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FERMENTED MULTI-VEGETABLE JUICES SUPPLEMENTED WITH *PROPIONIBACTERIUM* CELL BIOMASS

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Pasteurized multi-vegetable juices were fermented using the inoculum with the following composition: *L. plantarum, L. brevis* and *B. bifidum*. After the fermentation, centrifuged cells of *P. acidipropionici* and *P. jensenii* were added to juices at a dose of 10^9 cfu/cm^3 of juice. The juices were stored at a temperature of 5°C for 21 days, with periodical controls of the cell count of introduced bacteria, and subjected to a sensory analysis. In fresh juices, the numbers of all genera of lactic and propionic fermentation bacteria were similar (*ca*. 10^9 cfu/cm^3) and did not change until day 10 of storage. Deterioration of taste was proceeding gradually in consecutive days of storage and on the 21st day of storage the juices were rated 2.5-3.61, on average, in a five-point scale, Survivability of bacteria introduced into juices was good and their population numbers in the 21st day of storage were found to range from $3.9x10^7$ to $6.8x10^8$ cfu/cm^3. The inoculated biomass of *Propionibacterium* cells delivered vitamin B_{12} whose content in all fresh juices was alike and accounted for 8.62-8.71 μg/100cm^3, and was not changing over the storage period.

ITRODUCTION

Bacteria of the genus *Propionibacterium* deserve attention of food technologists and producers of novel, health-promoting food. They can serve beneficial functions as a supplement enriching the nutritive value and, simultaneously, as a type of a bio-preservative with antagonistic activity against a variety of bacteria and fungi. Those bacteria can be applied both in functional products addressed to a target consumer as well as in low-processed products [Świderski, 2003; Cieślak & Forkiewicz, 2001; Cygan *et al.*, 2003]. So far, the propionic bacteria have not been used in the production of foodstuffs with a short productive cycle. They are, usually, characterized by high susceptibility to acidic reaction, they proliferate relatively slowly, and medium acidification, *e.g.* by lactic fermentation bacteria, strongly inhibits their metabolism. Positive results were achieved while applying them in vegetable pickles, products with a long productive cycle [Warmińska-Radyko & Łaniewska-Moroz, 1999; Babuchowski *et al.*, 1999]. Relations between *Propionibacterium* and bacteria belonging to *Lactobacillus* and *Bifidobactreium* genera are diversified depending on strains. Selected strains of propionic bacteria have been found to exert a stimulating effect on the growth of *Bifidobactreium* [Kaneko *et al.*, 1994; Warmińska-Radyko *et al.*, 2002]. A number of *Propionibacterium* strains produce bacteriocins with antibacterial activity against Gram-negative and Gram-positive bacteria [Holo *et al.*, 2002]. Metabolites of propionic bacteria – volatile acids – are characterized by fungicidal activity mainly against moulds and, to a lesser extent, against yeast [Lind *et al.*, 2005]. A great interest is also aroused by probiotic properties of *Propionibacterium* bacteria. Studies carried out recently have pointed to the possibility of survival as well as colonization of the gastrointestinal tract of humans by those bacteria [Zarate *et al.*, 2002; Leverrier *et al.*, 2003; Huang & Adams, 2004]. Some strains are known to be capable of adapting in the gastrointestinal tract of animals, which has a beneficial effect on the general health condition of animals [Huang *et al.*, 2003, 2004; Mantre-Alhonen, 1995]. Strains of Propionibacterium are the most active producers of vitamin B_{12}. In specially selected culture media, they may synthesize and accumulate that vitamin in cells in high concentrations, over 20 mg/dm^3 of the medium [Schneider *et al.*, 1995; Piao *et al.*, 2004]. Vitamin B_{12} does not occur or occurs only in trace amounts in plants and is deficient in the case of a vegetarian diet.

The study was aimed at evaluating a health-promoting, fermented multi-vegetable juice enriched with vitamin B_{12} through the addition of *Propionibacterium* cell biomass during long-term storage.

MATERIALS AND METHODS

Juices were prepared from fresh cucumbers, sugar beets, celery and parsley. The juices were pasteurized at a temperature of 80°C/10 min, next mixed in appropriate proportions, thus obtaining 3 following compositions: juice No. 1 – 70% of sugar beet juice, 20% of fresh cucumber juice and 10% of celery juice; juice No. 2 – 70% of sugar beet juice, 20% of fresh cucumber juice and 10% of parsley juice; and juice No. 3 – 70% of sugar beet juice and 30% of fresh cucumber juice.

