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FRUIT AND VEGETABLE JUICE FERMENTATION WITH BIFIDOBACTERIA

P. Havas[a]*, Sz. Kun[a], G. Styevkó[a], V. Slačanac[b], J. Hardi[b] and J. Rezessy-Szabó[a]

[a]Brewing and Distilling Department, Faculty of Food Science, Corvinus University of Budapest, H-1118 Budapest, Ménesi út 45. Hungary
[b]Faculty of Food Technology, J.J. Strossmayer University, Franje Kuhača 18, 31 000 Osijek. Croatia

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Consumers are becoming more interested in healthy nutrition. To meet consumer requirements, the possibility of the fruit and vegetable juice fermentation by bifidobacteria was investigated. Sour cherry, orange, carrot, and tomato juice was fermented with five *Bifidobacterium* strains (from human origin and starter culture). The tested strains have grown well in orange, carrot, and tomato juices. The *B. longum* Bb-46 strain demonstrated the best growth activities. It was found that ratio of the produced acetic and lactic acids are dependent on the *Bifidobacterium* strain rather than on the fermentation medium. The most intensive inhibition was observed against the *Campylobacter jejuni* strain. In course of the fermentation the antioxidant capacities slightly decreased, except when the orange juice was fermented with *B. lactis* Bb-12 and *B. longum* A4.8. The obtained results may contribute to the design of a novel functional food product.

Keywords: *Bifidobacterium*, vegetable, fruit, antimicrobial effect

Activity of microorganisms is utilized to produce food and beverages for thousands of years. Food produced with spontaneous fermentation forms as a result of the activity of a complex microbiota. Due to the development of food and nutrition science, the potential probiotic strains have got great importance in the selection of industrial microorganisms, to be used in food processing. The most widely used probiotics are found among lactic acid bacteria (mostly lactobacilli and bifidobacteria) (Scardovi, 1981). The probiotic foods are a large segment of functional food products. Most probiotic preparations on the market are dairy products (based on milk and its derivates). The demand of consumers for non-dairy based probiotic products, like fermented soy-based products, vegetable and fruit juices, is increasing in recent years. Vegetables, fruit, and their juices have several beneficial effects for human health, because they are rich in antioxidants, vitamins, fibres, and minerals, and are consumed by a large percentage of the global population. Vegetable and fruit juices may also serve as media for cultivating probiotics. The bioactive components may be absorbed even better from juices than from plant tissues (Brandt et al., 2004; Luckow & Delahunty, 2004). The carrot is an excellent source of potassium and β-carotene, which is regarded as a provitamin for vitamin A; it contains vitamin C, vitamin B_6, thiamine, folic acid, and magnesium. Among the carotenoids, the β-carotene (2–10 mg/100 g) is the most important, and it gives carrots their distinctive orange colour (Herrmann, 2001). The tomato is the richest source of

* To whom correspondence should be addressed.
Phone:+36-1-482-6027; fax: +36-1-482-6558; e-mail: havas.bt@gmail.com

lycopene in the diet. Lycopene can act as antioxidant, enhance cell-to-cell communication, and modulate cell-cycle progression. Tomato also contains folate, vitamins C, A, and E, potassium, various other carotenoids, and phytochemicals, such as polyphenols (quercetin, naringenin). Orange is a very good source of vitamins, especially vitamin C. Oranges also contain a sufficient amount vitamin A, other flavonoid antioxidants (α and β-carotenes, β-cryptoxanthin, zeaxanthin, and lutein), B-complex vitamins (thiamin, pyridoxine, folates), potassium, and calcium. The sour cherry is rich in anthocyanins, vitamins C, A, B, and in calcium, potassium, magnesium, iron, and zinc. The anthocyanins and cyanidin isolated from sour cherries had antioxidant and anti-inflammatory properties (Pennington, 2002; Eskin & Tamir, 2006).

Protective and nutritive properties of these juices or drinks are improved through fermentation with probiotic bacteria (Saarela et al., 2011). Probiotic lactic acid bacteria, *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* species have health-promoting properties by maintaining an improved intestinal bacterial composition, stimulating the immune response, having antimutagenic effect, protecting against infection, etc. (Kaur et al., 2002; Mercenier et al., 2002). Among starter cultures, *Bifidobacterium lactis* Bb-12 (Chr. Hansen) is widely used in the dairy industry, because this strain has numerous physiological and technological advantages. In human studies, *B. lactis* Bb-12 has shown efficacy in prevention of traveller's diarrhoea, treatment of rotavirus diarrhoea, modulation of intestinal microbiota, and alleviation of atopic dermatitis symptoms in children (Alander et al., 2001).

