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ORIGINAL ARTICLE

Synbiotic functional drink from Jerusalem artichoke juice fermented by probiotic *Lactobacillus plantarum* PCS26

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Abstract A probiotic strain *Lactobacillus plantarum* PCS26 was used to ferment Jerusalem artichoke juice. Growth kinetics of the bacterial strain was followed during juice fermentation both in flask and in laboratory fermentor. Jerusalem artichoke showed to be an excellent source of nutrients for *L. plantarum* PCS26 growth. The culture grew very well reaching more than 10^{10} cfu/ml in just 12 h. The pH changed from the initial 6.5 to 4.6 at the end of fermentation. The culture hydrolyzed fructooligosaccharides present in the Jerusalem artichoke juice, yielding fructose which was presumably consumed along with the malic acid as energy and carbon source. Lactic acid was the main metabolite produced in concentration of 4.6 g/L. Acetic and succinic acid were also identified. Sensory evaluation of the fermented Jerusalem artichoke juice and its mixtures with blueberry juice showed that the 50/50 % *v/v* mixture would be very well accepted by the consumers. Above 80 % of the panelists would buy this drink, and over 60 % were willing to pay more for it. Culture survivability in the fermented juices during storage at 4–7 °C was assayed by the Weibullian model. The product shelf-life was extended from 19.70 ± 0.50 days of pure Jerusalem artichoke juice to 35.7 ± 6.4 days of the mixture containing 30 % blueberry juice.

Keywords *Lactobacillus plantarum* · Jerusalem artichoke · synbiotic beverage · probiotic · Weibullian model

Research highlights
- Probiotic *L. plantarum* PCS26 was used to ferment Jerusalem artichoke juice
- The culture reached 10^{10} cfu/ml in just 12 h lowering the pH from 6.5 to 4.6
- *L. plantarum* PCS26 hydrolyzed the fructooligosaccharides present in the J. artichoke juice
- Mixtures of fermented J. artichoke juice and blueberry juice showed good sensory properties
- Culture survivability was assessed by Weibullian model and shelf life of 36 days is expected

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Introduction

Probiotic foods and beverages are gaining in popularity as more and more consumers become aware of their health benefits. In addition to dairy products, in particular yogurt, fruit and vegetable juices were found to be good vehicles to deliver probiotic microorganisms. Therefore, the development of such products is one of the research priorities for food industry, particularly in developed countries, where demand for vegetarian probiotic products is boosting (Betoret et al. 2012). However, the survival of the health-conferring microorganisms in these media remains a main challenge for manufacturers. As reported, prebiotics, non-digestible ingredients in foods, can improve the survivability of probiotics, live microorganisms which confer health benefits on the hosts when consumed in adequate amounts (Rezaei et al. 2014). Furthermore, when probiotics and prebiotics had been used together, a higher advantage to the host was noticed due to their synergistic action (Vitali et al. 2010). Like probiotics, prebiotics are also involved in treatment of irritable bowel syndrome and inflammatory bowel diseases (Ghouri et al. 2014).

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Jerusalem artichoke (*Helianthus tuberosus L.*) is a perennial plant from the sunflower family *Asteraceae* cultivated in North America, Europe, Asia and Australia (Paseephol et al. 2007). Although by name it is a type of artichoke, neither it originates from Jerusalem nor does it belong to the same genus widely known as artichoke plant. It comprises a great nutritive value which comes primarily from its storage carbohydrates, among which inulin is present in the highest concentrations (Jovanovic-Malinovska et al. 2014). When consumed, inulin is not digested in the upper digestive tract, but rather fermented by native microflora of the colon. Therefore it is considered as prebiotic which can affect both the composition and/or activity of the gastrointestinal microflora that confers health benefits to host (Slavin 2013). Jerusalem artichoke has low caloric value since it does not contain fats and starch. It is a good source of B-group vitamins, trace elements, in particular iron, and is well known for decreasing the levels of blood cholesterol and triglycerides (Grela et al. 2014). There is a large number of publications dealing with the fermentation of Jerusalem artichoke and generating different products, starting from organic compounds for analytical purposes to single-cell proteins (Gao et al. 2007). Jerusalem artichoke has been often used as substrate for bio-fuel production like ethanol and butanol by yeast fermentation (Sarchami and Rehmann 2014). It is also known as a raw material for inulin, fructose and oligosaccharides production in the medical and pharmaceutical industry (Jovanovic-Malinovska et al. 2015). Concerning its raw consumption, it is still mostly used as animal feed. However, due to its dietary properties, the presence of Jerusalem artichoke in human diets gradually evolves.

