

Viability of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 in Rice Pudding

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Summary

The purpose of this study was to determine the survival of two probiotic micro-organisms (*Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12) in a rice pudding, the impact of these bacteria on hygienic quality, and to verify the perspectives of the product with regard to consumer sensorial acceptance. The products were monitored for the microbial population, pH, titratable acidity and consistency, during storage at 4 ± 1 °C for up to 21 days. Sensory preference was also tested. Even though the viability of the probiotic bacteria was reduced over 21 days of storage, the viable cell concentrations were still sufficient to obtain the desired therapeutic impact. The counts of yeasts-moulds and *Staphylococcus* spp. decreased in samples with added probiotic bacteria. The sensorial properties of probiotic rice pudding demonstrated similar acceptability to the control up to 14 days and declined thereafter. Rice pudding was considered suitable food for the delivery of probiotic micro-organisms, with sufficient viability and acceptable sensory characteristics.

Key words: rice pudding, viability, *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* BB-12

Introduction

Currently, the increased interest in healthy living motivates people to consume functional foods and dietary supplements. Foods that are containing probiotic bacteria come to the forefront as having positive effect on health. These benefits include improving the gut microbial balance, stimulation of the immune system, reduction of blood cholesterol level, and reduction in the incidence of cancer, cardiovascular diseases, diarrhea and osteoporosis (Holzapfel and Schillinger, 2002; Marteau and Boutron-Ruault, 2002; Sanders, 2003; Heenan et al., 2004; Madureira et al., 2005a; Samaržija et al., 2009). The survival of probiotic micro-organisms during processing and storage besides the acceptance of the product by the consumers is the major criterion to determine the efficacy and the market success of the probiotic product. The health benefits are not only dependent on the choice of micro-

organism with specific therapeutic properties, but it is also essential that these live micro-organisms are consumed in sufficient quantities to exhibit the desired metabolic effects. Several authors have suggested that ingestion of 10^6 - 10^9 viable cells g^{-1} in the product at the moment of consumption is the minimum necessary concentration to cause a beneficial result (Gomes et al., 1995; Rybka and Kailasapathy, 1995; Blanchette et al., 1996; Gomes and Malcata, 1999; Vinderola et al., 2000).

There are many studies on pioneering products containing probiotic bacteria, especially on dairy products such as yogurt, cheese, whey and fermented milk (Blanchette et al., 1996; Gomes and Malcata, 1998; Gomes et al., 1998; Oliveira et al., 2001; Boylston et al., 2004; Ferreira and Favaro, 2004; Malcata et al., 2005; Madureira et al., 2005b; Maity et al., 2008; Matijević et al., 2009). In recent years researches focus is on alterna-

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tive products such as milk-based desserts (Ravula and Shah, 1998; Helland et al., 2004; Favaro-Trindade et al., 2006; Ares et al., 2008; Cardarelli et al., 2008; Magariños et al., 2008). There is a wide range of ready-to-eat dairy desserts differing in texture, flavour and appearance (Verbeke et al., 2006). Concerning consumer preferences a pleasant taste and attractive texture are essential for dairy products, and hence, these differences could influence the acceptability of the product (Hekmat and McMahon, 1992; Tarrega and Costell, 2006).

The liveliness of probiotic bacteria in dairy desserts and their impact on chemical, textural and sensorial characteristics have been examined (Kleiss et al., 1995; Ravula and Shah, 1998; Heenan et al., 2004; Helland et al., 2004; Aragon-Alegro et al., 2007; Cardarelli et al., 2008). However, the information on the effect of probiotic bacteria on growth of pathogen bacteria during storage are very limited (Shimamura et al., 2006; Magariños et al., 2007; 2008). Milk-based desserts have always been important and distinctive elements of Turkish food culture. Rice pudding, made generally with milk, sugar and rice, has shown a great market potential. The combination of milk with rice increases the nutritive value of the final product (Ayok, 2002).

The aims of this study were to evaluate i) the survival of two probiotic micro-organisms, *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12, ii) to determine the impact of these probiotic cultures on growth of pathogen micro-organisms

during storage, and iii) to verify the perspectives of the product with regard to potential for consumer sensorial acceptance in rice pudding over 21 days of storage.

Materials and Methods

Probiotic strains

The micro-organisms used consisted of freeze-dried DVS-type cultures of *Lactobacillus acidophilus* La-5 from WISBY Starter Cultures and Media Niebüll, Germany and *Bifidobacterium bifidum* BB-12 from Chr. Hansen's Laboratorium, Denmark. The working cultures were prepared by activation of frozen cultures. Powdered skimmed milk, from SUTAS Co., Bursa, Türkiye, reconstituted to 10.7 % (w/v) of total solids and autoclaved, was used to activate and obtain the cultures.

