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Development of a Fructan-supplemented Synbiotic Cabbage Juice Beverage Fermented by *Bifidobacterium lactis* Bb12

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ABSTRACT

Since, prophylactic properties and status of healthy food is generally attributed to cabbage and probiotic *Bifidobacterium lactis* Bb12, scientific research is needed for simultaneous application of vegetable and fruit juices and probiotic cultures for development of novel functional drinks especially taking into consideration growing demand for non-dairy fermented beverages. There are few reports on fermentation of vegetable and fruit juices with bifidobacteria cultures and to our knowledge, this is first report on cabbage juice fermentation by bifidobacteria. The aim of this research was to develop novel functional beverage based on cabbage juice fermented with probiotic *B. lactis* Bb12. The probiotic *B. lactis* Bb12 grew well in cabbage juice at 25°C as the sole culture or in mixed starters, which is quite notable for bifidobacteria. Strains of bifidobacteria were relatively NaCl-tolerant at 5 g L^{-1}. The addition of fructan sources promoted *B. lactis* Bb12 survival in fermented cabbage juice during storage at 4°C for 2 weeks. The survival of *B. lactis* Bb12 was dependent upon the molecular weight of the fructan source and contributed to the extension of the shelf life of the fermented cabbage juice. In this study, it was shown that *B. lactis* Bb12 grows well in cabbage juice, achieving a concentration of 10^6 CFU mL^{-1}, the minimum required concentration for probiotic efficacy. It was stated that combination of probiotic strain *B. lactis* Bb12 and fructan additives is a prospective approach for cabbage juice-based fermented functional synbiotic beverage development.

Key words: Cabbage juice, probiotics, prebiotics, *Bifidobacterium lactis* Bb12

INTRODUCTION

Probiotic products are usually offered to consumers in the form of fermented milks or dietary supplements. However, with the increased prevalence of vegetarianism in developed countries and plant-based foods traditionally contributing the core of the daily food intake, there is also a growing demand for vegetarian probiotic products. Additionally, lactose intolerance, milk allergies and cholesterol content are major drawbacks related to fermented dairy products. Therefore, cereals, fruits and vegetable may be prospective fermentable substrates and carriers for healthy probiotic bacteria, both in the developed and developing countries (Vasudha and Mishra, 2013). The use of probiotic cultures for the fermentation of non-dairy substrates represents a great challenge for industry-targeted research for commercial production of these healthy products (Mattila-Sandholm *et al*., 2002).

Vegetable juices can be processed by lactic acid fermentation, which broadens the available fermentable beverage assortment to include items that have high nutritive value and bioavailable

vitamins and minerals (Karovicova and Kohajdova, 2002). Lactic acid fermentation of vegetable raw materials, which is used as a preservation method for the production of finished and half-finished products, is again considered an important technology and is being further investigated. The main reasons for this renewed interest are the nutritional, physiological and hygienic aspects of the obtained products and their production costs (Karovicova *et al*., 1999). Consumption of lactic acid-fermented vegetable juices has increased worldwide (Kohajdova *et al*., 2006). These juices are produced mainly from cabbage, red beets, carrots, celery and tomatoes and may be called 'new functional foods' (Karovicova *et al*., 2002). For example, cabbage is rich in minerals, vitamin C, dietary fibers and especially, phytochemicals (Chu *et al*., 2002). While *Leuconostoc mesenteroides*, *Lactobacillus brevis* and *Lactobacillus plantarum* are involved in the spontaneous fermentation of cabbage juice (Breidt *et al*., 2013), its fermentation has also reported by the use of defined starter cultures of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* and *Lactobacillus casei* (Kohajdova and Karovicova, 2004; Yoon *et al*., 2006; Buruleanu *et al*., 2012). During fermentation, organic acids (primarily lactic acid), aldehydes, flavor substances, some bacteriocins and other biocompounds are produced by Lactic Acid Bacteria (LAB), the intake of which reduces the risk of numerous diseases, making these fermented foods a very desirable part of a healthy diet (Kris-Etherton *et al*., 2002). Fermented vegetable juices could serve as healthy beverages for vegetarians and lactose-allergic consumers (Vasudha and Mishra, 2013).