Although lactic acid fermentation of Jerusalem artichoke tubers dates back to 1942 (Andersen and Greaves 1942), it still remains an interesting topic. Dao et al. (2013) evaluated the application of enzymes for inulin hydrolysis and fermented the hydrolyzed Jerusalem artichoke tubers by *Pediococcus acidilactici* DQ2 to generate lactic acid with high productivity. Most of the lactic acid bacterial fermentations of Jerusalem artichoke tubers were performed after inulin hydrolysis to reducing sugars and by supplementing the media with growth promoting components (Cheng et al. 2014). In contrast, Choi et al. (2012) demonstrated that certain *Lactobacillus* species can efficiently ferment fructooligosaccharides with degree of polymerization up to 13 without any pretreatment.

Lactobacillus plantarum PCS26 was isolated from traditional Slovenian cheese and studied for its probiotic properties. Its extracellular metabolites showed several positive effects on the gut epithelial cells during in vitro tests (Dimitrovski et al. 2014). Therefore, the primary aim of this study was to ferment Jerusalem artichoke juice, without any pretreatments or supplementations, with the probiotic bacterium, *Lactobacillus plantarum* PCS26 and to create a functional synbiotic drink with acceptable sensory properties. The indigenous presence of inulin in the Jerusalem artichoke juice and the probiotic organism would result in a synergistic action on human health. The second goal was to scale up the fermentation process and to evaluate the survivability of the culture using the Weibullian model.

Materials and methods

Preparation of Jerusalem artichoke juice

Jerusalem artichoke juice was prepared from fresh tubers obtained from local green market. After washing and roughly pealing, the tubers were cut into smaller pieces (1–2 cm^3) and fed to the kitchen juicer. The juice was filtered through cheese cloth to remove the debris and blanched in boiling water for 2 min. The soluble solids were measured by refractometer (around 20 % *w*/*v*) and adjusted to 10 % (*w*/*v*) by dilution (approximately 1:2) with sterile distilled water.

Strain and cultivation

Lactobacillus plantarum PCS26 (Deposited at Microbial Strain Collection of Latvia, accession number: PCS 26 (P 975)) (PathogenCombat 2011) was kept at −20 °C and revitalized by overnight growth in de Man, Rogosa and Sharpe (MRS) broth (Merck, Whitehouse station, New Jersey, USA) at 37 °C using semi-anaerobic conditions. Overnight preculture of *L. plantarum* PCS26 incubated in MRS broth at 37 °C was used as inoculum. Flask fermentations were carried out with 300 mL Jerusalem artichoke juice in a 500-mL Erlenmeyer flask placed on a rotary shaker (120 rpm) at 37 °C for about 35 h. Inoculation with 30 μL of the probiotic preculture was sufficient to result into initial viable count of about 10^5 cfu/mL in all fermentations.

Scaling up of the fermentation of the Jerusalem artichoke juice was carried out in 1-L laboratory fermentor (B. Braun Biotech International, Melsungen, Germany). The sterilized fermentor was filled with 800 mL Jerusalem artichoke juice and inoculated with 150 μL of overnight MRS preculture to reach initial viable count of 10^5 cfu/mL.

All fermentations were performed in triplicates. Samples were taken aseptically at appropriate time intervals to evaluate the viable count and pH value.

Sample analysis

The number of viable cells was measured by standard colony counting method. After a series of appropriate dilutions, samples were plated on MRS agar and incubated at 37 °C for 48 h before colony counting. pH of the fermentation broth was measured with a pH-meter (Sartorius PB-11, Göttingen, Germany).

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Agilent 1200 High Performance Liquid Chromatography (HPLC-Agilent Technologies, Inc., Santa Clara, USA) was used for quantification of reducing sugars in the samples. Separation of the sugars was done on Supelcosil LC-NH_2 column, 250 × 4.6 mm, 5 μm particle size, (Supelco analytical, Sigma Aldrich Group, Taufkirchen, Germany) using isocratic mobile phase acetonitrile/water =75/25 (*v*/*v*) at 40 °C (Muntean and Muntean 2010). Refractive index detector, thermostated at 40 °C, fed the software (Agilent ChemStation) with data for analysis. The run time was 15 min at 1.0 mL/min flow of the mobile phase.