Preparation of probiotic starter culture inocula

The cultures of each micro-organism were prepared according to Ravula and Shah (1998) and Magariños et al. (2007), using 1 g of lyophilized culture in 100 mL of milk. To facilitate the activation of *B. bifidum* Bb-12, 0.05 % L-Cys-HCl was added to diminish the oxidation-reduction potential of the medium. To stimulate the growth, 2 % glucose and 1 % yeast extract were added. *B. bifidum* Bb-12 cultures were incubated at 37 ± 1 °C for 18 h under anaerobic conditions by the Anaerobic System Anaerogen (Oxoid) (Lapierre et al., 1992), whe-

Table 1. Composition of experimental rice puddings produced with probiotic bacteria
Tablica 1. Sastav eksperimentalnih sutlijaša proizvedenih s probiotičkim bakterijama

Ingredients/Sastav	Treatment/Tretiranje		
	Control Kontrola (C)	Probiotic I Probiotik I (PI)	Probiotic II Probiotik II (PII)
Pasteurized Cows' Milk Pasterizirano kravlje mlijeko	X	X	X
Milk Cream/Vrhnje	X	X	X
Invert Sugar Syrup/Invertni šećerni sirup	X	X	X
Skimmed Milk Powder Obrano mlijeko u prahu	X	X	X
Rice Flour/Rižino brašno	X	X	X
Rice/Riža	X	X	X
<i>L. acidophilus</i> LA-5		X	
<i>B. bifidum</i> BB-12			X

reas *L. acidophilus* LA-5 was incubated at the same temperature and time under aerobic conditions. The necessary inoculum, to give approximately 6 or 7 log cfu⁻¹ in rice pudding after inoculation, was calculated.

Production of rice pudding

Three pilot-scale rice pudding formulations denoted C, PI and PII were produced in triplicate. The ingredients used for the production of the three formulations are shown in Table 1.

Each batch of rice pudding was produced in amounts to obtain 4 kg of the final product. For this purpose, after weighing all ingredients individually, they were all mixed together, heated 80-85 °C until the powdered ingredients dissolved completely in a scraped surface heat exchanger, and cooled to 40 °C in an ice bath with continuous stirring. As soon as the mixture reached the desired temperature, calculated amounts of probiotic suspensions were added in trials PI and PII, in order to achieve concentrations of approximately 7 log cfu g⁻¹ in rice pudding at the beginning of the storage time (0 day). The inoculum of probiotic strains was evenly distributed in the rice pudding by mixing with a sterile domestic mixer for 50 s (model K1433, Arçelik Inc., Türkiye). The final product obtained was packaged in individual plastic cups, each one containing approximately 200 g of rice pudding, cooled and then stored at 4±1 °C for up to 21 days.

Analyses

Triplicate rice pudding samples from each batch were taken for microbiological, physico-chemical and sensory analysis at the following stages: immediately after inoculating the probiotic cultures in the dessert (0 day) and during the products' storage at 7, 14 and 21 days. The average cell counts of starter probiotic cultures were determined by plate count to evaluate the viability of cultures in the rice pudding.

The significance of differences among samples was determined by analysis of variance (ANOVA), using 99 % confidence intervals on random parcel. Trial pattern was run on each of the microbiological, physico-chemical and sensorial variables to disclose possible differences among the samples for the two factors "probiotic micro-organism used (treatment)" and "the time of storage". All analyses were

performed using the Minitab for Windows (Version 14) Statistical Software Package (Minitab Inc., State College, PA).

Physico-chemical analysis

The pH of rice pudding was measured by means of a digital pH-meter (Analyzer model 315i/SET, WTW, Germany). Titratable acidity was determined according to the method described by Kurdal et al. (2008) and expressed in terms of lactic acid concentration in normal solution (g/100 g). The consistency values of rice pudding were determined at 4±1 °C using a Bostwick Consistometer (Christison Particle Technologies Ltd., UK).

Microbiological analysis

The viability of probiotic micro-organisms were assessed by plate count method. *L. acidophilus* LA-5 was enumerated according to Vinderola and Reinheimer (1999) on MRS-Bile Agar (Ox bile Dried Pure, Merck, Germany) after 3 days of aerobic incubation at 37±1 °C. The population of *B. bifidum* BB-12 was enumerated on MRS-LP Agar (Lithium chloride, Sodium propionate) after 3 days of anaerobic incubation (Anaerobic System Anaerogen, Oxoid) at 37±1 °C as reported by Lapierre et al. (1992).

