroot juice and brewer's yeast autolysate in the ratio 0.5:0.5:1.
The values given above are means of three determinations ±SD.

Table 3
The contents of minerals in the fermented vegetable juices and brewer's yeast autolysate

Minerals	Fermented product 1	Fermented product 2	Fermented product 3
Ca (g/l)	0.180 ± 0.01	0.580 ± 0.03	0.310 ± 0.01
Mg (g/l)	0.236 ± 0.02	0.270 ± 0.02	0.241 ± 0.03
Na (g/l)	0.625 ± 0.04	0.500 ± 0.04	0.625 ± 0.04
K (g/l)	1.88 ± 0.06	1.72 ± 0.05	1.85 ± 0.07
Fe (mg/l)	7.22 ± 0.03	9.50 ± 0.04	8.36 ± 0.04
P (g/l)	0.595 ± 0.03	0.952 ± 0.04	0.714 ± 0.03

Fermented bioproduct 1 – fermented beetroot juice and brewer's yeast autolysate in the ratio 1:1.
Fermented bioproduct 2 – fermented carrot juice and brewer's yeast autolysate in the ratio 1:1.
Fermented bioproduct 3 – fermented mixture of carrot and beetroot juice brewer's yeast autolysate in the ratio 0.5:0.5:1.
The values given above are means of three determinations ±SD.

and brewer's yeast autolysate are richer in Ca, Fe, P. All the samples have a favourable ratio of K and Na (approx. 3–3.5:1). The contents of K, Na and Fe are satisfactory in all fermented bioproducts. The content of Mg in all bioproducts satisfies 80% of recommended daily intakes, while Ca satisfies 80% of the same only in product 2 (FAO, 2002). The content of P is satisfactory, and, in bioproduct 2, it satisfies recommended daily intakes.

The contents of betanin and vitamins are shown in Table 4. The content of betanin is naturally highest in the beetroot juice-based bioproduct. Betanin is the most important beetroot pigment that, as proven by investigations of many authors, has antitumorous effects (Kapaida et al., 2003; Patkai, Barta, & Varsanyi, 1997). Its content is satisfactory, as it is known that the values for betanin in beetroot extracts range from 0.3% to 1% (Adams, von Elbe, & Amundson, 1976) and that betanin is highly sensitive to temperature (Attoe & von Elbe, 1981). The results show that the contents of tested vitamins in the bioproducts were satisfactory. The contents of vitamin C, thiamine, riboflavin in analyzed products can satisfy recommended daily intakes of an adult (FAO, 2002). Vitamin C is the major water-soluble antioxidant and acts as a radical scavenger. The intake of fresh vegetables, containing vitamin C along with many other vitamins and micronutrients, can reduce incidence of various cancers (Kakizoe, 2003). Vitamin C in these products mainly originates from vegetables. Its content is satisfactory in fermented bioproducts (82.5–103 mg/l). Vitamin C content was higher in juice with beetroot than with carrot juice, and corresponds to the content of vitamin C in fresh beetroot. The content of vitamins of the B group in fermented bioproducts mainly originates from the brewer's yeast, as their presence in fresh beetroot and carrot is negligible. Lower values were observed for the content of pyridoxine, the values being 10–20 times lower than the content in the extract of brewer's yeast. Therefore, these products should be enriched with pyridoxine, as vitamins from the B group manifest their effects only if they are quantity-balanced. The content of β-carotene is highest in carrot juice and brewer's yeast autolysate (fermented bioproduct 2) and was 3.6 mg/l. Thus the fermented products 2 and 3 satisfy recommended daily intakes of 2 mg. The products obtained in this work can be used as functional additives of beverages after pasteurization, or else may be dried and used in the form of powder or tablets. Our previous investigations showed that similar products could be obtained using the technique of fluidized bed drying, when about 30% of bacterial cells of genera Lactobacillus and Bifidobacterium survive (Dimitrijevic-Brankovic & Baras, 2001).

Table 4
The contents of betanin and vitamins in the fermented vegetable juices and brewer's yeast autolysate

Vitamins/pigments	Fermented product 1	Fermented product 2	Fermented product 3
Betanin (%)	0.384 ± 0.05	0.095 ± 0.02	0.227 ± 0.04
Vitamin C (mg/l)	103 ± 1.05	82.5 ± 0.92	91.6 ± 0.95
Vitamin B_1 (mg/l)	1.77 ± 0.09	1.70 ± 0.08	1.66 ± 0.08
Vitamin B_2 (mg/ml)	1.84 ± 0.02	2.12 ± 0.02	2.12 ± 0.03
Vitamin B_6 (mg/l)	0.26 ± 0.02	0.29 ± 0.01	0.28 ± 0.02
β-Carotene (mg/l)	1.30 ± 0.03	3.60 ± 0.05	2.60 ± 0.05

Fermented bioproduct 1 – fermented beetroot juice and brewer's yeast autolysate in the ratio 1:1.
Fermented bioproduct 2 – fermented carrot juice and brewer's yeast autolysate in the ratio 1:1.
Fermented bioproduct 3 – fermented mixture of carrot and beetroot juice brewer's yeast autolysate in the ratio 0.5:0.5:1.
The values given above are means of three determinations ±SD.

4. Conclusions

The fermentation of beetroot and carrot juices, with addition of brewer's yeast autolysate, was carried out in order to improve their nutritive values. Brewer's yeast autolysate contributes to the increase of the number of viable cells of lactic-acid bacteria during the fermentation and to better production of lactic acid. In addition, the fermented products also have high contents of minerals, vitamins and pigments, in accordance with the contents of these components in the initial raw material. Especially, a mixture of beetroot and carrot juices with brewer's yeast autolysate (fermented bioproduct 3) has optimum proportions of pigments, vitamins and minerals. This balanced material represents a valuable product as far as nutrition and health are concerned.

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