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ORIGINAL RESEARCH PAPER

FUNCTIONAL CHARACTERISATION OF FERMENTED BEVERAGE BASED ON SOYMILK AND SEA BUCKTHORN SYRUP

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In the last decade, there is an increasing interest in using nondairy ingredients as substrates for certain strains of bifidobacteria to deliver the benefits of probiotics to a wider group of consumers. This research aimed to explore the use of soymilk and sea buckthorn syrup as substrates for bifidobacteria fermentation. Microbial population, pH, and titratable acidity were measured during the fermentation period while the viability, pH, titratable acidity and water holding capacity were determined during the storage time at 4°C ± 1°C within 14 days. Survival and stability of *Bifidobacterium bifidus* (*Bb-12®, Bb*) inoculated into a beverage when exposed to simulated gastrointestinal tract conditions, were assessed. The *Bb-12®* strain exhibited the highest viable cell numbers when exposed to simulated gastrointestinal tract conditions.

Keywords: fermented probiotic beverage, soy milk, sea buckthorn syrup, lactic fermentation, *Bifidobacterium bifidus* (Bb-12®, Bb)

Introduction

In the last few years, intensive research has been conducted on the possibilities of designing new dietary fruit beverages containing either milk or milk-derived functional products. Fruit beverages are a source of antioxidants, which are often commercially supplemented with milk, vitamins and/or minerals to improve their nutritional value and to provide bioactive food components. These beverages could be helpful in complying with the dietary intake recommendations related to fruits and antioxidants, with a view to preventing several diseases caused by oxidative stress (Laparra *et al*., 2008).

Soybean (*Glycine max*) has been an important protein source for millions of people for over five thousand years (Mathur, 2004). The flavor associated with soybean is regarded as one of the important factor limiting its use in food products

(Deshpande *et al.,* 2008). The milk white fluid obtained from soybean by soaking, grinding and filtering is called "Soymilk" (Lo *et al*., 1968). Soymilk contains some major and minor components like protein, fat, carbohydrate, calcium, iron, sodium, carotene, vitamin-E and riboflavin (Deshpande *et al*., 2008). Soymilk is used in various products like paneer, yogurt, cheese, tea and coffee whiteners, shrikhand, rasogolla and various indigenous milk sweets, confectionary, etc. (Wang *et al*., 2001). During the last years, soy beverages consumption has gradually increased due to their significant concentration of health-promoting compounds, such as isoflavones. Epidemiological and clinical studies suggest that consumption of a diet rich in isoflavones is associated with low risk of the so-called Western diseases (Adlercreutz and Mazur, 1997), such as coronary heart disease (Anderson *et al*., 1995), osteoporosis, menopausal symptoms, hormone-dependent cancers (Peeters *et al*., 2003) obesity, and diabetes. The "Beany" flavor is indeed the main inconvenience of traditional soymilk. This objectionable flavor comes from some ketones and aldehydes, particularly hexanals and heptanals, produced through lipoxidase-catalyzed oxidation of soybean oil. These compounds are not present in sound, dry soybeans but are produced as soon as the beans are wetted and ground. Several approaches have been used to overcome the problem of off flavors in soymilk (Kale *et al*., 2011).

In recent years, due to the changes in consumer preference towards natural products with functional properties, the use of sea buckthorn (*Hippophaë rhamnoides L*.) berries as a natural food ingredient has been increasing. Sea buckthorn contains a large variety of substances which possess strong biological activity. The berries are especially rich in vitamins and flavonoids, both of which are natural plant pigments (Gao, *et al*., 2000).

Recently, numerous *Bifidobacterium* species are being used in industries for the production of probiotic dairy products such as *B. animalis* subsp. *lactis*, *B. bifidum*, *B. longum*, and *B. breve*. The potential application of the above bacteria is highly dependent on their physiological and technological properties including growth and viability, oxygen and acid tolerance, utilization of carbohydrates and metabolites, results of clinical tests, etc. that vary from strain to strain. One of the most widespread industrially used *Bifidobacterium* strains is *B. lactis Bb-12* (Chr. Hansen A/S, Hørsholm, Denmark), because this strain has numerous physiological and technological advantages such as clinically proven physiological properties (Saxelin *et al*., 1999), oxygen and acid tolerance (Meile *et al*.,1997; Hoier, 1999), even it does not derive from human sources. *Bifidobacterium spp*. can reduce the off-flavor of n-hexanal (Scalabrini *et al*., 1998) and ferment sucrose, raffinose and stachyose (Desjardins *et al*., 1990).

The main goal of this work was to combine soymilk with different percentages of sea buckthorn syrup and fermented with a culture of *Bifidobacterium bifidus* (*Bb-12*®) in order to produce a novel fermented lactic acid beverage. An additional objective was to test the viability of *Bifidobacterium bifidus* (*Bb-12*®) strain in simulated gastric transit conditions (pH 2.0 gastric juices) and the viability in simulated intestinal transit conditions (pH 8.0, with 4.5% bile salts).

Materials and methods

Materials

The **soymilk** *Dr. Oetker* used in this study is a sterilized product –Soy Beverage Inedit from
Company, Romania – obtained from selected ingredients and certified as organic. According to the information given on the product label, it contains 1.1% proteins, 0.0 % sugars and 1, 9 % lipids.
Sea buckthorn syrup available at Plafar market in Galati, Romania was used in these experiments. The soluble solids content of untreated syrup was 6° Brix, and its pH was 3.10. All samples used in the experiment were obtained from a single syrup batch.
Probiotic lactic acid bacteria. *Bifidobacterium bifidus* was provided by Chr. Hansen, Denmark, as a freeze-dried commercial starter with commercial name *Bb-12®, Bb*. The storage and maintenance of the culture was carried out as per the recommendation of the manufacturer.