Identification of inulin (degree of polymerization >10) and other fructooligosaccharides with lower degree of polymerization was carried out using thin layer chromatography according to Jovanovic-Malinovska et al. (2014) with minor adjustments. The samples were diluted (1:5) and applied in quantity of 2 μL on a silica gel 60 F_{254} plates (aluminium sheets 20 × 20 cm, Merck, Darmstadt, Germany). Aqueous solutions of 1 % *w*/*v* inulin, fructose, glucose and sucrose were used as standards. Solvent system acetonitrile/water (75:25 *v*/*v*) was used as mobile phase to separate the carbohydrates at room temperature. Since this mobile phase did not result into satisfactory separation of fructose and glucose, another mobile phase with higher concentration of acetonitrile (85:15 *v*/*v*) was also tested, and it successfully separated the monosaccharides. Unfortunately, with this mobile phase the separation of fructooligosaccharides deteriorated. Therefore, these two mobile phases of acetonitrile/water, 75:25 *v*/*v* and 85:15 *v*/*v*, were used on two separate plates to analyze the components. Spots were visualized by spraying the plates with 5 % *w*/*v* phenol in 10 % *v*/*v* sulphuric acid followed by heating at 120 °C for 5 min. The analysis was done in triplicate.

The concentration of the organic acids was determined using a Shimadzu Prominence Liquid Chromatography (Shimadzu corp., Kyoto, Japan). The separation of the compounds was achieved at 55 °C using Aminex HPX-87 H column, 300 mm × 7.8 mm ID, 5 μm particle size (Bio-Rad Laboratories, California, USA) according to the manufacturer instructions. Isocratic elution was applied with a mobile phase consisting of 2.5 mM H_2SO_4 at a flow rate of 0.6 mL/min. The wavelength selected for detection purposes was 214 nm and the measured data were processed by Class VP 7.3 software.

The concentrations of the measured components were calculated from the peak areas using appropriate standard curves generated with external standards. The presented data are average values of three independent measurements and the standard deviations are shown as error bars.

Sensory evaluation

Sensory evaluation of the fermented Jerusalem artichoke juices was performed in two parts. In the first part the preliminary sensory analysis was carried out in two sets of tests. The first set of test consisted of evaluating the taste, color and smell of the pure JA juice and its mixtures with a wide variety of commercial 100 % vegetable and fruit juices, such as: beet root, carrot, apple, orange, lemon, blueberry, pineapple, cherry, raspberry and pear, all purchased from the local grocery store. The tasting and the evaluation was done by 3 trained judges, food engineers, working on development of new commercial drinks. After the first set of tests, the number of possible commercial juices for mixing was reduced to 5. After tasting different ratios of blueberry, pineapple, cherry, raspberry and beet root juice with the JA juice, the blueberry (100 % fruit) was chosen as a best option for the mixtures.

In the second part, mixtures of fermented Jerusalem artichoke juice with blueberry juice (100 % fruit) were evaluated for their sensory quality in a standardized test room. Three mixtures were prepared containing different percentage of Jerusalem artichoke juice: 50 %, 60 % and 70 %. Each of them was served in a randomized order in a white plastic cup labeled with three digit random number. The panel consisted of 19 random panelists selected from a larger group of possible testers due to their declaration as frequent juice drinkers willing to pay more than the average cost for juice, 1.5 ± 0.5 euros/L, for products that satisfy their needs and taste. Among the 19 panelists, 10 were women and 9 were men in the age range from 30 to 60 years. Before the tasting, the juices were introduced to them and they were briefed on the attributes that should be evaluated. The acceptance of color, smell and taste as well as overall acceptance were graded from 1 (not acceptable) to 5 (very good). Afterwards, two more questions with offered answers followed: 1. would you buy this product? (yes/no) and 2. are you ready to pay more for it than the regular price for juice? (yes/no).

L. plantarum PCS26 viability during storage of fermented Jerusalem artichoke juice

The pure fermented Jerusalem artichoke juice and its mixtures with blueberry juice were refrigerated (4-7 °C) at the moment when *L. plantarum* PCS26 reached the maximal cell density. Samples were collected at regular time intervals during the following 16 days and viable count was determined. Weibull distribution, nonlinear survival model (Equation 1) (van Boekel 2009), was fitted to the data and used to estimate the storage time by which the probiotic juice would retain viable count above 10^6 cfu/mL.

$$log\frac{N}{No} = -b \cdot t^n \quad (1)$$

N and No represent the viable count at time t and at $t = 0$, respectively; $b = \frac{1}{2.303} \cdot \left(\frac{1}{\alpha_w}\right)^{\beta_w}$, and $n = \beta_w$ where α_w and β_w are the two parameters of the distributions: α_w is a scale

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parameter (a characteristic time) and β_w is the so-called shape parameter.