The juices were inoculated by introducing 3% of basic inoculum with the following composition: *Lactobacillus plantarum* 6M, *Lactobacillus brevis* 2M, and *Bifidobacterium*

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bifidum 557, at a 2:1:1 ratio. Fermentation was run for 24 h at a temperature of 30°C. After the fermentation, 10% (v/v) biomass of *P. acidipropionici* 117 and *P. jensenii* 128 strains suspended in a physiological saline was added to particular juices. The juices were kept at a temperature of 5°C. Numbers of particular genera of bacteria were analysed after 1, 3, 7, 10, 14 and 21 days of storage: *Lactobacillus* on MRS culture medium (Merck), *Bifidobacterium* on Garche's medium, and *Propionibacterium* on lactate medium. Simultaneously, pH was controlled and a sensory analysis of fermented juices was carried out using a 5-point scale according to Baryłko-Pikielna [1975].

Fresh juices and juices after 21 days of cold storage were determined for the content of vitamin B_{12} using the method of high-performance liquid chromatography according to Hiroshi & Ichiro [1997], applying an HP 1050 chromatograph on a LiChrosper column RP-18e 250x4.6 mm.

RESULTS AND DISCUSSION

The inoculum delivered into particular juices from 4.5×10^7 to 2.1×10^8 cfu/cm^3 of *Lactobacillus* and from 7.7×10^7 to 2.2×10^8 cfu/cm^3 of *Bifidobacterium* rods. After 24-h fermentation, the cell count of both genera of lactic fermentation bacteria increased over hundredfold and reached 3.25–3.8×10^9 cfu/cm^3 (Figures 1–3).

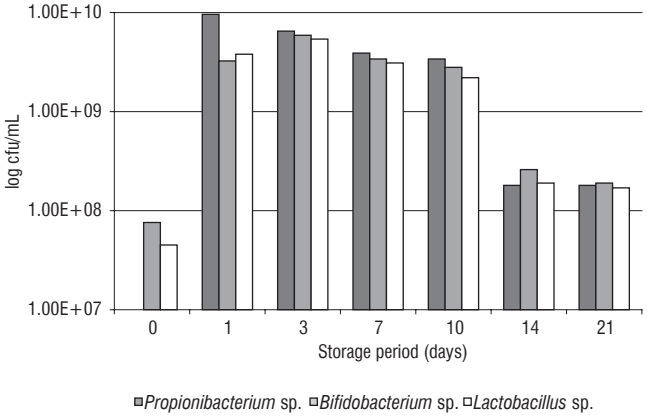

FIGURE 1. Changes in the number of introduced lactic fermentation bacteria during storage od fermented tree-component juice No. 1.

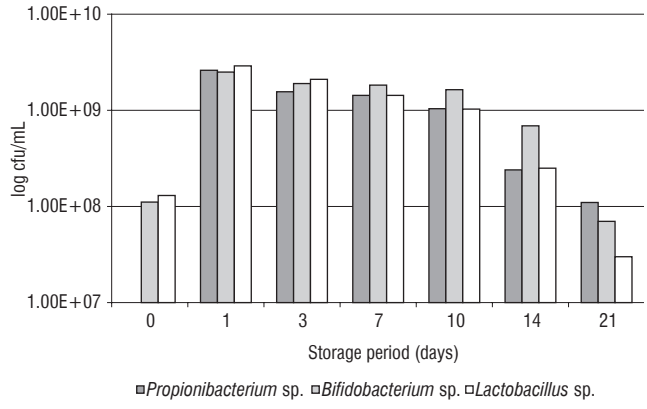

FIGURE 2. Changes in the number of introduced lactic fermentation bacteria during storage od fermented tree-component juice No. 2.

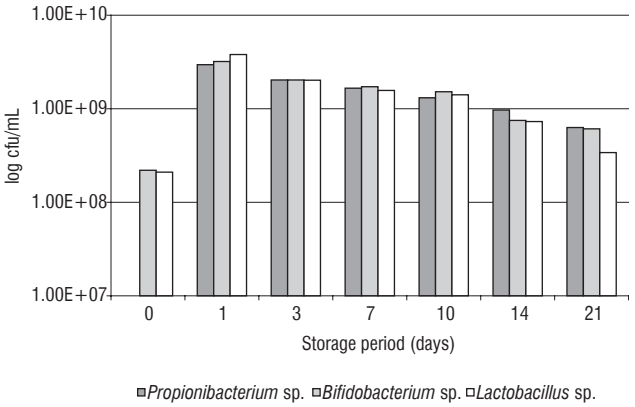

FIGURE 3. Changes in the number of introduced lactic fermentation bacteria during storage od fermented two-component juice No. 3.