To achieve fast and controlled fermentation of vegetable products, pure or mixed starter cultures of lactic acid bacteria are used. Strains of the *Lactobacillus* genera improve the aroma and taste of juices, allow a rapid decrease in pH, produce mainly lactic acid, allow for reduce concentrations of nitrates and nitrites and decrease the concentration of biogenic amines (Drdak *et al*., 1994). LAB cultures that improve organoleptic properties and rapidly decrease the pH are required for industrial production of fermented juices (Karovicova *et al*., 1999).

Certain *Lactobacillus* and *Bifidobacterium* strains are characterized as probiotic and their natural habitat is the human and animal gastrointestinal tract and therefore, they can be used as components in probiotic preparations and functional foods (Gomes and Malcata, 1999; Mattila-Sandholm *et al*., 2002; Mountzouris *et al*., 2002). The development of functional food stuffs is associated, to a large extent, with products containing probiotics and prebiotics, which contribute to the normalization of the gut microbiota (Mattila-Sandholm *et al*., 2002; Mountzouris *et al*., 2002). It was reported that, in order to achieve detectable health results, at least approximately 10^8-10^9 live microbial cells should be consumed daily (Gomes and Malcata, 1999; Irena, 1999). The survival of probiotics in food matrices during the shelf life of a product depends on many factors, including: Structure of the food matrix, properties of the strains used, pH, storage temperature, oxygen levels and the presence of competitive microorganisms, inhibiting and protective substances (Lee *et al*., 1999; Yoon *et al*., 2005; Goderska *et al*., 2007; Pereira *et al*., 2011). The survival of probiotic bacteria on the effective level in fermented juices during product shelf life has been reported (Karovicova *et al*., 1999).

Prebiotics are indigestible ingredients that selectively stimulate the growth and/or activity of certain bacteria (probiotic) in the host organism's intestine, thus, improving the host's health (Gibson and Roberfroid, 1995). The substrates required for the growth of probiotic bacteria in the gastrointestinal tract should not be absorbed in its upper parts (Schrezenmeir and de Vrese, 2001). Most of the studied prebiotic substrates are soluble dietary fibers, such as fructans and fructo-oligosaccharides from plant or microbial origin. Synbiotics are pro- and prebiotic combinations that improve the survival of microbiological origin ingredients in the digestive system and thus,

can positively influencing the host organism (Gibson and Roberfroid, 1995; Schrezenmeir and de Vrese, 2001). By simultaneous application of probiotics and fructan sources in one product, it is possible to improve the efficiency of such synbiotic products in the human gut, as well as increase survival of probiotics during storage (Bielecka *et al*., 2002).

In addition to possessing beneficial properties, probiotic cultures that are being considered for the production of fermented foods should posses a number of important technological characteristics, allowing for the manufacture of products with desirable sensory qualities. Fermentation of non-dairy materials by probiotic cultures is still a challenge for both scientists and in industry. Fruits, such as oranges, grapes, pomegranates, etc. (Marhamatizadeh *et al*., 2012) and vegetables, such as tomatoes, cucumbers, carrots, pumpkin and courgette (Kohajdova *et al*., 2006; Buruleanu *et al*., 2011, Tamminen *et al*., 2013), have been suggested to be prospective substrates for fermentation by probiotic bacteria belonging to lactobacilli (*Lactobacillus plantarum* (Kohajdova *et al*., 2006), *L. paracasei*, *L. rhamnosus* (Tamminen *et al*., 2013) and *L. acidophilus* (Gorghiu *et al*., 2011)) and bifidobacteria *B. lactis* (Tamminen *et al*., 2013), *B. breve*, *B. longum*, *B. infantis* (Koh *et al*., 2010) and *B. bifidum* (Kun *et al*., 2008). Probiotic-fermented fruit and vegetable juices are evaluated by consumers based on their attractiveness and taste (Irena, 1999; Tannock, 2002). However, the survival of probiotics in fruit- and vegetable-based matrices more difficult than in dairy products due to the more aggressive influence of the acidic conditions on the probiotics survival (Schrezenmeir and de Vrese, 2001).