Lactic fermentation and analytical assays

Four beverages were prepared in duplicate, by adding to the soymilk the following proportions of sea buckthorn syrup: 5.0% (v/v), 10.0% (v/v), 15.0% (v/v) and 20.0% (v/v). *Bifidobacterium bifidus* was cultivated in 100 ml of sterilized soymilk with different amounts of sea buckthorn syrup at 30°C and 37°C for 12 h. During incubation, samples were taken at 0, 2, 4, 6, 8, 10, and 12 h in order to test the pH, culture growth and titratable acidity. The pH of the sample was measured with a pH meter (MP2000, Mettler Toledo, Greifensee, Switzerland). Titratable acidity was determined with 0.1 N NaOH solution and expressed in grams of lactic acid per 100 ml of fermented product.

After 12 h of fermentation, the fermented samples were stored at 4°C ± 1°C for 14 days and the viability of probiotic bacteria, pH, titratable acidity and water holding capacity were measured during the whole storage time.
Soluble solid content (Atago RX-1000 refractometer, Atago Company Ltd., Japan) and electrical conductivity (InoLab Multilevel 1 conductivimeter, Senton, GmBh, Germany) of the beverages were determined for its physico-chemical characterization immediately after preparation.

Probiotic bacteria counting

Viable cell counts were determined by preparing serial decimal dilutions with 0.1% (w/v) peptone water (Merck) which were subsequently plated on MRS agar (Merck) on Petri dishes. The plates were incubated in anaerobic jar (Merck) with Anaerocult® A kit (Merck), for 48 h, at 37°C. Plates containing 25– 250 colonies were selected and CFU ml^{-1} fermented product was recorded. All plate counts were carried out in duplicates.

Simulation of conditions in the gastrointestinal tract

Simulated gastric juice (SGJ) was prepared according to the procedure of the USP, National Formulary: 2.0 g NaCl, 3.2 g pepsin and 3.0 ml concentrated HCl diluted to 1 L and adjusting the pH to 2.0 with concentrated HCl or sterile 0.1 mol l^{-1} NaOH. Simulated intestinal juices (SIJ) were prepared by suspending pancreatin USP (P-1500) in sterile sodium chloride solution (0.5%, w/v) to a final concentration of 1 g L^{-1}, with 4.5% bile salts (Oxoid, Merck, Germany) and adjusting the pH to 8.0 with sterile 0.1 mol L^{-1} NaOH. Both solutions were filtered for sterilization through a 0.22 µm membrane. 0.2 ml fermented beverage have been taken and homogenized with 10 ml of simulated gastric juice and incubated for 5, 30, 60 and 120 minutes for viability of probiotic bacteria in SGJ and 60, 90 and 120 minutes, respectively for viability in SIJ at 37°C with constant agitation at 50 rpm. Surviving bacteria were counted by pour plate techniques in MRS agar by anaerobic incubation at 37°C, for 3 days. The data is expressed as means from three independent experiments with two replicates.

Water holding capacity

Water holding capacity of the beverage was determined through the centrifugation procedure. Approximately 10 g of beverage was transferred into a 20 ml glass tube and was centrifuged at 2500 rpm for 10 min at 20°C (modified method of Pyo and Song, 2009). The water holding capacity was estimated as the percentage of the released whey over the initial beverage weight and was an average of three determinations:

Water holding capacity, % = (weight of supernatant/weight of beverage) x 100 (1)

Rheological measurements

Beverage samples were gently stirred before rheological analysis. Rheological measurements were carried out in duplicate by means of a RHEOTEST-2 type rotating viscometer manufactured by VEB-MEDINGEN, Germany. Due to the medium viscosity of the samples the coaxial cylinder device S3 was used and 50 g of sample was tested. The working frequency was 50 Hz and the shear rate (γ) varied from 0.1667 to 145.6 s^{-1}. The apparent viscosity (η) was calculated as:

$$\eta = \frac{\tau}{\gamma} \quad (2)$$

Statistical analysis

Statistical analysis was performed using Statgraphics plus v.5.1 package (Manugistics Inc., Rockville, MA, USA). Data were analysed by multifactor analysis of variance and a Duncan multiple-range test was applied to determine differences among means, with a significance level of 0.05.

Results and discussion

Microbiological and physico-chemical analysis during fermentation

The counts for *Bifidobacterium bifidus* (*Bb-12®, Bb*) strain in fermented beverages are shown in Figure 1. The culture strain of *Bb-12®* was able to grow in all beverages without nutrient supplementation. From an initial concentration of $4.8 \cdot 10^7$ CFU·ml^{-1} the *Bb-12®* strain increased exponentially to around 10^9 CFU·ml^{-1} during the 12 hours of fermentation for both temperatures (Figure 1a and b). After a short lag phase the multiplication rate increased progressively for all beverages ($p<0.05$) and was significantly high for the sample with 20% sea buckthorn syrup. Compared to the data published in the literature, our results demonstrate the ability of *Bb-12®* strain to grow in beverages based on soymilk and sea buckthorn syrup. Production of bifidobacteria in soymilk was studied intensively by various authors. For example, Garro *et al.* (2004) and Kamaly (1997) reported that maximum cell concentration from bifidobacteria varied from 10^7 CFU·ml^{-1} to 10^9 CFU·ml^{-1}. The different fermentation rates could be attributed both to strain specificities and differences of concentration of sea buckthorn syrup.

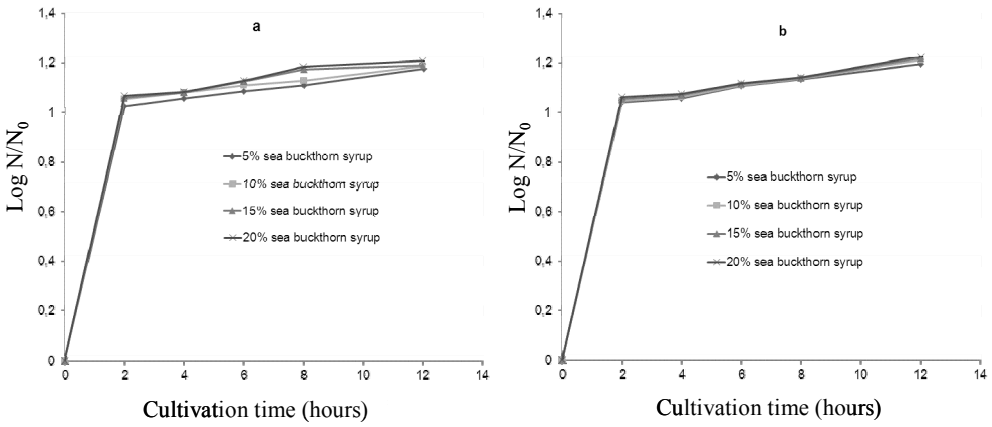

Figure 1. Growth kinetic of *Bb-12®* during fermentation
a – Fermentation at 30°C; b – Fermentation at 37°C