The populations of yeasts and moulds (Beuchat and Cousin, 2001), total *Staphylococcus* (Shimamura et al., 2006), total *Coliform* group bacteria and *Escherichia coli* (Kornacki and Johnson, 2001) were determined for all samples throughout storage. The colonies were counted and the results were expressed in logarithm of colony forming units per gram of product (log cfu g⁻¹).

Sensory evaluation

Ten panellists, selected depending on their availability and willingness to participate in the study, from staff of Department of Food Engineering, Uludag University, evaluated the organoleptic characteristics of the rice pudding samples, after preliminary training sessions. The sensorial evaluation sessions were carried out in individual booths between 10:00 and 11:00 a.m., under fluorescent light. Samples were presented in white plastic cups, containing approximately 200 g rice pudding per cup,

already removed from the refrigerator. The samples were coded with three-digit random numbers. The judges were asked to evaluate the coded samples (C, PI, and PII, all of them from the same replicate), using a 9-point balanced hedonic scale (1: dislike extremely - 9: like extremely) based on texture, firmness, aroma, colour, taste, and overall impression of each sample, described by Lawless and Heymann (1999) and Cardarelli et al. (2008). The samples were presented at random, and the evaluations were made in triplicate. Water was provided to judges to cleanse their palates between samples.

The panel was asked to note three terms for texture (mouth feel, viscosity, smoothness), two terms for firmness (uniform, syneresis), seven terms for aroma (natural, milky, creamy, fruity, sweet, rancid, acidic), three colour properties (white, cream-white, yellowish), and eight attributes encompassing taste (milky, creamy, fruity, sweet, rancid, acidic, astringent, metallic).

Results and discussion

The average cell counts of mother starter cultures of *L. acidophilus* LA-5 and *B. bifidum* BB-12, expressed as log cfu g⁻¹, reached values of 8.46±0.037 and 8.68±0.042, respectively (0*). The concentrations are the basic amount in probiotic inoculates added to rice puddings.

The variation of the logarithm of the probiotic bacteria in rice pudding samples and the standard deviation during 21-day storage at 4±1 °C were summarized in Fig. 1. The dilution of starter probiotic cultures in rice pudding samples resulted in a reduction of 0.98 logarithmic cycles in the case of *L. acidophilus* LA-5 and 1.21 log cycles for *B. bifidum* BB-12 in comparison to inoculated amounts.

The statistical analysis showed significant differences between viable cell counts of these two probiotic bacteria over time (P<0.01). One may observe that *B. bifidum* BB-12 cultures had a higher concentration during the period of storage than *L. acidophilus* LA-5, nevertheless, the viability of *L. acidophilus* (89.44 %) was less affected than *B. bifidum* BB-12 (87.40 %) due to the sensitivity of this strain to air during storage (Fig. 1).

Several studies have shown the diminution of at least one logarithmic cycle for probiotic bacteria in dairy desserts after 21 days of storage at 4±1 °C

(Hekmat and McMahon, 1992; Ferreira and Favaro, 2004; Helland et al., 2004; Magariños et al., 2008).

Recommendations for the minimum suggested viable cell counts for probiotic bacteria in the food at time of consumption are quite variable. In general, the food industry has applied the recommended level of 10⁶ cfu g⁻¹ at the time of consumption for *L. acidophilus* to Bifidobacteria and other probiotic bacteria (Roy, 2001). The minimum therapeutic daily dose for Bifidobacteria is usually considered as 10⁸-10⁹ viable cells, which could be achieved with a daily consumption of at least 100 g of fermented bioproduct containing between 10⁶ and 10⁷ viable cells g⁻¹ (Rasic and Kurman, 1983; Blanchette et al., 1996).

As it could be observed from Fig. 1, both probiotic bacteria maintained a population above this count during the whole storage period. Results indicated that probiotic rice puddings pioneered in the present study could have a potential to be used as carriers of *L. acidophilus* LA-5 and *B. bifidum* BB-12 in food systems, as similar to the literature depending on viability and activity of probiotic bacteria incorporated into dairy desserts (Helland et al., 2004; Heenan et al., 2004; Aragon-Alegro et al., 2007).