The growth of particular bacteria proceeded alike, similar was also the acid-forming activity in three juices differing in composition. It was indicated by both changes in juices pH after the fermentation as well as gains in population numbers of the bacteria introduced. The reaction of juices measured before the fermentation accounted for pH 5.86–6.0 and after the 24-h fermentation changed to pH 3.86–3.90, which indicates a good acid-forming activity of inoculum bacteria. The acidity of juices obtained remained at the same level over the entire storage period, which was stated based on pH measurements. The introduced biomass of centrifuged cells of *Propionibacterium* contained $2.61–2.96\times10^9$ cfu/cm^3 juice of live cells, hence all genera of the bacteria occurred in the same numbers in the first day of storage. In the medium of fermented vegetable juices, all genera of the bacteria well survived storage at a temperature of 5°C. Numbers of their populations changed negligibly within the same log cycle in all juices up to the 10th day of storage (Figures 1–3). Since the 14th day, cell counts in all juices reached 10^8 cfu/cm^3 for all genera of the bacteria and such populations were remaining unchanged until day 21. Only in juice No. 2 was the number of *Lactobacillus* and *Bifidobacterium* bacteria lower on that day, around 10^7 cfu/cm^3 (Figure 2). Similar changes in bacterial counts during fermentation and storage of juices were observed by Babuchowski *et al.* [1999] and Yoon *et al.* [2005].

Fermented juices from sugar beet were investigated during 4-week storage by Yoon *et al.* [2005]. The authors evaluated the survivability of *Lactobacillus sp.* bacteria as good, since after such a long time of storage their number decreased by as little as from 1 to 3 log cycles and reached from 10^6 to 10^8 cfu/cm^3. It was shown that storage temperature of juices ranging from 4°C to 6°C was the most favorable as it facilitated the survivability of lactic fermentation bacteria and maintaining appropriate sensory attributes.

The sensory assessment of juices No. 1 and No. 2, carried out immediately after the fermentation and after the introduction of propionic bacteria biomass, demonstrated their similar quality. Both juices were evaluated with points whose averaged value for taste, aroma and colour accounted for 4.78 and 4.56, respectively (Tables 1 and 2). The two-component juice No. 3 immediately after the fermentation demonstrated taste with a distinct cucumber-like flavour, which was negatively associated with a typical beetroot colour, thus

TABLE 1. Sensory quality of juice No. 1 during cold storage.

Storage period (days)	Aroma	Taste	Colour	Mean score
	Units of a 5-point scale			
1	4.50	4.83	5.00	4.78
3	4.00	4.66	5.00	4.55
7	3.50	4.00	4.83	4.11
10	3.50	4.00	4.63	4.04
14	3.33	3.16	4.67	3.72
21	3.00	3.16	4.67	3.61

TABLE 2. Sensory quality of juice No. 2 during cold storage.

Storage period (days)	Aroma	Taste	Colour	Mean score
	Units of a 5-point scale			
1	4.17	4.50	5.00	4.56
3	4.00	4.33	5.00	4.44
7	4.00	4.17	4.83	4.33
10	3.93	4.17	4.67	4.22
14	3.50	3.66	4.67	3.94
21	3.17	2.67	2.83	2.89

TABLE 3. Sensory quality of juice No. 3 during cold storage.

Storage period (days)	Aroma	Taste	Colour	Mean score
	Units of a 5-point scale			
1	3.66	3.83	4.67	4.05
3	3.33	3.83	4.50	3.89
7	3.33	3.66	4.50	3.83
10	3.17	2.33	4.50	3.33
14	2.50	2.17	4.17	2.95
21	1.83	1.50	4.17	2.50

TABLE 4. Content of vitamin B_{12} in fresh juices and after 21 days of storage.

Juice No.	Vitamin B_{12} content (μg/100 cm^3)	
	Fresh juices	Stored jucies
1	8.81	8.62
2	8.76	8.69
3	8.79	8.71

it was rated a lower average score, 4.5 point (Table 3). That attribute appeared to intensify in the period of storage and that juice obtained the lowest scores, as little as 2.5 points on average in the 21st day of storage.

The colour of juices was the most stable attribute during storage and in all juices obtained the highest scores. According to Klewicka *et al.* [2004], a decrease in juice pH to 4.0 and less as a result of lactic fermentation permanently stabilizes colour as well as affects its partial reconstruction in the case of a change into brown as affected by thermal treatment.