As shown in Figure 2a and b, the pH values of all beverages dropped from the initial pH of 6.12, 5.71, 5.46 and 5.32 to 4.86, 4.81, 4.75 and 4.74 for the beverages fermented at 30°C. For beverages fermented at 37°C, the pH values after 12 hours of fermentation were 4.65, 4.62, 4.58 and 4.55. The drop in pH is due to the production of organic acids. Our results agree with the results reported by Rozada-Sánchez *et al.* (2008) who studied the evaluation of *Bifidobacterium spp*. for the production of a potentially probiotic malt-based beverage and observed that after 14 hours of fermentation, the pH ranged between 4.40 and 4.6. Angelov *et al.* (2005) reported that the pH of a fermented beverage must be between 4 and 4.5, which could mean that fermentation with *Bifidobacterium spp*. over a period of 12–14 h could produce an unacceptable product.

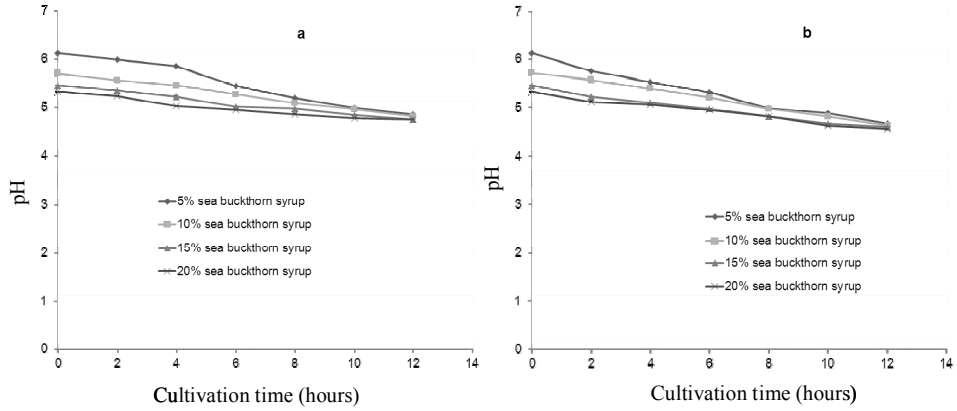

Figure 2. pH changes in beverages during fermentation
a – Fermentation at 30°C; b – Fermentation at 37°C

During fermentation for both temperatures the titratable acidity increased with the decrease of pH (Figure 3a and b) for all beverages. It is known that lactic acid increases the nutritional value of fermented products by engendering taste and structure (Kun *et al*., 2008). Upon the completion of fermentation, about 0.55 – 0.63 g lactic acid·100 ml^{-1} and 0.73 – 0.88 g lactic acid·100 ml^{-1} were obtained in case of fermentation at 30°C and 37°C, respectively. Farnworth *et al*. (2007) found after 12 hours at fermentation with bifidobacteria in a soy beverages a titratable acidity of 0.38 – 0.39 g·100 ml^{-1}. Gardner *et al.* (2001) reported that the concentration of lactic acid varied in the range of 0.3 g·ml^{-1} to 1.5 g·ml^{-1} using mono and mixed cultures of lacto-bacteria for fermented vegetable juice. Kwon *et al*. (2000) and Nancib *et al*. (2001) reported that numerous studies dealing with nutrients necessary for lactic acid fermentation have established that the more nitrogenous components are added, the higher concentration of lactic acid is produced. Our results showed that the bifidobacteria compete with lactic acid bacteria in the production of lactic acid.

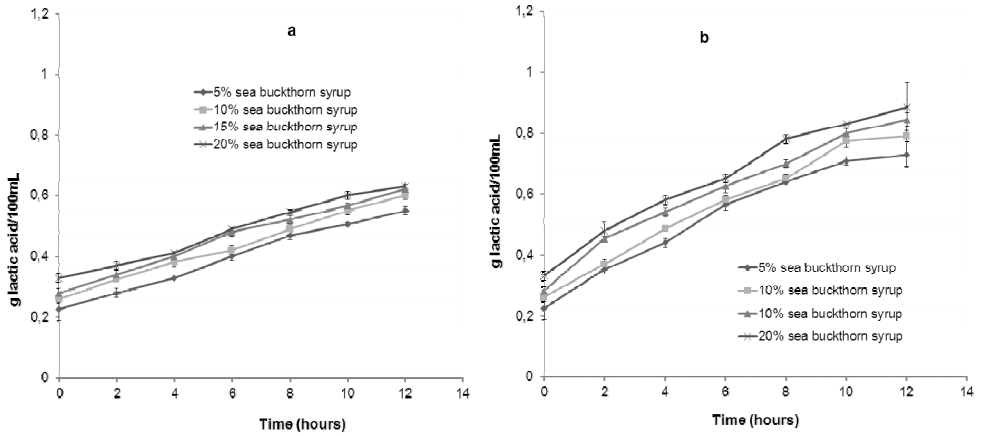

Figure 3. Titratable acidity changes in beverages during fermentation
a – Fermentation at 30°C; b – Fermentation at 37°C

In Table 1 and Table 2 were presented results obtained with respect to physico-chemical and rheological characteristics of a novel fermented beverage based on soymilk and sea buckthorn syrup. As seen in Table 1 and Table 2 with the increase in syrup concentration percent acidity, soluble solids and viscosity increased, while pH and electrical conductivity decreased. An important characteristic of fermented milk beverages, as non-Newtonian fluids, is viscosity (Malbaša *et al*., 2009). The sugar composition for the four beverages is likely the factor that can explain the differences in rheological properties and electrical conductivity.