The impact of using probiotic culture on microbiota of rice pudding samples was evaluated by determining the population of the yeasts and moulds, total *Staphylococcus*, total *Coliform* group bacteria and *E. coli* during storage (Table 2). *E. coli* was not detected during the whole storage time. Yeast and moulds were determined at a level of 1.98-2.06 log cfu g⁻¹ immediately after inoculation. For PI and PII the counts of these micro-organisms decreased up to 14th day of storage in comparison to C, thereafter the counts increased probably due to reduction in counts of viable probiotic cells in rice puddings or reduction in formation of anti-microbial substances produced by probiotic strains. Several authors have previously reported that cumulative effects of anti-microbial agents, i.e. organic acids, hydrogen peroxide, various antibiotics and bacteriocins are responsible for inhibitory activity of probiotic cultures (Servin, 2004; Makras and Vyust, 2006; Parada et al., 2007). The administration of anti-microbial substances alters the microbial balance within a product.

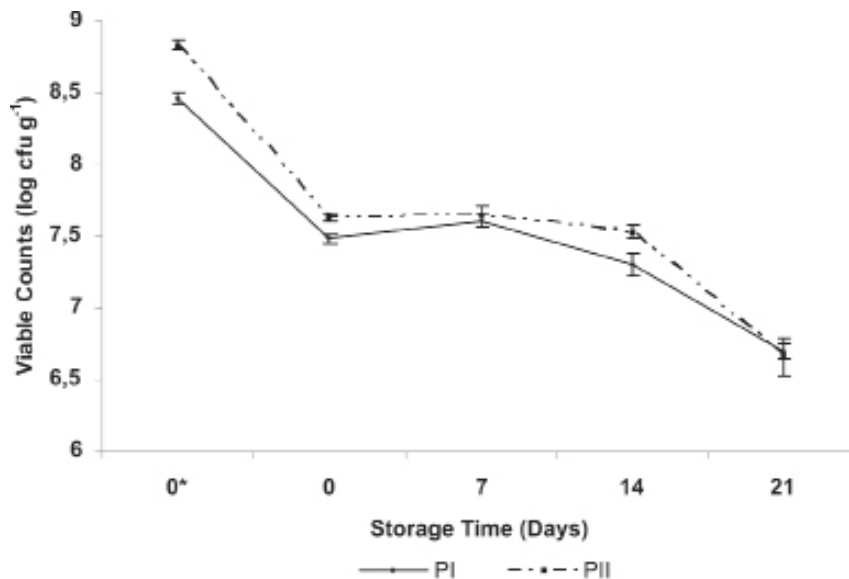


Fig. 1. The effect of dilution and storage of rice pudding on the viable counts of probiotic bacteria (log cfu g⁻¹); 0* indicates the viable cell counts' of mother probiotic cultures of *L. acidophilus* LA-5 (PI) and *B. bifidum* BB-12 (PII)

Slika 1. Utjecaj razrjeđivanja i skladištenja sutlijaša na broj živih probiotičkih bakterija (log cfu g⁻¹); 0* pokazuje broj živih bakterija u matičnoj probiotičkoj kulturi *L. acidophilus* LA-5 (PI) i *B. bifidum* BB-12 (PII)

Table 2. The population of micro-organisms in rice pudding samples during 21 days of storage (log cfu g⁻¹) (n=18)

Tablica 2. Populacija mikroorganizama u uzorcima sutlijaša tijekom 21 dana čuvanja (log cfu g⁻¹) (n=18)

	Treatments Tretiranje	Storage time (Days) Vrijeme skladištenja (dani)			
		0	7	14	21
Yeasts and moulds Kvasci i plijesni	C	1.98±0.003 ^{aC}	2.00±0.071 ^{aC}	3.30±0.283 ^{aB}	4.46±0.085 ^{aA}
	PI	2.00±0.071 ^{aAB}	1.92±0.028 ^{aB}	1.89±0.127 ^{bC}	2.08±0.113 ^{bA}
	PII	2.06±0.014 ^{aAB}	2.00±0.042 ^{aB}	1.98±0.085 ^{bB}	2.14±0.198 ^{bA}
<i>Staphylococcus</i> Total/Ukupno	C	1.74±0.028 ^{aB}	1.48±0.113 ^{aB}	1.55±0.071 ^{aB}	2.44±0.197 ^{aA}
	PI	1.00±0.283 ^{bA}	ND	ND	1.00±0.062 ^{bA}
	PII	1.70±0.042 ^{aA}	ND	ND	1.06±0.034 ^{bB}
<i>Coliform</i> Total/Ukupno	C	ND	ND	ND	1.35±0.122 ^{aA}
	PI	ND	ND	ND	ND
	PII	ND	ND	ND	1.13±0.116 ^{bA}
<i>E. coli</i>	C	ND	ND	ND	ND
	PI	ND	ND	ND	ND
	PII	ND	ND	ND	ND