Fermentation of cabbage juice by LAB starter cultures or by spontaneous fermentation has been widely reported (Karovicova and Kohajdova, 2002; Karovicova *et al*., 2002; Yoon *et al*., 2006), but there are no reports on cabbage juice fermentation by bifidobacteria. Most strains of bifidobacteria are unable to grow in vegetable juices and require complex growth factors presented in e.g., milk whey, yeast extract or tomatoe juice (Poch and Bezkorovainy, 1988). Bifidobacteria have been used as starter culture for carrot (Kun *et al*., 2008; Tamminen *et al*., 2013), tomato (Koh *et al*., 2010) and green pepper (Buruleanu *et al*., 2012) juice fermentation. *B. lactis* Bb12 is widely used in probiotic-containing fermented milks due to its technological advances i.e., oxygen and acid tolerance (Meile *et al*., 1997). The probiotic properties of *B. lactis* Bb12 are well documented.

In this study the development of a *B. lactis* Bb12 fermented synbiotic fructan-containing cabbage juice-based drink was investigated.

MATERIALS AND METHODS

Strains: Seven probiotic strains (*Bifidobacterium lactis* Bb12, *Bifidobacterium animalis*, *Bifidobacterium lactis* OX, *Lactobacillus acidophilus* T20, *Lactobacillus acidophilus* La5, *Lactobacillus acidophilus* 33 and *Lactobacillus reuteri* 12) and five starter cultures (*Lactobacillus bulgaricus*, *Lactobacillus plantarum* KL, *Lactobacillus plantarum* 8014, *Lactobacillus casei var. alactosus* and *Pediococcus pentosaceus*; Strain Collection of Institute of Microbiology and Biotechnology University of Latvia) were used in this study.

Media and cultivation conditions: MRS (Man, Rogosa and Sharpes medium for cultivation of lactobacilli) growth medium (De Man *et al*., 1960) containing (gL^{-1}) 10.0 peptone, 8.0 beef extract, 5.0 sodium acetate, 4.0 yeast extract, 2.0 ammonium citrate, 2.0 KH_2PO_4, 1.0 Tween-80, 0.1 $MgSO_4 \times 7H_2O$, 0.05 $MnSO_4 \times 5H_2O$ and 20.0 glucose (pH 6.0) was used for the maintenance and propagation of the cultures.

Cabbages (*Brassica oleracea var. capitata*) were from a Latvian local market and juice was obtained using a mechanical squeezer. Fresh cabbage juice was pasteurized at 60°C for 20 min. Cabbage juice contained 4.8% d.w., 13.4 gL^{-1} glucose, 15.8 gL^{-1} fructose and 8.9 gL^{-1} sucrose. The initial PH was 6.05, titrable acidity 23 T. Bacteria strains were grown at 25 or 37°C in sealed 250 mL Erlenmeyer flasks.

Cabbage juice was supplemented with 20 gL^{-1} of various fructan sources and 5-30 gL^{-1} NaCl, when appropriate.

The sources of fructan were:

- The Jerusalem artichoke (*Helianthus tuberosus*) concentrate, containing (% of the dry mass): 94.6% solids; 63.7% total carbohydrates, including 45.0% fructans, 8.5% sucrose, 3.4% fructose, 0.8% glucose and 4.9% other carbohydrates, 17.1% proteins, 1.9% lipids and 2.1% nucleic acids The average fructan molar mass was ~1.5 kDa (Bekers *et al.*, 2007)
- Fructooligosaccharides Nutraflora FOS® (Twin Laboratories inc. Ronkonkoma, New York 11779 USA), for which the fructan average molar mass was ~0.6 kDa
- Raftiline® ST (93.9% inulin, 1.1% glucose+fructose and 5% saccharides), which had an average fructan molar mass ~3 kDa
- Raftilose® L60/75 (FOS >60% of the dry mass; Orafti, Belgium), with an average fructan molar mass ~0.6 kDa
- Levan (β-2,6-fructosan) exopolysaccharide was synthesized by *Zymomonas mobilis* and had an average molar mass ~2000 kDa
- *P. pentosaceus* exopolysaccharide complex (1:3 fructans and glycans mix), which had an average molar mass ~2000 kDa (Semjonovs and Zikmanis, 2008)

The average molecular mass and composition of fructan sources was detected as previously reported.

Analytical measurements

Optical density measurements: The growth (biomass concentration) of strains of *L. reuteri* was monitored by spectrophotometric measurements of optical density at 550 nm (Helios Gamma, Thermo Scientific, UK).

The dry matter of the samples was determined gravimetrically after dewatering at 105°C.