Table 1. Physico-chemical characteristics of beverages

Sample	Soluble solids (°Bx)	pH	Acidity	Electrical conductivity ($\mu s\ cm^{-1}$)
5% sea buckthorn syrup	6.05 ± 0.007^{a}	6.12 ± 0.007^{a}	0.22 ± 0.035^{a}	5.73 ± 0.007^{a}
10% sea buckthorn syrup	9.15 ± 0.021^{b}	5.71 ± 0.007^{b}	0.26 ± 0.014^{b}	5.35 ± 0.014^{b}
15% sea buckthorn syrup	12.05 ± 0.007^{c}	5.46 ± 0.000^{c}	0.28 ± 0.014^{bc}	5.06 ± 0.035^{c}
20% sea buckthorn syrup	14.00 ± 0.035^{d}	5.33 ± 0.007^{d}	0.33 ± 0.014^{d}	4.55 ± 0.014^{d}

Mean and standard deviation for n = 3. Means with different letters within the same row are statistically significant ($p<0.05$) according to Duncan multiple-range test.

Table 2. Rheological characteristics of beverages

Sample	Viscosity (Pa s^{-1})	
	Fermentation at 30°C	Fermentation at 37°C
5% sea buckthorn syrup	0.24 ± 0.014^{a}	0.28 ± 0.035^{a}
10% sea buckthorn syrup	0.32 ± 0.007^{b}	0.34 ± 0.00^{b}
15% sea buckthorn syrup	0.35 ± 0.007^{c}	0.38 ± 0.007^{c}
20% sea buckthorn syrup	0.57 ± 0.007^{d}	0.63 ± 0.014^{d}

Mean and standard deviation for n = 3. Means with different letters within the same row are statistically significant ($p<0.05$) according to Duncan multiple-range test.

Microbiological and physico-chemical analysis during cold storage

After 14 days of storage at 4 ± 1°C, the cell numbers of *Bb-12*® in the fermented beverages were $3.6 \cdot 10^{9}$ CFU·ml^{-1} and $5.8 \cdot 10^{9}$ CFU·ml^{-1}, for the sample with 20% sea buckthorn syrup fermented at 30°C and 37°C, respectively (Figure 4a and b). In this experiment, the start point of evaluation was considered the end of the fermentation time, after 12 h of incubation. As shown in Figure 4, after 7 days of storage, the cell number of *Bb-12*® increased slightly for all samples at both temperatures of fermentation. Also, after 14 days of storage, the viable cell population of *Bb-12*® strain decreased slightly for all samples ($p<0.05$). Generally, the results presented here support data obtained by others. For example, Lin *et al.* (2004) reported that after 14 days of storage the viability of *Bifidobacterium*

longum in a fermented beverage based on milk, soymilk and *L. chinense* Miller juice was $6.3 \cdot 10^8$ CFU·ml^{-1}. Chou and Hou (2000) found that the cell numbers of *Bifidobacterium infantis*, in fermented soymilk were lower when stored at 25°C than at 5°C. Regarding the functionality of probiotics, Shah (2001) reported that it is thought that in order to exert beneficial effects, they must be viable and available at a high concentration, typically at least 10^8–10^9 per gram of product. The results obtained in these experiments show that *Bb-12*® strain was capable to maintain well in a novel fermented beverage without nutrient supplementation. Our results demonstrate that *Bb-12*® strain can be used as a probiotic culture for obtaining fermented beverages, based on soymilk and sea buckthorn syrup. Also, other authors reported satisfactory probiotic viability when producing probiotic fresh food (Cardarelli *et al*., 2008, Wanita *et al.,* 2009, Malbaša *et al*., 2009). Therefore, the novel fermented beverage based on soymilk and sea buckthorn syrup fermented with *Bb-12*® strain seems to be the most promising concerning functional food.

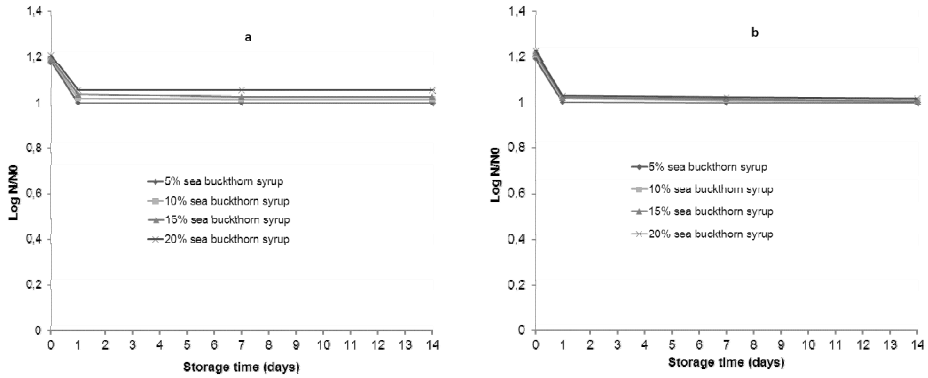

Figure 4. Variation in cell numbers of *Bb-12*® in the fermented beverages during storage
a – Fermentation at 30°C; b – Fermentation at 37°C

The pH evolution during cold storage for all samples studied is shown in Figure 5a and b ($p<0.05$). After 14 days of storage, the pH ranges between 4.68 – 4.58 and 4.51 - 4.46 for the sample fermented at 30°C and 37°C, respectively. The decrease in pH is due to the production of organic acids. Lin *et al.* (2004) and Rozada-Sánchez *et al*. (2008) reported similar results.

Lactic acid, one of the metabolites associated with nutritive and functional characteristics of fermented beverages, was determined. The values for all samples after 14 days of storage are shown in Figure 6a and b ($p<0.05$). The greatest content of lactic acid was found in beverage with 20% sea buckthorn syrup (0.74 and 0.88 g·100 ml^{-1} for temperature of fermentation at 30°C and 37°C, respectively). As expected, during storage the acidity increased. Lin *et al.* (2004) reported similar results. Caplice *et al*. (1999) observed that the presence of lactic acid in fermented foods is advantageous due to their antimicrobial properties, preventing spoilage by other microorganisms.