Statistical analysis

The growth curves were plotted using representative results from several fermentations where data were average values of triplicate measurement of the samples. The presented data of the HPLC analysis are average values of three independent fermentations. The standard deviations are shown as error bars. Sensory evaluation data were statistically analyzed using Anova with Tukey post-hoc test (SPSS software, IBM Corporation, Armonk, New York, USA) for determination of statistically significant difference between the two populations of values at 95 % confidence interval. The equality of variances was checked by the Levine's test. Binary logistic regression was used to test which sensory attribute influenced the panelists' decision the most, in terms of buying and paying more for the synbiotic drink compared to regular juices.

Results and discussion

Flask fermentation

Cell concentration and pH

The viable count and pH development during the *L. plantarum* PCS26 growth in Jerusalem artichoke juice is presented in Fig. 1. Two growth phases can be noticed separated by a very short lag phase between 11.5 and 12.5 h from the start of the fermentation. This, what seems to be a diauxic growth, started after 7 h lag phase during which the culture adapted to the environment. Throughout the first growth phase the culture grew 4 log cycles in 4.5 h, reaching 6.3×10^9 cfu/mL with specific growth rate (μ) of 0.85 h^{-1}. Minor change of the pH value, from 6.7 to 6.2, was noticed at this phase. The second growth phase was characterized by significantly slower change of the viable count and ended after 19.5 h from the start of the fermentation. The culture grew 2 log cycles in 7 h reaching 3.1×10^{11} cfu/mL with μ of 0.24 h^{-1}. After relatively short stationary phase of 4 h, ending in total fermentation time of 23.5 h, the culture started decreasing its viable count. During the second growth phase a rapid and pronounced decrease of pH was noticed reaching pH 5.3. The pH decreasing trend continued all through the stationary phase at a slower rate, attaining final value of pH 4.7 at the end of the fermentation.

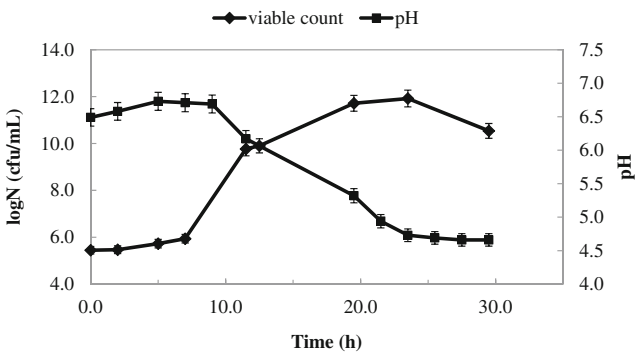

Fig. 1 Bacterial viable count and pH during flask fermentation of Jerusalem artichoke juice by *L. plantarum* PCS26

The diauxic growth of *L. plantarum* when cultivated on complex carbohydrates was also noticed by Hasan and Durr (1974). Goh et al. (2007) observed the same growth pattern for *L. paracasei* in the presence of fructooligosaccharides and limited amount of glucose in the medium (0.1 %). They concluded that oligosaccharides are hydrolyzed extracellularly with the cell wall associated enzyme β-fructosidase, induced by the presence of inulin, sucrose and fructose in the medium. Recently, several studies were published about different *Lactobacillus* species that utilize fructooligosaccharides as energy source (Endo et al. 2012; Zubaidah 2013). The fructooligosaccharides consumption by *L. plantarum* WCFS1 was studied in detail by Saulnier et al. (2007). They found that the main mechanism includes phosphoenolpyruvate transport system, β-fructofuranosidase, fructokinase and α-glucosidase. Trisaccharide 1-kestose was preferentially utilized in comparison to the tetrasaccharide nystose and the pentasaccharide fructofuranosylnystose.

Inulin and other fructooligosaccharides

The analysis of the Jerusalem artichoke juice by thin layer chromatography indicated a complex composition of the carbohydrates (Fig. 2). As can be noticed from Fig. 2a (lines 1 and 2), Jerusalem artichoke juice consists of sucrose, fructooligosaccharides of different degree of polymerization and inulin. Minor amounts of fructose and glucose, not detectable by thin layer chromatography, might be present as well. There was no major change in the carbohydrate composition as a result of the blanching process. Similar results for the carbohydrate composition of the Jerusalem artichoke were obtained by Jovanovic-Malinovska et al. (2014). The samples of diluted Jerusalem artichoke juice (10 % *w*/*v*), fermenting Jerusalem artichoke juice during the exponential phase and fermenting Jerusalem artichoke juice during stationary phase were analyzed in lines 3, 4 and 5, respectively. It can be observed that during the fermentation certain amounts of monosaccharides were generated. To separate fructose and glucose, the latter three samples (lines 3, 4 and 5) were additionally analyzed using solvent system acetonitrile/water in ratio 85/15 *v*/*v*. The resulting thin layer chromatogram revealed that mostly fructose was generated (Fig. 2b).