Cell count: The viable cell count was monitored by the spread-plate method using the agarized MRS medium.

B. lactis in the mixed culture with LAB was selectively enumerated on the agarized MRS medium supplemented by (gL^{-1}) 3.0 LiCl, 0.015 nalidixic acid, 0.10 neomycin sulfate and 0.125 paramomycin sulfate (Sigma Aldrich GmbH) (Charteris *et al.*, 1997). To determine bacteria viability during storage, after fermentation the samples were stored in the dark at +4°C for 2 weeks.

Total acidity measurement: Total acidity was determined by alkaline titration (0.1 mol L^{-1} NaOH) of the samples using phenolphthalein as the indicator and expressed in Thörner degrees (T).

Organic acid determination: The concentrations of organic acids (lactic, acetic, gluconic, succinic and citric) were quantified by HPLC (Agilent 1100, HP, USA) with a refraction detector, Shidex SH 1011 column, a column temperature of 50°C, a mobile phase consisting of 0.01 N H_2SO_4 and a flow rate of 0.6 mL min^{-1}.

Carbohydrates assay: The concentrations of carbohydrates (glucose, fructose and sucrose) were quantified by HPLC (Agilent 1100, HP, USA) with a spectrophotometric detector (wavelength of 210 nm). Data was analyzed using the Agilent chemistation program. The content of the fructans was determined according to the AOAC methods AOAC-99.03 and AACC32.32.

Data processing and analysis: All procedures were performed on cells from at least three independent cultivations. All analytical measurements for each sample were performed in at least triplicate. The data were processed by multivariate analysis of variance (MANOVA) and linear and nonlinear regressions using Statgraphics ®Plus (Manugistics, Inc., US) and SPSS 11.0 for Windows (SPSS Inc. Ill., US). To evaluate the statistical significance of the regression models, the F-test was performed.

RESULTS AND DISCUSSION

It was found that potentially probiotic cultures (*L. acidophilus*, *B. lactis*, *B. animalis* and *L. reuteri*), which are not traditionally used for vegetables or vegetable juice fermentation, can grow in cabbage juice (Table 1), achieving significant cell counts (10^7-10^8 CFU mL^{-1}) that are particularly important for probiotic-containing products (Table 1). It is known that, in order to be considered a probiotic, the minimum number of probiotic cells in fermented products is 10^6 CFU mL^{-1} (Shah, 2001). Additionally, it was found that probiotic strains could rapidly decrease the pH value of juice during fermentation (Table 1).

The fermentation of cabbage juice using different LAB starter cultures or spontaneous fermentation has been widely reported (Karovicova and Kohajdova, 2002; Karovicova *et al*., 2002; Yoon *et al*., 2006), but there are no reports for generally recognized probiotics-bifidobacteria-application for cabbage juice fermentation (Lee *et al*., 1999). *Bifidobacterium* strains are fastidious for growth factors in the substrate and they are characterized by relatively low growth rates in food substrates in comparison to commercially available LAB starter cultures that are suitable for vegetable substrates (Gomes and Malcata, 1999). It is useful to include certain *Bifidobacterium* strains in defined starter cultures to improve a product's probiotic properties. The use of *bifidobacteria* strains in mixed starter cultures is recognized as a successful method to improve the functional value of the product. However, it is important to evaluate the compatibility of the probiotic strain and the substrate that will undergo fermentation (De Vuyst, 2000).

The differences in the growth of various cultures in cabbage juice (Table 1) can be explained by the presence or absence of strain-specific growth factors or their level in the substrate. Hence, the data shown in Table 1 should be taken into consideration for selecting appropriate strains for the fermentation of certain food substrates.

For sauerkraut or fermented cabbage juice production, it is necessary to ensure a rapid decrease in the pH during the early stages of fermentation (Viander *et al*., 2003; Holzapfel *et al*., 2003). In comparison with heterofermentative LAB, homofermentative LAB could more rapidly decrease the pH during the cabbage fermentation (Breidt *et al*., 2013; Holzapfel *et al*., 2003). However, using only homofermentative strains in sauerkraut or cabbage juice fermentation, it is impossible to

Table 1: pH, total acidity (°T) and culture cell-count (log CFU mL^{-1}) of cabbage juice fermented by different *Lactobacillus* and *Bifidobacterium* cultures (48 h, 37°C)