After 14 days of storage, water holding capacity ranges between 74.39 – 72.44% for the sample fermented at 30°C and between 74.74 – 72.74% for the sample fermented at 37°C (Figure 7a and b).

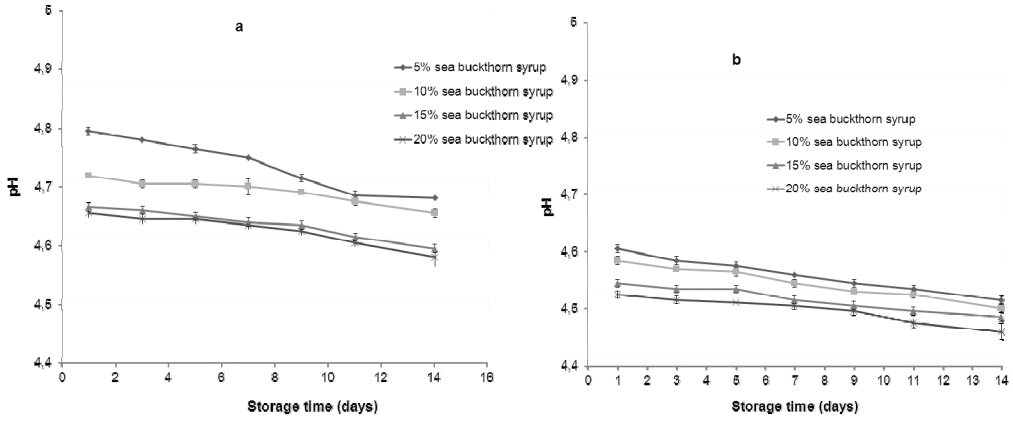

Figure 5. pH changes in beverages during storage
a – Fermentation at 30°C; b – Fermentation at 37°C

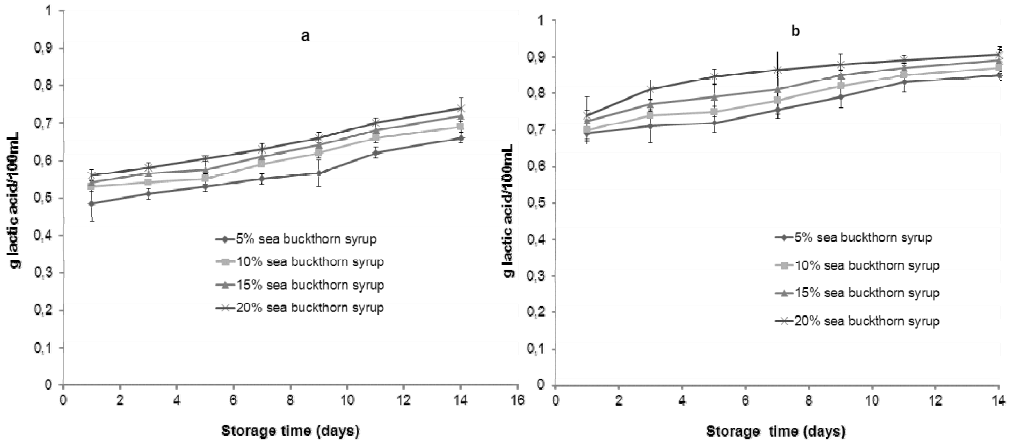

Figure 6. Titratable acidity changes in beverages during storage
a – Fermentation at 30°C; b – Fermentation at 37°C

Kovalenko and Briggs (2002) found 84.1-96% of water holding capacity in soy-based desserts. Mocanu *et al*. (2009) reported that the water holding capacity was 65.2 % in a sample with milk and sea buckthorn extract.
During storage, viable cell counts and acidity increased, while pH and water holding capacity decreased with the increase in syrup concentration percent at both temperatures.

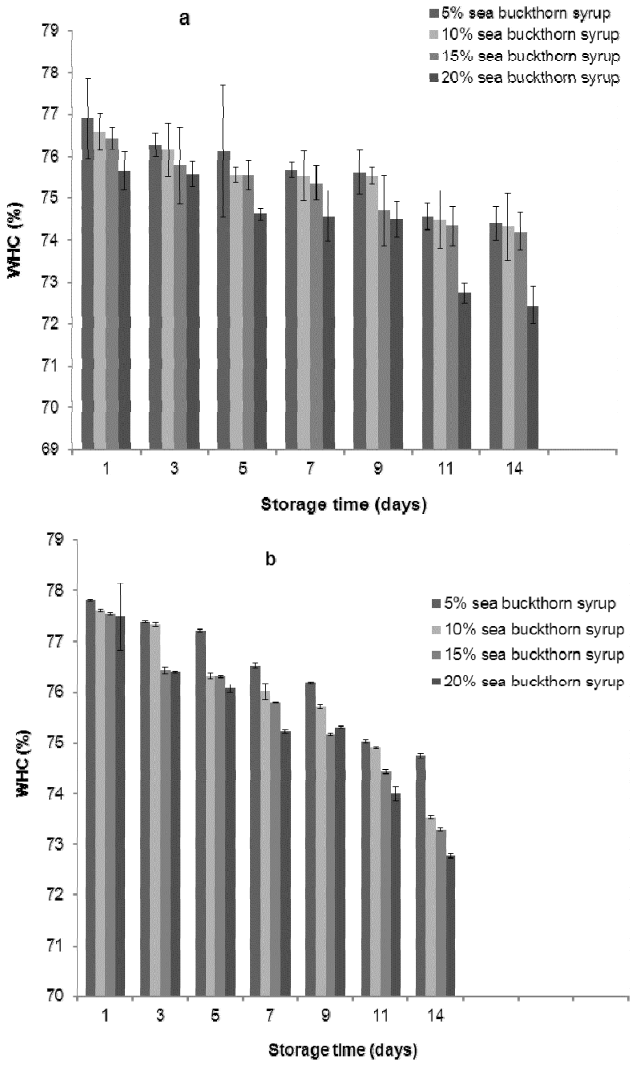

Figure 7. Water holding capacity of the beverages during storage
a – Fermentation at 30°C; b – Fermentation at 37°C