The goal of this study was to assess suitability of raw material from plant origin as a substrate for growth of selected probiotic bacteria, and to make trials for preparation of non-dairy products with these strains, because raw materials do not contain dairy allergens that might prevent usage by certain segment of the population (Netzel et al., 2002; Lavermicocca, 2006).

1. Materials and methods

1.1. Micro-organisms and their maintenance

The following *Bifidobacterium* and test strains were used in this study: *Bifidobacterium lactis* Bb-12 (Chr. Hansen, Denmark), *B. bifidum* B3.2 (human isolate, Mayer et al., 2003), *B. longum* A4.8 (human isolate, Mayer et al., 2003), *B. longum* Bb-46 (Chr. Hansen), *B. bifidum* NCFB (National Collection of Food Bacteria) 1454, and *Staphylococcus aureus, Salmonella enteritidis, Campylobacter jejuni, Candida albicans* (Institute of Public Health in Osijek: Collection of digestive and urinary pathogens).

Bifidobacteria were grown anaerobically (in Bugbox anaerobic chamber, Ruskin Technology) at 37 ºC for 48 h in TPY (Trypticase-Phytone-Yeast) medium (Mayer et al., 2003). Test strains were grown on Mueller-Hinton plates, incubated at 37 °C for 24 h before analysis (Slačanac et al., 2004).

Media and raw materials: Fermentation experiments were carried out in fruit (Happy Day Orange and Sour cherry by Rauch) and vegetable (Happy Day Tomato by Rauch and Naturpur Carrot by Spar) juices. The juices contained no preservatives and were purchased in retail store. The initial pH was set to 6.10–6.20.

1.2. Applied methods

1.2.1. Conditions of fermentation. Fermentation was initiated with defined concentration of the relevant *Bifidobacterium* strains. All trials were carried out under anaerobic conditions in Anaerobe Jar+GasPak System (OXOID) or in Bugbox anaerobic chamber at 37 °C. Fermentation was followed by counting the colony forming units (CFU) and measuring the pH. In some cases the spectrum of organic acids and the quantities of typical acids were also determined.

1.2.2. Determination of colony forming units. Beeren's agar was used to determine the concentration of Bifidobacteria (Beeren's, 1990). Serial tenfold dilutions were prepared from the ferment broth. The diluted samples were transferred into Petri dishes and mixed with the appropriate medium. The plates were incubated under anaerobic conditions in Anaerobe Jar+GasPak System or in Bugbox anaerobic chamber at 37 °C. The colonies were counted after 48 or 72 h incubation.

1.2.3. Determination of antagonistic activity. The measurements were done according to Tagg and McGiven (1971).

1.2.4. Determination of organic acids by HPLC. The samples were centrifuged at 14 000 r.p.m. for 10 min and the supernatants were filtered through 0.45 µm polyvinylidene difluoride membrane (Waters, Milford, MA, USA) before injection. Acetic and lactic acid contents were measured with HPLC equipment with the following technical data: Detector: PDA detector (210 nm); column: Aminex HPX-87H; mobile phase: 5 mM H_2SO_4; flow rate: 0.5 ml min^{-1}; column and detector temperature: 45 °C.

1.2.5. Determination of antioxidant capacity. The Blois's method was applied, which is based on the binding of DPPH radicals (Blois, 1958).

2. Results and discussion

2.1. Ability of growth in fruit and vegetable juice

Tested *Bifidobacterium* strains utilized sour cherry and orange juice well, but to varying degree. By the 24th hour of fermentation, the cell concentration increased two orders of magnitude, in some cases three orders of magnitude. The best growth ability was achieved by *B. longum* Bb-46 in orange juice, reaching 0.344 h^{-1} growth rate. Cell count increased three orders of magnitude: from the initial 6.2×10^6 CFU ml^{-1} to 1.8×10^9 CFU ml^{-1} by the end of the fermentation. The weakest growth activity was shown by the *B. bifidum* NCFB 1454 strain both in sour cherry and orange juice, which is well reflected in the growth rate values (0.108 h^{-1} and 0.14 h^{-1}). Comparing the changes of cell concentration in sour cherry and orange juice, it can be stated that the strains had shown better growth and metabolic activity in orange juice. It was demonstrated through the growth rate and generation time (Tables 1 and 2). Furthermore, the pH values confirmed, that initial pH values (pH=6.2) of the orange juice decreased to pH=4.15–4.73 after the 24th h of fermentation. In the sour cherry fermentation the pH value was reduced approximately to 5.0.