Cultures	pH±S.E	Total acidity, T°±S.E	Cell count (log CFU mL^{-1}±S.E)
B. animalis 001	5.50±0.01	28±2	7.50±0.13
B. lactis Bb12	5.30±0.01	35±2	7.01±0.12
L. acidophilus La5	4.80±0.01	50±2	7.90±0.10
B. lactis OX	4.20±0.01	60±2	7.40±0.11
L. reuteri 12	4.15±0.01	104±3	8.03±0.16
L. plantarum 8014	4.05±0.01	69±2	7.80±0.08
L. acidophilus T20	4.30±0.01	62±2	8.05±0.10
L. delbrueckii 03	3.95±0.01	87±4	8.25±0.10
L. casei var. *alactosis* 002	3.75±0.01	110±4	8.39±0.11
L. plantarum KL	3.70±0.01	115±2	8.40±0.09

S.E: Square error

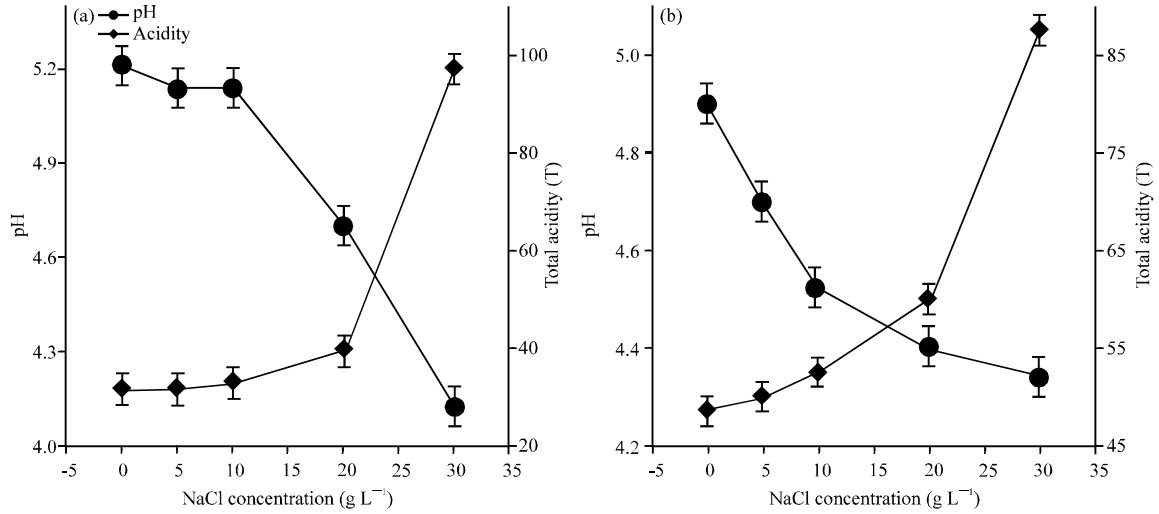

Fig. 1: Effect of NaCl concentration on pH and total acidity during cabbage juice fermentation for 48 h at 37°C by (a) *Bifidobacterium lactis* Bb12 and (b) *Bifidobacterium animalis*

attain products with good organoleptic properties. Consequently, to attain a good quality final product, it is necessary to use both homo and heterofermentative strains for fermentation. As shown (Table 1), both of these can grow in cabbage juice.

It is known that salt (NaCl) in sauerkraut and cabbage juice fermentation is commonly used for suppression of undesirable microflora, improvement of organoleptic properties and facilitation of juice release (Viander *et al*., 2003; Holzapfel *et al*., 2003). NaCl also affects the metabolism and growth of LAB, which are involved in the fermentation process (Chikthimmah *et al*., 2001; Johanningsmeier *et al*., 2012). It was reported that the presence of a higher level of NaCl (5 gL^{-1}) inhibited the growth of LAB and, in turn, increased pH level (Chikthimmah *et al*., 2001).