Survival of Bb-12® strain under gastric conditions

Taking into account the results previously presented, the survival in simulated gastric juice of the *Bb-12*® strain, after 7 days of storage of fermented products has been analyzed. Resistance to adverse gastrointestinal tract conditions and the ability to adhere to human epithelial intestinal cells are some of the *in vitro* tests recommended according to the guidelines of the FAO/WHO (2006), for the

selection of probiotic bacteria before studying their *in vivo* functionality by means of animal models and/or human intervention trials. The effect of simulated gastric juices on the viability of *Bb-12*® strain is presented in Figure 8a and b. The *Bb-12*® strain had high survival rates in simulated gastric juice for both temperatures at fermentation ($p<0.05$). The results showed that in the highest survival effect was identified in case of the beverage with 20.0% sea buckthorn syrup fermented at 37°C. As it can be seen in Figure 8, the entire beverage showed progressive reduction in viability during 120 min of simulated gastric transit. Viability decreased from 100.0% to 78.0% and to 77.0% relative to the initial concentration for the incubation temperature of 37°C (Figure 8 a) and 30°C (Figure 8 b), respectively. These results are comparable to the findings of Madureira *et al.* (2005) who studied the survival of probiotic bacteria in a whey cheese vector submitted to gastrointestinal tract conditions and indicated that *B. animalis Bo* and *B. animalis Bb-12* strains were the least affected when exposed to artificial gastric juice. Grimoud et al. (2010) reported that the bifidobacteria had high survival rates (about 95.0%) in simulated gastric juice. Reyes-Gavilán *et al.* (2011) found that *Bifidobacterium animalis* tolerated gastric juice, whereas *Bifidobacterium longum* showed poor survival under these conditions. This current study has demonstrated that the viability of *Bb-12*® strain is not affected by pH 2.0. The literature shows that a low final pH during bacterial growth induces an acid tolerance response (Lorca, & Font de Valdez, 2001). Van de Guchte *et al.* (2002) reported that the induction of pH stress response may protect probiotic bacteria not only from acid challenge but also from other stresses such as heat, osmotic or oxidative shocks.

Survival of Bb-12® strain under intestinal conditions

Figure 8a and b depicts the evolution of bifidobacteria counts during simulated intestinal juice. *Bb-12*® strain was resistant to the intestinal conditions (0.5% pancreatin and 4.5% bile salts). During simulated intestinal juice after 120 min, the rate of survival of *Bb-12*®strain decreased from 100% to 75.5% for the beverage with 20.0% sea buckthorn syrup and fermented at 30°C (Figure 8a). In Figure 8b it can be seen that for the same beverage fermented at 37°C, the rate of survival decreased from 100% to 75.6% ($p>0.05$).

Data from this study suggest that after 7 days storage in the beverage with soymilk and sea buckthorn syrup would not affect sensitivity of probiotics to bile or pancreatic enzymes. The results obtained in this study are in accordance with those reported by Grimoud et al. (2010) who found that the strains *B.bifidum 02*, *B. bifidum 20*, *B. breve R0070* and *B. pseudocatenulatum 14* had high survival rates in intestinal conditions except for *Bifidobacterium longum*, which showed a loss of viability >50% after treatment by intestinal fluid. Madureira *et al.* (2005) reported that the best viability profiles throughout the period of exposure to the bile salts was obtained for *B. animalis Bo* and *B. animalis Bb-12* strains.

The current study was based on an original approach that combined the effect of exposure to gastric juice, followed by the effect of exposure to bile salts on the viability of *Bb-12*® strain incorporated in a food matrix. This approach simulated

the two situations that prevail during transit through the gastrointestinal tract: passage through the stomach, followed by release of bile salts in the small intestine.

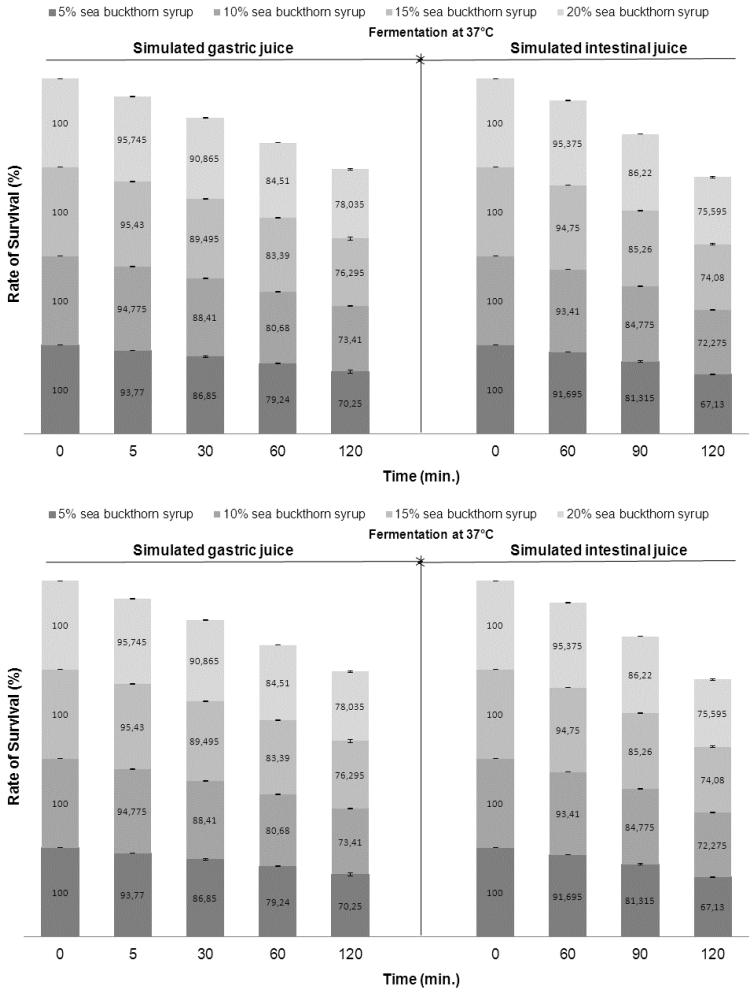

Figure 8. Survival of *Bb-12*® under gastric and intestinal conditions
a – Fermentation at 30°C; b – Fermentation at 37°C