Table 1. Change of cell concentration, pH, growth rate, and generation time of *Bifidobacterium* strains in fermented sour cherry juice

Bifidobacterium strains	Cell concentration (log[CFU ml^{-1}])		pH		μ_{max} (h^{-1})	t_g (h)
	Initial	Final	Initial	Final		
A4.8	6.13±0.11	8.04±0.20	6.20	5.10	0.222	3.12
Bb-12	6.91±0.18	8.71±0.14	6.20	4.52	0.198	3.50
Bb-46	6.42±0.13	8.49±0.43	6.20	4.66	0.257	2.69
B3.2	6.92±0.21	8.59±0.29	6.20	5.14	0.167	4.15
NCFB1454	6.51±0.16	7.50±0.16	6.20	5.34	0.108	6.40

Values are averages of triplicate results ± standard deviation

Table 2. Change of cell concentration, pH, growth rate, and generation time of *Bifidobacterium* strains in fermented orange juice

Bifidobacterium strains	Cell concentration (log [CFU ml^{-1}])		pH		μ_{max} (h^{-1})	t_g (h)
	Initial	Final	Initial	Final		
A4.8	6.30±0.15	8.41±0.31	6.20	4.73	0.266	2.60
Bb-12	7.14±0.21	8.73±0.15	6.20	4.15	0.151	4.59
Bb-46	6.79±0.19	9.25±0.39	6.20	4.25	0.344	2.01
B3.2	7.20±0.44	9.51±0.17	6.20	4.28	0.309	2.24
NCFB1454	6.97±0.11	8.51±0.52	6.20	4.63	0.140	4.94

Values are averages of triplicate results ± standard deviation

To compare the growth of applied strains in different natural media, fermentations in vegetable juices were also performed. Results showed that vegetable juices provided better growth media for the tested strains. The best growth was shown by the *B. lactis* Bb-12, *B. longum* Bb-46, and *B. longum* A4.8 strains in carrot juice. The highest cell concentrations (8.12×10^8 and 1.25×10^9 CFU ml^{-1}) were detected in the 12 to 20 h range, and the growth rates were between 0.264 h^{-1} and 0.339 h^{-1} (Table 3). In tomato juice, the highest growth rate and viable cell number at the end of fermentation were found in samples fermented with *B. longum* Bb-46, *B. bifidum* B3.2, and *B. longum* A4.8 strains. The cell concentration of *B. longum* Bb-46 and *B. bifidum* B3.2 increased by about 3 log CFU ml^{-1} and the specific growth rate was 0.323 h^{-1} and 0.275 h^{-1} (Table 4.). KAMALY (1997) reported similar results in the case of *B. longum* and *B. bifidum* when it was grown in soy milk. The intensive metabolic activity was demonstrated by the evolution of pH values. The pH values were pH=4.31–4.92 in the 24th of fermentation both in carrot and in tomato juices.

Table 3. Change of cell concentration, pH, growth rate, and generation time of *Bifidobacterium* strains in fermented carrot juice

Bifidobacterium strains	Cell concentration (log[CFU ml^{-1}])		pH		μ_{max} (h^{-1})	t_g (h)
	Initial	Final	Initial	Final		
A4.8	6.43±0.20	8.94±0.27	6.10	4.39	0.339	2.04
Bb-12	6.43±0.09	8.64±0.16	6.10	4.64	0.264	2.63
Bb-46	6.55±0.16	8.82±0.47	6.10	4.64	0.264	2.63
B3.2	6.41±0.10	8.74±0.36	6.10	4.92	0.202	3.43
NCFB1454	6.69±0.12	8.17±0.34	6.10	4.64	0.200	3.47

Values are averages of triplicate results ± standard deviation.

Table 4. Change of cell concentration, pH, growth rate, and generation time of *Bifidobacterium* strains in fermented tomato juice

Bifidobacterium strains	Cell concentration (log[CFU ml^{-1}])		pH		μ_{max} (h^{-1})	t_g (h)
	Initial	Final	Initial	Final		
A4.8	6.96±0.11	8.71±0.27	6.10	4.60	0.371	1.87
Bb-12	6.35±0.10	8.56±0.15	6.10	4.39	0.151	4.59
Bb-46	6.94±0.29	9.17±0.48	6.10	4.31	0.323	2.15
B3.2	6.83±0.26	9.11±0.18	6.10	4.40	0.275	2.52
NCFB1454	6.55±0.37	7.81±0.46	6.10	4.56	0.234	2.96

Values are averages of triplicate results ± standard deviation.