These results suggested that *L. plantarum* PCS26 possesses an extracellular enzymatic system able to hydrolyze the fructooligosaccharides into their building monomers. This finding

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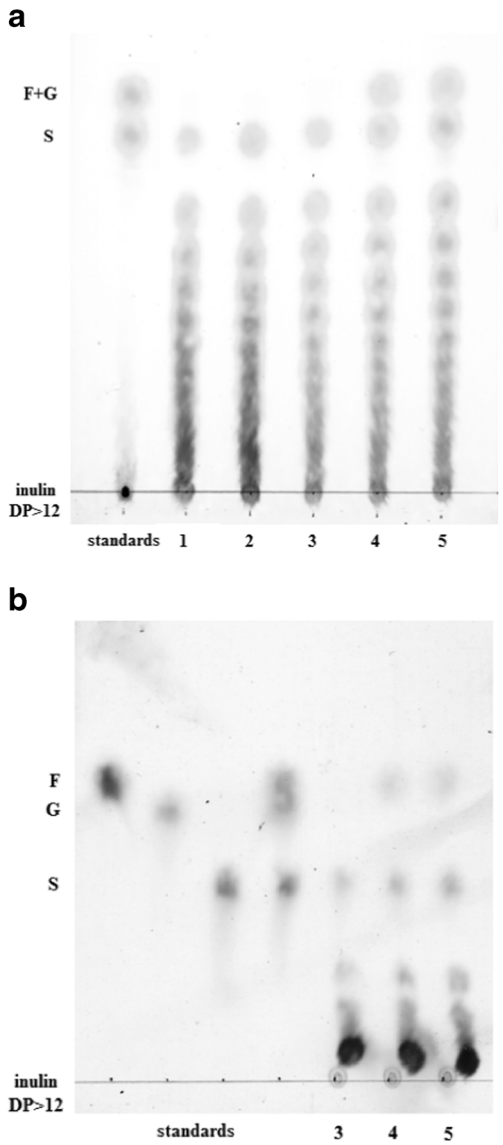

Fig. 2 Thin layer chromatography of Jerusalem artichoke juice carried out with two acetonitrile/water solvent systems: **a** 75/25 *v/v* **b** 85/15 *v/v*. 1 – before blanching, 2 – after blanching, 3 – after dilution, 4 – during fermentation (exponential phase), 5- during fermentation (stationary phase). F- fructose, G – glucose, S – sucrose, DP – degree of polymerization

is in agreement with a behavior of other *Lactobacillus* species which could ferment Jerusalem artichoke tubers without acidic or enzymatic inulin hydrolysis prior to fermentation (Choi et al. 2012). The preference between different fructooligosaccharides such as 1-kestose (GF_2) and nystose (GF_3) was studied for different *Lactobacillus* species by Endo et al. (2012).

Organic acids production during fermentations

The concentrations of malic and lactic acid during the fermentation of Jerusalem artichoke juice by *L. plantarum* PCS26 are depicted in Fig. 3. The concentration of malic acid, initially

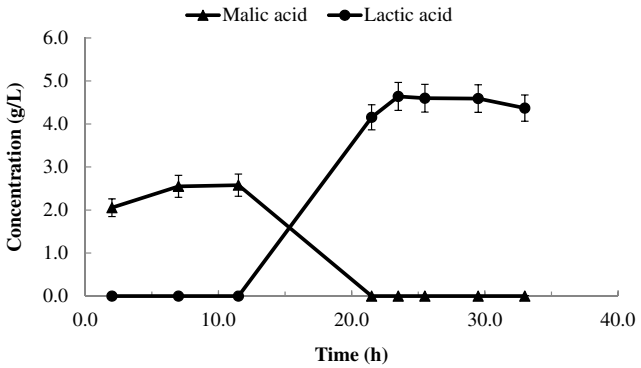

Fig. 3 Malic and lactic acid concentration during the fermentation of Jerusalem artichoke juice by *L. plantarum* PCS26

present in the Jerusalem artichoke juice in total of 2.1 ± 0.2 g/L, started decreasing after 11.5 h, while the concentration of lactic acid started increasing. The onset of these changes was concomitant with the initiation of the second growth phase of *L. plantarum* PCS26. After 21 h, the concentration of malic acid was below the detection limit of the analytical technique whereas lactic acid sustained its increase until the 24th h reaching concentration of 4.6 ± 0.3 g/L. Theoretically, taking into account that during malolactic conversion 1 mol of malic acid converts into 1 mol of lactic acid, approximately 1.6 g/L of lactic acid could be generated by malolactic conversion alone. The remaining 3.0 g/L of lactic acid were presumably produced by consumption of the hydrolyzed fructooligosaccharides. The metabolism of fructooligosaccharides as energy and carbon sources can be undertaken by two major pathways: glycolysis (Embden-Meyerhof pathway), which is mostly used by the homofermentative lactic acid bacteria, and 6-phosphogluconate/phosphoketolase pathway mostly used by the heterofermentative bacteria (Axelsson 1998).