The three-component juices No. 1 and 2 immediately after the fermentation were characterized by pleasant taste and aroma resulting from the contribution of parsley and celery which masked the taste of cucumber. During storage, negative taste and olfactory sensations were observed to intensify gradually. The sensory attributes of juices, their durability and changes during storage depend, to a great extent, on the inoculum applied, the appropriate selection of strains constituting it. Investigations of Klewicka *et al*. [2004] and those of Babuchowski *et al*. [1999] confirmed that the appropriate combination of strains applied to run fermentation of vegetable raw materials guarantees achieving desirable sensory traits.

All juices were also determined for the content of vitamin B_{12}. The introduction of *Propionibacterium* cell biomass enriched the multi-vegetable juices with that vitamin, usually deficient in vegetable products (Table 4). Analyses were carried out two times: immediately after the introduction of propionic bacteria biomass and on the 21st day of storage. When introducing equal volumes of the cell biomass into all juices examined, similar results were obtained, the concentration of vitamin B_{12} fluctuated between 8.6 and 8.8 μg/100 cm^3. The content o vitamin delivered with propionic bacteria cells remained almost unchanged during storage of juices. This points to the stability of that vitamin in juices with reaction around pH 3.80 and in the presence of considerable numbers of live cells of lactic fermentation bacteria and *Propionibacterium*. Investigations of Warmińska-Radyko & Łaniewska-Moroz [1999] demonstrated the possibility of enriching fermented cabbage with vitamin B_{12} through applying *Propionibacterium* inoculum. The growth of those bacteria in fermented vegetable mass proceeded very slowly and the content of vitamin was observed to increase negligibly to reach almost twofold higher concentration after 28 days, as compared to traditional pickled products. By appropriately adjusting active strains and by introducing biomass with an appropriate concentration of cells it is possible to enrich vegetable products with vitamin, lending them additional health-promoting properties.

CONCLUSIONS

1. The multi-vegetable juices subjected to fermentation by appropriately selected strains of lactic fermentation bacteria gain new sensory and nutritional values.

2. The concentrate of *Propionibacterium* cells containing strains, selected in terms of the activity towards biosynthesis of vitamin B_{12}, may be a natural method of enriching fermented vegetable juices with vitamins. The biomass of live and dead cells provided 8.7 μg of vitamin B_{12} /100 cm^3 of juice.

3. Immediately after the fermentation and supplementation with *Propionibacterium* bacteria, in the sensory analysis the juices obtained from 4.78 to 4.05 points depending on the composition of vegetable constituents.

4. Until day 10 of cold storage, sensory attributes of all juices were remaining unchanged or changed only to a negligible extent. The three-component juices No. 1 and No. 2 were characterised by more stable sensory attributes and obtained better scores during cold storage, as compared to the two-component juice No. 3.

5. The survivability of all strains used for the production of juices was evaluated as good, their population numbers remained at a level of 10^9 cfu/cm^3 up to the tenth day of storage, and after three weeks some live cells were still observed and their number ranged from 10^7 to 10^8 cfu/cm^3 of juice.

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FERMENTOWANE SOKI WIELOWARZYWNE SUPLEMENTOWANE BIOMASĄ KOMÓREK *PROPIONIBACTERIUM*

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Pasteryzowane soki wielowarzywne fermentowano stosując szczepionkę o składzie: *L. plantarum, L. brevis* i *B. bifidum*. Po fermentacji do soków wprowadzono odwirowane komórki *P. acidipropionici* i *P. jensenii* o liczebności 10^9 jtk/cm^3 soku. Soki przechowywano w temperaturze 5°C przez 21 dni okresowo kontrolując liczebność komórek wprowadzonych bakterii oraz dokonywano oceny sensorycznej. W świeżych sokach liczebność wszystkich rodzajów bakterii fermentacji mlekowej i propionowej była podobna w granicach 10^9 jtk/cm^3 i nie uległa zmianie do 10 dnia przechowywania (rys. 1–3). Cechy sensoryczne soków w tym okresie zmieniły się nieznacznie (tab. 1–3). Pogorszenie smaku następowało stopniowo w kolejnych dniach przechowywania i w 21 dniu soki oceniono średnio na 2,5–3,61 punktów w pięciopunktowej skali. Przeżywalność zastosowanych bakterii w fermentowanych sokach była dobra i liczebność w granicach $3{,}9\times10^7$–$6{,}8\times10^8$ jtk/cm^3 wszystkich rodzajów bakterii stwierdzono w 21 dniu przechowywania. Wprowadzona biomasa komórek *Propionibacterium* wniosła witaminę B_{12}, której zawartość we wszystkich świeżych sokach była podobna 8,62–8,71 μg/100 cm^3 i w czasie przechowywania soków nie zmieniła się (tab. 4).