2.2. Change of acetic and lactic acid contents

It is well known that bifidobacteria produce mainly acetate and lactate from carbohydrate catabolism by the bifidus pathway, yielding 2 mol of lactic acid and 3 mol of acetic acid per 2 mol of glucose in synthetic medium (Biavati et al., 2000). The production of these acids was determined to evaluate the metabolic activity of the applied strains. Table 5 shows the acetic acid to lactic acid ratio. *B. lactis* Bb-12 showed the lowest acetic to lactic acid ratios (0.05–0.88), while *B. bifidum* NCFB 1454 the highest ones (9.6–12.28). Based on these data it was found that ratio of the two acids are dependent on the *Bifidobacterium* strain rather than on the fermentation medium. Scalabrini and co-workers (1998) observed the same phenomenon when they fermented soy milk with 13 different *B. longum* and 4 different *B. bifidum* strains. According to several reports, it is desirable that fermented products contain low quantities of acetic acid, because of its disagreeable taste. The favourable acetic to lactic acid ratio that was produced by *B. lactis* Bb-12 in all juices of plant origin gives one more evidence of being one of the best industrial strains.

Table 5. Acetic acid to lactic acid ratio at the end of fermentation

Bifidobacterium strains	Acetic acid/Lactic acid rate			
	Orange	Sour cherry	Carrot	Tomato
A4.8	2.99	1.03	1.83	1.33
Bb-12	0.18	0.88	0.23	0.05
Bb-46	3.52	1.97	0.41	0.15
B3.2	1.81	1.36	2.77	0.13
NCFB1454	9.60	2.87	2.38	12.28

2.3. Inhibition effect of fermented vegetable and fruit juices

The antimicrobial effects of fruit and vegetable juices fermented by *Bifidobacterium* against entero- and uropathogenic microorganisms (*Staphylococcus aureus, Salmonella enteritidis, Campylobacter jejuni, Candida albicans*) were determined. They are sources of danger from the clinical aspect to the human body. Table 6 shows the antagonistic activity of control (unfermented) fruit and vegetable juices, juice with adjusted pH (6.2), and juices fermented with *Bifidobacterium* strains.

The most intensive inhibition was observed against the *Campylobacter jejuni* strain (Fig. 1). Probably due to acidity, natural orange juice exerted a strong inhibition effect, which was not reduced even after the fermentation with *B. bifidum* B3.2 strain. In case of the original sour cherry juice and the one fermented with *B. longum* A4.8 strain, a wide range of inhibition was observed, but in other cases the inhibition effect was reduced. The antagonistic effect of carrot juices fermented by *B. lactis* Bb-12 and *B. longum* A4.8 was increased compared to the control sample. These are certainly reassuring results. Another good result is that the tomato juices fermented by bifidobacteria showed increased inhibiting effect except in case of *B. bifidum* B3.2. Antagonistic activity of fermented orange-, carrot-, and tomato juices against *Salmonella enteritidis* was remarkable, whereas the raw material did not show such activity against the test strains. The improved inhibition effect was due to the metabolism of *Bifidobacterium* strains. The strongest inhibition against the *Salmonella* strain was obtained

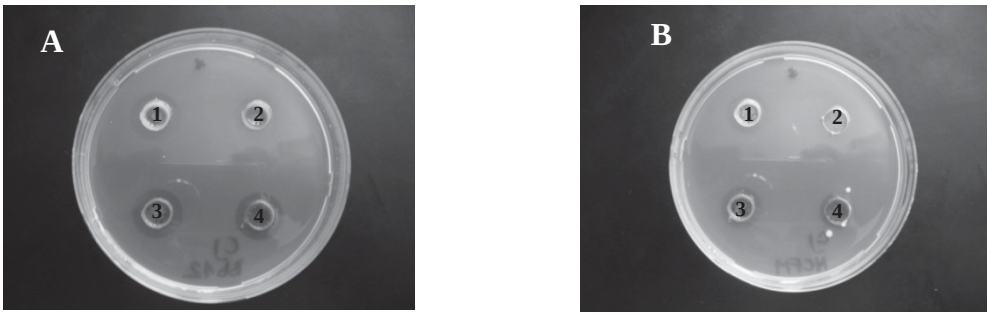

Fig. 1. Inhibition effect against *Campylobacter jejuni* strain of fermented orange (1), sour cherry (2), carrot (3), and tomato (4) juice by *B. lactis* Bb-12 (A) and *B. bifidum* NCFB 1454 (B) strains