To be considered as probiotic, microorganisms had to meet some selection criteria. Among all the in vitro parameters defined: human origin for human use, survival in gastrointestinal conditions, pathogen inhibition, adhesion to intestinal epithelial cells, etc. (Kalliomaki *et al.*, 2008) we chose to test the resistance to acidic and bile salt conditions along the digestive tract. Liong and Shah (2005) reported that the exposure to gastric and intestinal fluids along the digestive tract is the main stress that could decrease the viability of ingested probiotics. Sanders (2003) found that optimum delivery of viable microorganisms to the distal gut is critical for intestinal probiotic effects and acid resistance is required for food applications. Thus we

investigated the probiotics' resistance through a protocol simulating gastric and intestinal conditions as already described and *Bb-12*® strain was resistant to gastric conditions and to artificial intestinal fluid. The results obtained in the current study provide us with a first-level relevant selection criterion, highlighting the strongest resistance effects of the bifidobacteria in the present study.

Conclusions

In this study, a novel probiotic beverage based on soymilk and sea buckthorn syrup fermented with *Bifidobacterium bifidus* (*Bb-12*®, *Bb*) was produced. The growth rate, pH, titratable acidity during 12 h of fermentation as well as viability of selected strain, pH, titratable acidity, water holding capacity during storage at 4°C and survival of *Bb-12*® strain under simulated gastric and intestinal juices, were monitored. Results showed that the increase in syrup concentration caused the increase of viable cell counts and acidity and the decrease of pH during fermentation and during storage period viable cell counts and acidity increased, while pH and water holding capacity decreased with the increase in syrup concentration percentage at both temperatures.
Also, this study indicates that *Bb-12*® strain was able to survive *in vitro* in human gastrointestinal tract and the results obtained reveled that *Bb-12*® strain is a potential probiotic which can be used in a beverage based on soymilk and sea buckthorn syrup. The next step will be to verify whether these *in vitro* findings also apply to the *in vivo* situation in which potential probiotics must compete for mucosa receptors and nutrients with a plethora of intestinal microorganisms. This research proves that there are opportunities to develop a new beverage and a market of functional foods based on fermented soy milk and sea buckthorn syrup that incorporates health benefits.

Acknowledgments

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References

Adlercreutz, H. and Mazur, W. 1997. Phyto-estrogens and Western diseases. *Annals of Medicine*, **2**, 95-120.

Anderson, J.W., Johnstone, B.M., and Cook-Newell, M.E. 1995. Meta-analysis of the effects of soy protein intake on serum lipids, *The New England Journal of Medicine*, **333**, 276-282.

Angelov, A., Gotcheva, V., Hristozova, T., and Stoyanka, G. 2005.Application of pure and mixed probiotic lactic acid bacteria and yeast cultures for oat fermentation. *Journal of the Science of Food and Agriculture*, **85**, 2134–41.

Caplice, E. and Fitzgerald, G.F. 1999. Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, **50**, 131–149.

Cardarelli, H.R., Buriti, F.C.A., Castro, I.A., and Saad, S.M.I. 2008. Inulin and oligofructose improve sensory quality and increase the probiotic viable count in potentially synbiotic petit-suisse cheese. *LWT - Food Science and Technology*, 41, 1037–1046.

Chou, C.C., Hou, J.W. 2000. Growth of bifidobacteria in soymilk and their survival in the fermented soymilk drink during storage. *International Journal of Food Microbiology*, **56**, 113–121.

Chr. Hansen A/S, Hørsholm, Denmark: www.chr-hansen.com/, *accessed in 21.03.2012.*

Deshpande, S., Bargale, P., and Jha, K. 2008. Suitability of soymilk for development of shrikhand, *Journal of Food Science and Technology*, **45**(3), 284-286.

Desjardins, M.L., Roy, D., Goulet, J. 1990. Growth of bifidobacteria and their enzyme profiles. *Journal of Dairy Science*, **73**, 299–307.

Farnworth, E.R., Mainville, I., Desjardins, M.-P., Gardner, N., Fliss, I., and Champagne, C. 2007. Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. *International Journal of Food Microbiology*, **116**, 174–181.

FAO/WHO, 2006. Probiotics in Food. *Health and Nutritional Properties and Guidelines for Evaluation, FAO Food and Nutritional*, paper No. **85**, (ISBN 92-5-105513-0).

Gao, X., Ohlander, M., Jeppsson, N., Björk, L., and Trajkovski, V. 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophaë rhamnoides* L.) during maturation. *Journal of Agricultural and Food Chemistry*, **48**, 1485−1490.

Gardner, N.J., Savard, T., Obermeier, P., Caldwell, G., and Champagne, C.P. 2001. Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *International Journal of Food Microbiology*, **64**, 261–275.

Garro, M.S., De Valdez, G.F., and De Giori, G.S. 2004. Temperature effect on the biological activity of *Bifidobacterium longum CRL 849* and *Lactobacillus fermentum CRL 251* in pure and mixed cultures grown in soymilk. *Food Microbiology*, **21**, 511–518.

Grimoud, J., Durand, H., Courtin, C., Monsan, P., Ouarné, F., Theodorou, V., and Roques, C. 2010. *In vitro* screening of probiotic lactic acid bacteria and prebiotic glucooligosaccharides to select effective synbiotics. *Anaerobe*, **16**, 493–500.

Hoier, E. 1992. Säure- und gallentoleranz von *Lactobacillus acidophilus* und *Bifidobakterien*. *Lebensmittelindust Milchwirtsch*; **26**, 770–2.

Kale, R.V., Pandhare, G.R., Satwase, A.N., and Goswami, D. 2011. Effect of Different Concentration of Orange Juice on Quality Characteristics of Soya Milk Blended Beverage. *Journal of Food Processing & Technology*, **2**:140.

Kalliomaki, M, Salminen, S, and Isolauri, E. 2008.Positive interactions with the microbiota: probiotics. *Advances in Experimental Medicine and Biology*, **635**, 57-66.

Kamaly, K. M. 1997. Bifidobacteria fermentation of soybean milk. *Food Research International*, **30**, 675–82.