The addition of NaCl has different effects on the acidification of cabbage juice during its fermentation by different bifidobacteria (Fig. 1). The growth of *B. lactis* Bb12 and *B. animalis* 001 in cabbage juice slowly decreased in the presence of >5 gL^{-1} NaCl or higher and was followed by

Table 2: Changes in pH, total acidity and probiotic cultures cell count during cabbage juice fermentation at 25°C

	*pH±S.E		Total acidity (**° T±S.E)		Cell count (log CFU mL^{-1}±S.E)
Cultures	26 h	48 h	26 h	48 h	48 h
B. lactis Bb12	4.12±0.01	3.98±0.01	95±3	114±2	7.72±0.03
B. animalis 001	4.39±0.01	4.19±0.01	67±2	84±2	8.18±0.08
L. acidophilus T20	4.33±0.01	3.73±0.01	48±2	104±3	8.31±0.08
L. acidophilus 33	4.32±0.01	4.10±0.01	73±4	90±3	8.22±0.04
L. acidophilus La5	4.47±0.01	4.20±0.01	60±3	78±2	8.14±0.08
L. reuteri 121	3.67±0.01	3.82±0.01	135±2	163±4	7.90±0.03

*pH before fermentation = 6.84, **° T before fermentation = 22°T, S.E: Square error

an increase in pH and decrease in total acidity (Fig. 1) indicating a suppressive influence of NaCl on the acidification of the substrate. Considering that the addition of NaCl to sauerkraut or cabbage juice fermentation is required, at least for the improvement of the products organoleptic characteristics (Viander *et al*., 2003; Holzapfel *et al*., 2003), the optimal NaCl concentration that inhibits the probiotic bifidobacteria the least is = 5 gL^{-1}.

The temperature for sauerkraut fermentation should not exceed 25°C to prevent decreased quality of the final product (Holzapfel *et al*., 2003). It is known that the optimal growth temperature for sauerkraut LAB starter cultures, such as *L. plantarum* and *L. casei alactosus*, is 18-26°C. However, the majority of probiotic strains e.g., *B. lactis* Bb12 and *L. acidophilus* La5, do not grow well at these temperatures (Bezkorovainy and Miller-Catchpole, 1989). It was found that all of the studied probiotic cultures could rapidly grow at relatively low (25°C) temperatures, decreasing the pH to technologically required levels<4.0 (Table 2). It points out well documented probiotic *B. lactis* Bb12 (Ringel-Kulka *et al*., 2009; Wildt *et al*., 2011) as a perspective candidate for cabbage or other vegetable fermented juices production.

The prebiotic properties of fructans are widely documented (Gibson and Wang, 1994; Kaplan and Hutkins, 2000; Semjonovs *et al*., 2004). It could be useful to supplement cabbage juice with fructans in combination with probiotics to enhance its functional value also by sources of prebiotics. The essential prerequisite for fructan-containing synbiotic (containing both prebiotic substances and probiotic strains) (Schrezenmeir and de Vrese, 2001) product development is the ability of the involved strain to metabolize the fructans as the sole carbon source. Strains actively growing in cabbage juice were tested for fructooligosaccharides (FOS) assimilation as the sole carbon source (Fig. 2). It was shown that probiotic strains (*B. lactis* Bb12), as well as technological cultures (*L. plantarum* KL), could assimilate fructans as the sole carbon source.

The viability of LAB cultures during product storage is the most important probiotic product quality indicator. It was reported that the viability of probiotic bacteria depends on the level of oxygen in products, storage time and storage temperature (Shah, 2001) and is also affected by product acidity, postacidification at the storage stage and the food matrix being fermented (De Vuyst, 2000). During fermentation of cabbage juice by *L. casei* to pH 4.0, the viable cell count decreased significantly within the first week of storage (Yoon *et al*., 2006). It was reported that fructan additives positively influence the viability of probiotics during storage (Lukacova *et al*., 2005; Goderska *et al*., 2007). It was shown that the addition of fructans improved the viability of probiotic bacteria in fermented cabbage juice during storage at 4°C (Fig. 3) and correlated directly with the molecular weight of the polysaccharide (Fig. 3).