using *B. lactis* Bb-12, *B. bifidum* B3.2, and *B. longum* Bb-46 for the fermentation. In case of *Staphylococcus aureus* test strain, only partial inhibition was detected. Against the *Staphylococcus* strain, clear inhibition was observed only in case of orange juice fermented by *B. lactis* Bb-12 and carrot juice fermented by *B. longum* Bb-46. *Candida albicans* was resistant against the samples. Table 6 summarizes the sensitivity that was shown by different test strains against the *Bifidobacterium* strains.

Table 6. Inhibition effect against test strains in control, adjusted pH (6.2), and fermented juices

	Sample	*Staphylococcus aureus*	*Salmonella enteritidis*	*Campylobacter jejuni*	*Candida albicans*
Orange juice	Raw material	±	–	+++	–
	Adjusted pH	±	–	+++	–
	Bb-12	++	++	+++	–
	B3.2	–	+	–	–
	NCFB 1454	±	±	+++	–
	Bb-46	+	++	+++	–
	A4.8	±	–	+++	–
Sour cherry juice	Raw material	++	±	+++	–
	Adjusted pH	±	±	±	–
	Bb-12	±	+	++	–
	B3.2	±	±	±	–
	NCFB 1454	±	–	±	–
	Bb-46	±	++	±	–
	A4.8	±	–	++	–
Carrot juice	Raw material	–	–	++	–
	Adjusted pH	±	–	–	–
	Bb-12	++	++	+++	–
	B3.2	+	++	+	–
	NCFB 1454	±	±	++	–
	Bb-46	++	++	++	–
	A4.8	±	±	+++	–
Tomato juice	Raw material	±	–	+	–
	Adjusted pH	±	–	–	–
	Bb-12	+	++	+++	–
	B3.2	±	++	±	–
	NCFB 1454	±	±	++	–
	Bb-46	±	++	++	–
	A4.8	±	±	++	–

–: no inhibition; ±: partial inhibition; +: difficult measured inhibition; ++ : clear, good measured inhibition (<15 mm); +++: wide zone of inhibition

2.4. Changes in antioxidant capacity of fermentation

Fruit and vegetable are rich in functional components such as minerals, vitamins, fibre, and antioxidant compounds. The selected fruits and vegetables are rich sources of flavonoid and carotenoid compounds that are bioactive compounds, so they have high antioxidant capacities. These arguments have led us to explore the extent to which antioxidant activity changes during the fermentation. In most cases – though slightly – the antioxidant capacity decreased during fermentation with bifidobacteria (Table 7). Exceptions were orange and carrot juices fermented with *B. lactis* Bb-12 and *B. longum* A4.8. The other exception was the tomato juice fermentation, because the antioxidant activities decreased only in case the juice was fermented by *B. lactis* Bb-12. This result is encouraging, because these compounds are able to maintain their functionality in the fermented juice as well.

Table 7. Changes in antioxidant potential of fermented juices

	Antioxidant potential (mg TE (trolox equivalent) ml^{-1})			
	Orange juice	Sour cherry juice	Carrot juice	Tomato juice
Initial value	2.512±0.070	4.675±0.133	1.125±0.980	1.086±0.308
A4.8	3.517±0.133	3.536±0.007	0.838±1.260	1.279±0.595
Bb-12	2.705±0.105	3.036±0.084	2.863±1.610	0.828±0.028
Bb-46	2.601±0.392	2.729±0.210	0.591±0.014	1.234±0.924
B3.2	2.650±0.154	3.106±0.182	0.987±0.126	1.774±0.077
NCFB1454	2.566±0.105	4.135±0.112	0.571±0.014	2.417±0.399

Values are averages of triplicate results ± standard deviation

3. Conclusions

Our studies were directed towards the application of fruit- and vegetable-based raw material to develop probiotic products. The orange, carrot, and tomato juices served as appropriate medium for cultivating bifidobacteria. The best growth was shown by *B. longum* Bb-46 in the fruit and vegetable juices. This strain is a dairy starter culture with good techno-functional properties. Against entero- and uropathogenic microorganisms inhibition was observed, which is considered a positive functional property of the applied strains. The antioxidant capacity of juices slightly changed during the fermentation. Based on our results, the development of probiotic fruit and vegetable juices may be prognosticated.

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