Other components were also detected during the fermentation, of which acetic and succinic acid were identified. Their concentrations, however, were below the quantification limits. The production of succinic acid is often found in heterofermentative lactobacilli due to the metabolism of the terminal glucose in inulin and the citrate (Axelsson 1998; Kaneuchi et al. 1988). These acids could be responsible for the small pH drop from 6.7 to 6.2 before the 11.5th h, when no lactic acid production was observed.

Cheng et al. (2014) tested the fermentative activity of *L. plantarum* CX-15 on Jerusalem artichoke extracts. They succeeded in increasing the cell growth and producing lactic acid in concentration of approximately 12 g/L at the end of the fermentation. This was achieved by medium supplementation with Mn^{+2}, which is an essential growth factor for lactic acid bacteria. Metabolites developed during fermentation of Jerusalem artichoke juice with different *Lactobacillus* strains (*L. plantarum*, *L. paracasei*, *L. casei*, *L. rhamnosus* and *L. curvatus*) were analyzed by Zalán et al. (2011). They found only three organic acids produced by the tested strains: lactic,

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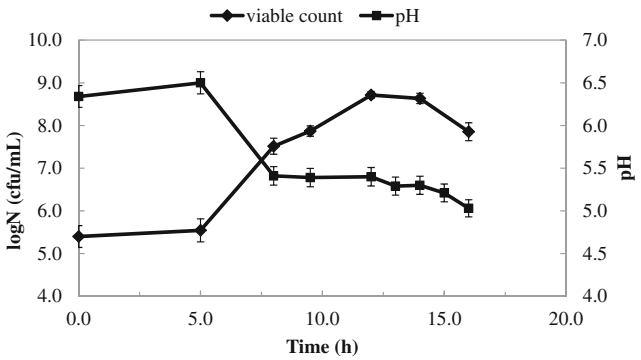

Fig. 4 Bacterial viable count and pH during the fermentation of Jerusalem artichoke juice by *L. plantarum* PCS26 in a laboratory fermentor

Fig. 5 Panelists that would buy and pay more for the drink prepared by mixing fermented Jerusalem artichoke juice with blueberry juice

acetic and succinic acid. All strains produced lactic acid, one strain did not produce acetic acid (*L. paracasei* subsp. *casei* SF1), and three strains produced succinic acid including *L. plantarum* 2142. Acetoin and diacetyl were also found to be produced in different concentrations. These two metabolites are produced in small concentrations during the citrate metabolism and they are very important flavoring components in the fermented products (Bassit et al. 1993).

Fermentation in a laboratory bioreactor

Scaling up of the Jerusalem artichoke juice fermentation for producing a synbiotic drink was conducted as a batch process in 1-L laboratory bioreactor. The viable count and pH changes during the fermentation of the juice are given in Fig. 4. Although the overall shape of the growth curve was similar, the growth kinetics was different from flask fermentation. After 5 h of lag phase the culture started increasing the viable count reaching 5.0×10^8 cfu/mL at the 12th h from the beginning of the fermentation. This growth of three log cycles was also divided into two growth phases observed by the change in the slope (specific growth rate) at the 8th h. Specific growth rates of 0.65 h^{-1} and 0.30 h^{-1} were calculated for the first and the second growth phase. The stationary phase lasted 2 h (12th - 14th h), and afterwards the cell concentration declined.

Unlike flask fermentation where pH had a decreasing trend throughout the diauxic growth and the stationary phase of the bacterium, the pH value in the fermentor broth dropped sharply from 6.4 to 5.4 within 3 h of the first growth phase (5th to 8th h).

It can be concluded that the culture in the fermentor had decreased growth when compared to the flask fermentation resulting into lower growth rates and lower maximal viable count. The results suggested that the production of lactic acid, as the major metabolite influencing the pH change, was also affected by the scaled up conditions in the reactor. It has been frequently reported that scaling up fails to achieve the same results concerning the biomass yield and other metabolites associated with its growth (Enfors et al. 2001). Part of the mechanisms responsible for these changes are the different systems of agitation and heating responsible for the homogeneity and the heat distribution in the fermentor (Hewitt and Nienow 2007).