It is to say that *P. pentosaceus* P773 exopolysaccharides that consist of 1:3 β-fructan and α-glycans mixture, has the same average molar mass (~2000 kDa) as levan synthesized by

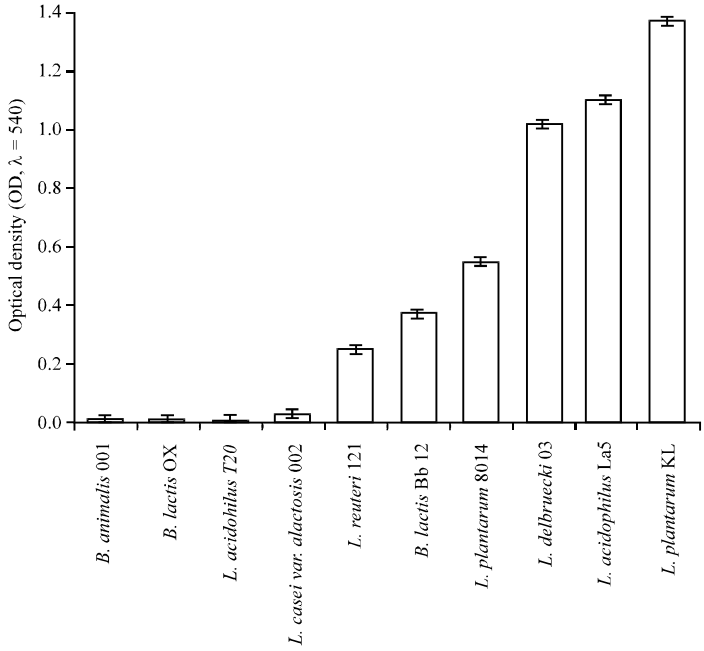

Fig. 2: Biomass concentration of different lactic acid bacteria and *Bifidobacterium* strains after the growth (48 h, 37°C) with fructooligosaccharides (20 g L^{-1}) as the sole carbon source

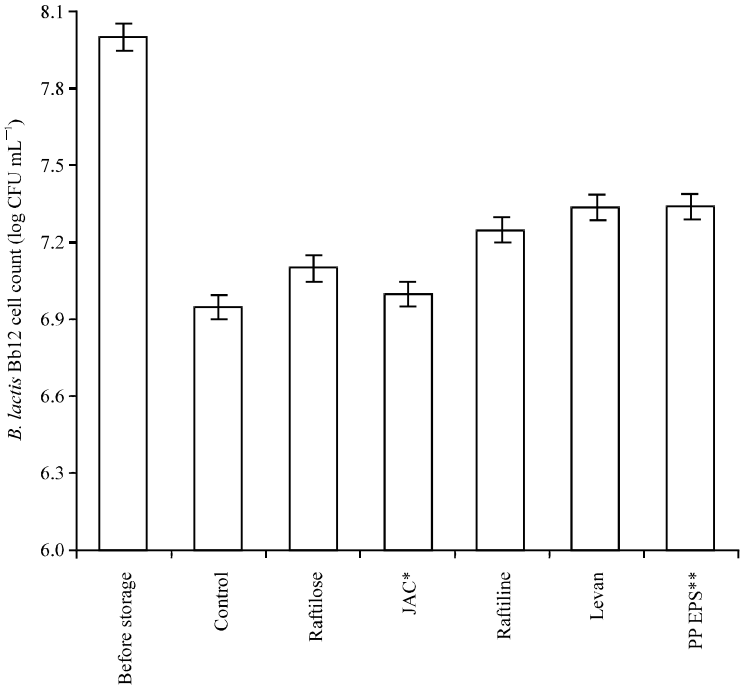

Fig. 3: Effect of fructan sources on *Bifidobacterium lactis* Bb12 viability during fermented cabbage juice storage (4°C, 2 weeks), *Jerusalem artichoke (*Helianthus tuberosus*) concentrate ******Pediococcus pentosaceus* P773 exopolysaccharides (Semjonovs and Zikmanis, 2008)