Kovalenko, I.V. and Briggs, J.L. 2002. Textural characterization of soybased yogurt by the vane method. *Journal of Texture Studies*, **33** (2), 105-118.

Kun, S., Rezessy-Szabó, J.M., Nguyen, Q.D., and Hoschke, Á. 2008. Changes of microbial population and some components in carrot juice during fermentation with selected *Bifidobacterium* strains. *Process Biochemistry*, **43**, 816–821.

Kwon, S., Lee, P.C., Lee, E.G., Chang, Y.K., and Chang, N. 2000. Production of lactic acid by *Lactobacillus rhamnosus* with vitamin-supplemented soybean hydrolysate. *Enzyme and Microbial Technology*, **26**, 209–215.

Laparra, J.M., Alegría, A. Barberá, R., and Farré, R. 2008. Antioxidant effect of casein phosphopeptides compared with fruit beverages supplemented with skimmed milk against H_2O_2-induced oxidative stress in Caco-2 cells. *Food Research International*, **41**, 773–779.

Lin, Fu-Mei, Chiu, Chiu-Hsia, and Tzu-Ming P. 2004. Fermentation of a milk–soymilk and *Lycium chinense* Miller mixture using a new isolate of *Lactobacillus paracasei* subsp. *paracasei NTU101* and *Bifidobacterium longum*. *Journal of Industrial Microbiology and Biotechnology,* **31**, 559–564.

Liong, M.T. and Shah, N.P. 2005. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains, *Journal of Dairy Science*, **88**, 55-66.

Lo, W.Y., Steinkraus, K.H., and Hand, D.B. 1968. Soaking soybeans before extraction and it affects chemical composition and yield of soymilk. *Food Technology*, **22**, 1188-1190.

Lorca, G.L. and Font de Valdez, G. 2001. A low-pH-inductible, stationary-phase acid tolerance response in *Lactobacillus acidophilus CRL 639*, *Current Microbiology*, **42**, 21–25.

Madureira, A.R., Pereira, C.I., Truszkowska, K., Gomes, A.M., Pintadoa, M.E., and Malcata, F.X. 2005. Survival of probiotic bacteria in a whey cheese vector submitted to environmental conditions prevailing in the gastrointestinal tract. *International Dairy Journal*, **15**, 921–927.

Malbaša, R.V., Milanović, S.D., Lončar, E.S., Djurić, M.S., Carić , M.-D., Iličić, M.D., and Kolarov, L. 2009. Milk-based beverages obtained by Kombucha application, *Food Chemistry*, **112**, 178–184.

Mathur, S., 2004. Soybean the wonder legume. *Beverage Food World*, **31**(1), 61-62.

Meile, L., Ludwig, W., Rueger, U., Gut, C., Kaufmann, P., Dasen, G., *et al.* 1997. *Bifidobacterium lactis* sp. *nov*., a moderately oxygen tolerant species isolated from fermented milk. *Systematic and Applied Microbiology*, **20**, 57–64.

Mocanu, G.D., Rotaru, G., Botez, E., Vasile, A., Gitin, L., Andronoiu, D., and Nistor, O. 2009. Research concerning the production of a probiotic dairy product with added medicinal plant extracts. *The Annals* of the University Dunarea de Jos of Galati Fascicle VI – *Food Technology*, New Series Year III (XXXII). Paper presented at the International Symposium *Euro - aliment 2009*, 9th – 10th of October 2009, Galati – ROMANIA.

Nancib, N., Nancib, A., Boudjelal, A., Benslimane Blanchard, C.F., and Boudrant, J. 2001. The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei* subsp. *rhamnosus*, *Bioresource Technology*, **78**, 149–153.

Peeters, P.H., Keinan- Boker, L., Van Der Schouw, Y.T., and Grobbee, D.E. 2003. Phytoestrogens and breast cancer risk: Review of the epidemiological evidence. *Breast Cancer Research and Treatment*, **77**, 171-183.

Reyes-Gavilán, C.,G., Suárez, A., Fernández-García, M., Margolles, A., Gueimonde, M., and Ruas-Madiedo, P. 2011. Adhesion of bile-adapted *Bifidobacterium* strains to the HT29-MTX cell line is modified after sequential gastrointestinal challenge simulated in vitro using human gastric and duodenal juices. *Research in Microbiology*, **162**, 514-519.

Rozada-Sánchez, R., Sattur, A.P., Thomas, K., and Pandiella, S.S. 2008. Evaluation of *Bifidobacterium spp.* for the production of a potentially probiotic malt-based beverage. *Process Biochemistry*, **43**, 848–854.

Sanders, M.E. 2003. Probiotics: considerations for human health. *Nutrition Reviews*, **61**,91-99.

Saxelin, M., Grenov, B., Svensson,U., Fonde´n, R., Reniero, R., and Mattila-Sandholm, T. 1999. The technology of probiotics. *Trends in Food Science and Technology*, **10**, 387–92.

Scalabrini, P., Rossi, M., Spettoli, P., and Matteuzzi, D. 1998. Characterization of *Bifidobacterium* strains for use in soymilk fermentation. *International Journal of Food Microbiology*, **39**, 213–219.

Shah, N.P., 2001. Functional foods from probiotics and prebiotics. *Food Technology*. **55,** 46–53.

USP, National Formulary: www.uspnf.com, accessed 12.03.2012.

Van de Guchte, M., Serror, P., Chervaux, C., Smokvina, T., Ehrlich, S., and Maguin, E. 2002. Stress responses in lactic acid bacteria. *Antonie van Leeuwenhoek*, **82**, 187–216.

Wang, B., Xiong, Y., Wang, C. 2001. Physicochemical and sensory characteristics of flavoured soymilk during refrigeration storage. *Journal of Food Quality*, **24**, 513-526.

Wanita, Y.P., Hatmi, R.U., Djaafar, T.F., and Rahayu, dan S. 2009. Karakteristik fisikokimia yogurt kedelai dan kacang tanah (*Physic-chemical characterization of soybean and peanut yogurts)*. Proceeding Seminar Lactic Acid Bacteria and Culture Collection, 16-17 January, 2009. Yogyakarta. P 121-130.