Sensory evaluation of fermented Jerusalem artichoke juices

After preliminary sensory analysis (data not shown) it was inferred that product properties such as color, smell and taste of the Jerusalem artichoke juice need improvement. Therefore, it was decided to mix the fermented Jerusalem artichoke juice with a fruit or vegetable juice that will cover up some of its earth-like flavors and change the indigenous brown color into a more attractive one. The presence of lactic acid in the fermented juice was not mentioned as off-taste by any of the panelists. After several different juices (blueberry, pineapple, cherry, raspberry, beet root) had been tested, blueberry juice

Table 1 Intensity of sensory attributes of fermented Jerusalem artichoke juice mixed with different concentrations of blueberry juice*

Fermented Jerusalem artichoke juice/blueberry juice (%, *v/v*)	Color	Smell	Taste	Overall acceptance
0/100	4.98 ± 0.71^{a}	4.80 ± 0.95^{a}	4.01 ± 0.79^{a}	4.22 ± 0.91^{a}
50/50	4.68 ± 0.67^{a}	4.11 ± 1.02^{b}	4.17 ± 0.86^{a}	3.97 ± 0.86^{a}
60/40	4.16 ± 0.90^{ab}	3.72 ± 0.96^{b}	3.78 ± 1.06^{ab}	3.42 ± 0.93^{ab}
70/30	3.74 ± 0.99^{b}	2.78 ± 1.06^{c}	3.17 ± 1.25^{b}	2.75 ± 1.21^{b}

*the reported data are mean values ± standard deviation of 19 panelists. Different letters in a column designate significantly different means by Tukey test ($p < 0.05$)

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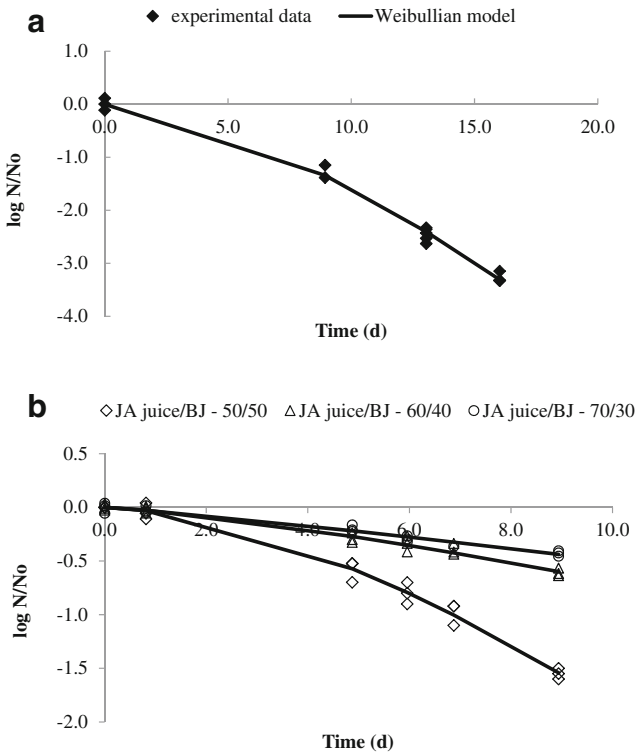

Fig. 6 Weibullian model fit to the viable count data of *L. plantarum* PCS26 cells in **a** Jerusalem artichoke juice and **b** mixed drinks prepared from Jerusalem artichoke (JA) juice and blueberry juice (BJ), during refrigerated storage at 4–7 °C

(100 % fruit) was selected as the best option. The results of the sensory evaluation of the blueberry juice and the three mixtures containing 50 %, 60 % and 70 % fermented Jerusalem artichoke juice are presented in Table 1. The addition of blueberry juice enhanced the overall acceptance of the juices. The mixture with 50 % blueberry juice had significantly higher grades for all attributes compared with the mixture containing 30 % blueberry juice ($p < 0.05$). This is a good indication that the mixture with 50 % blueberry juice, having grades of 4 and above, could be well accepted by consumers.

The readiness of the panelists to buy the synbiotic mixed juices is presented in Fig. 5. Bearing in mind the fermentation process, it is believed that the price for these drinks would be higher than the price of any regular commercial juice. Most of the panelists chose the 50/50 mixture as the one they would preferably buy over the other mixtures. Over 80 % of the panelists answered that they would buy this drink and above 60 % were ready to pay more compared to regular fruit juice. It was interesting to notice smaller differences between the panelists who would buy the other two mixtures and those who would be willing to pay more for them. Around 63 % of the panelists stated that they would buy the drink with 40 % blueberry juice and 58 % would be prepared to pay more for it. For the mixture with 30 % blueberry juice, only 21 % of the panelists would buy and pay more for it. It can be assumed that for this group the health benefits of the drink were more important than its sensory attributes and price.