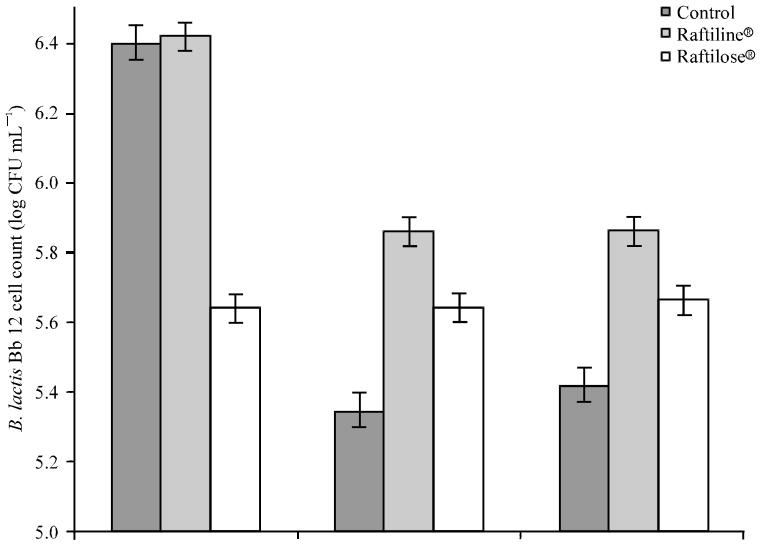

Fig. 4: Effect of fructans (20 gL^{-1}) on *Bifidobacterium lactis* Bb12 cell count during cabbage juice fermentation (48 h, 25°C) by *B. lactis* Bb12 in combination with different technological cultures

Zymomonas mobilis (Semjonovs and Zikmanis, 2008) and during storage promote LAB and *B. lactis* Bb12 survival equally (Fig. 3). This suggests that glycan polysaccharides may also have a protective effect that should be investigated further.

An essential prerequisite of a high quality fermented product is the ability of starter cultures to rapidly decrease the pH during the early stages of fermentation (Holzapfel *et al.*, 2003). It has been shown that probiotic strains grew in cabbage juice, but the growth was significantly lower in comparison to traditionally used starter cultures. Therefore, it is useful to combine probiotic strains and technological cultures in multistrain starters. However, the standard evaluation for strain mutual interaction should be performed (De Vuyst, 2000). Our results indicate that highest cell concentration of *B. lactis* Bb12 was obtained when this strain was grown in combination with *L. reuteri* 121 and *L. casei alactosus* (Fig. 4). While growth promoting effects of fructans (Raftiline® and Raftilose®) on *B. lactis* Bb12 were observed (Fig. 4), the highest *B. lactis* Bb12 cell count was reached when it was combined with *L. plantarum* KL.

Taking into account the well documented probiotic properties of *B. lactis* Bb12 (Gomes and Malcata, 1999), the beneficial influence of fructans on human health (Gibson and Wang, 1994; Menne and Guggenbuhl, 2000; Shah, 2001; Roberfroid, 2007) and *B. lactis* Bb12 growth promoting properties (Semjonovs *et al.*, 2004), the combination of probiotic *B. lactis* Bb12 with a fructan source (Raftiline®) could be advantageous for cabbage juice-based synbiotic beverage development (Table 3). As shown in this study, *B. lactis* Bb12 can grow well in cabbage juice, achieving 10^6 CFU mL^{-1}, which is the minimally required concentration for probiotic product efficacy (Minelli and Benini, 2008). *B. lactis* Bb12 as the sole starter culture or *B. lactis* Bb12 in combination with the appropriate technological culture could be used for cabbage juice fermentation.

Table 3: Characteristics of cabbage juice fermented by *Bifidobacterium lactis* Bb12 (72 h, 25°C) with the fructan additive Raftiline® (20 g L^{-1})

Parameter	Values
Dry weight (g L^{-1})	8.0
Fructans (g L^{-1})	16.0
NaCl (g L^{-1}	0.50
pH	3.9
Total acidity (°T)	110
Lactic acid (g L^{-1})	7.50
Acetic acid (g L^{-1})	2.50
Gluconic acid (g L^{-1})	0.30
Succinic acid (g L^{-1})	1.80
Citric acid (g L^{-1})	0.20
Glucose (g L^{-1})	31.1
Fructose (g L^{-1})	24.3
Sucrose (g L^{-1})	3.50
B. lactis Bb12 cell count (log CFU mL^{-1})	6.0±0.5

CONCLUSION

It was shown that studied LAB and bifidobacteria strains, including the well documented probiotic *B. lactis* Bb12, developed well in cabbage juice also at a reduced temperature (25°C), which is quite notable for bifidobacteria. As it is known, this is the first report on cabbage juice fermentation by bifidobacteria. The addition of fructan sources improved *B. lactis* Bb12 viability during fermented cabbage juice storage (4°C) for 2 weeks. The protective effect of fructans was in direct correlation to their average molecular weight. Combination of probiotic strains and fructan additives is a perspective strategy for cabbage juice-based fermented functional synbiotic beverage development.

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