From the statistical results of the binary logistic regression it was evident that only taste had statistically significant influence on the willingness of the panelists to buy and pay more for the mixed juices. Color and smell were statistically not important in terms of influencing panelists' choice. It can be concluded that the added blueberry juice could cover earth-like flavor and brownish color of Jerusalem artichoke fermented juice, providing a synbiotic drink that could be well accepted by consumers.

L. plantarum PCS26 viability during cold storage

The survivability of the probiotic bacterium *L. plantarum* PCS26 during cold storage of fermented Jerusalem artichoke juice and its mixtures with blueberry juice is displayed in Fig. 6. The Weibullian model for the survival of the culture was fitted to the experimental data, and the parameters n and b were determined by least-squares method. The kinetic parameters as well as the storage time (t_{st}) estimates during which the probiotic concentration would stay above 10^6 cfu/mL, are presented in Table 2. As can be noticed, the drink prepared by mixing the fermented Jerusalem artichoke juice with blueberry juice in ratio 70/30 % *v*/*v*, would have the longest storage time of 35.7 ± 6.4 days. Blueberry juice, with pH 3.0, contributed to decreasing the initial pH value of the fermented Jerusalem artichoke juice (pH 5) into 3.8, 4.1 and 4.3 in the mixtures with 50 %, 40 %, and 30 % blueberry juice, respectively. Some components in the juices, like proteins, dietary fibers and metabolizable sugars can enhance the culture survival while others, like phenolic compounds, can have a negative influence on survival during cold storage (Corcoran et al. 2005; Nualkaekul and Charalampopoulos 2011). The presence of acids and the pH drop have also been found to significantly increase the sensitivity of probiotic cultures in fruit and

Table 2 Parameters of the Weibullian models for different Jerusalem artichoke juices

Juice/parameters		b (day)	n	t_{st} (day)
Fermented Jerusalem artichoke juice		0.05 ± 0.01	1.55 ± 0.09	19.70 ± 0.50
Fermented Jerusalem artichoke juice/blueberry juice, (%*v*/*v*)	70/30	0.04 ± 0.01	1.15 ± 0.13	35.70 ± 6.40
	60/40	0.03 ± 0.01	1.30 ± 0.14	22.50 ± 2.70
	50/50	0.04 ± 0.01	1.64 ± 0.10	10.50 ± 0.24

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vegetable juices (Sheehan et al. 2007). It can be tentatively concluded that a combination of these factors influenced the survivability of *L. plantarum* PCS26 and resulted in shorter storage times of the drinks with 50 % and 40 % blueberry juice compared to that with 30 % blueberry juice.

Probiotic milk products like yogurt are usually used for storage time comparison. According to the guidelines, these products should have commercial storage time of 30 days (Ibarra et al. 2012). Cruz et al. (2010) implemented a new methodology for determining the shelf-life of yogurt supplemented with probiotic bacteria. The key factor was the overall sensory acceptability of the product by consumers. They found out that a probiotic fruit yogurt with *Bifidobacteirum animalis* DN 173010 W would be acceptable for 75 % of the tested population even after 38 days. In our research, the criterion for the storage time determination was the concentration of the probiotic culture to be maintained above 10^6 cfu/mL, which according to the Codex guidelines is a minimum requirement for a probiotic product (Codex Alimentarius Commission 2003).

Conclusion

Probiotic bacterium *Lactobacillus plantarum* PCS26 was successfully cultivated in Jerusalem artichoke juice without any pretreatment i.e. enzyme/acid hydrolysis and supplementation. The presence of inulin, a well known prebiotic, in the fermented juice classified this drink as a synbiotic one. The culture attained 10^{10} cfu/mL in just 12 h of diauxic growth. Carbohydrate analysis showed that fructooligosaccharides were hydrolyzed by bacteria yielding fructose. Lactic acid was the main growth metabolite reaching 4.6 ± 0.3 g/L, though acetic and succinic acids were also produced in minor concentrations.

For the 50/50 % *v*/*v* mixture of Jerusalem artichoke juice and blueberry juice the best sensory properties were attained, as judged by a group of panelists. From the panelists, 80 % would buy this drink and over 60 % would even pay more than for a regular fruit juice. The culture survival during cold storage was estimated by the Weibullian model. Concentration of live bacteria in the fermented Jerusalem artichoke juice would retain the critical value of 10^6 cfu/mL for about 20 days. Addition of 30 % blueberry juice extended this time to 36 days.

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