ND - not detected/nije detektirano; ^{a,b}Different superscript lowercase letters denote significant differences (P<0.01) between different treatments/Različita mala slova označavaju značajne razlike (P<0,01) između različitih tretiranja; ^{A,B}Different superscript capital letters denote significant differences (P<0.01) between different times of storage/Različita velika slova označavaju značajne razlike (P<0,01) između različitih vremena skladištenja; C - control samples/kontrolni uzorci; PI - samples with LA-5/uzorci s LA-5; PII - samples with BB-12/uzorci s BB-12

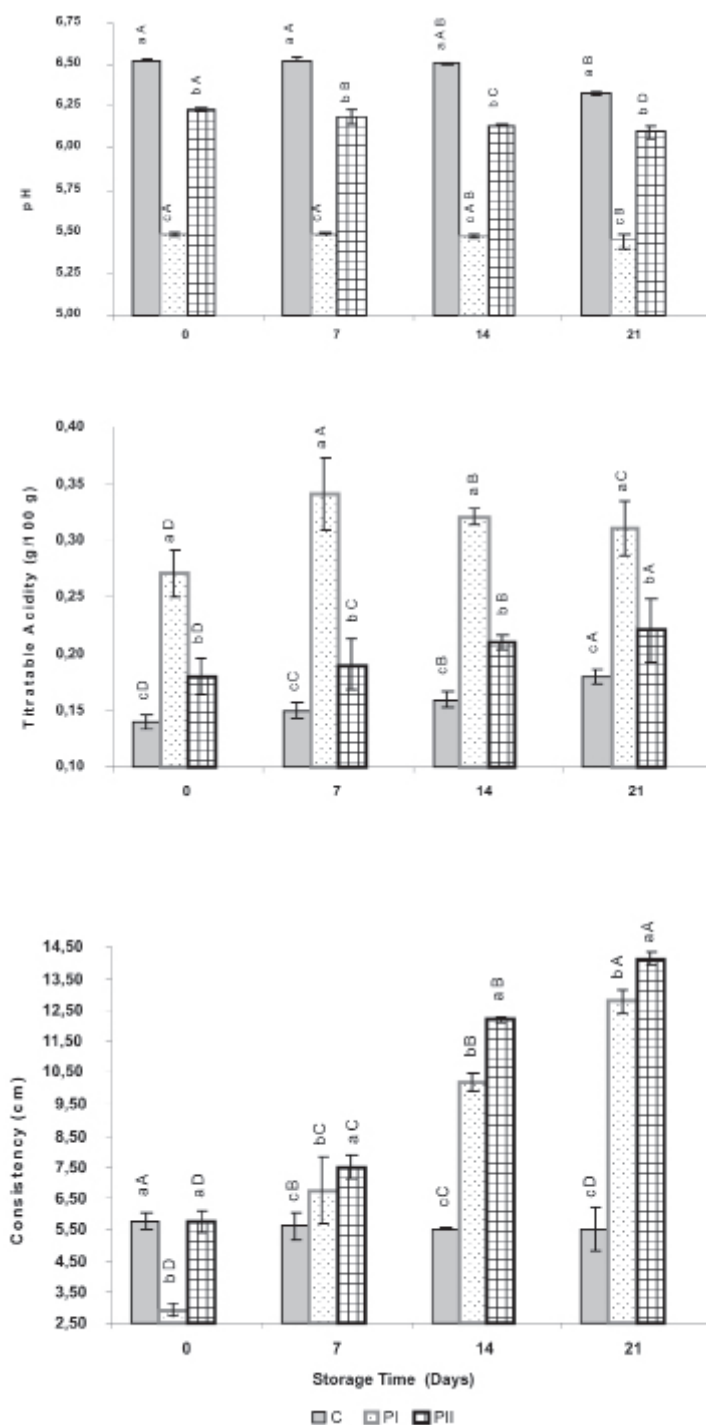


Fig. 2. Changes in (a) pH, (b) titratable acidity and (c) consistency of rice pudding during storage
Slika 2. Promjene u (a) pH vrijednosti, (b) titracijskoj kiselosti i (c) konzistenciji sutlijaša tijekom skladištenja

^{a,b}Different lowercase letters denote significant differences ($P < 0.01$) between different treatments/Različita mala slova označavaju značajne razlike ($P < 0.01$) između različitih tretiranja; ^{A,B}Different capital letters denote significant differences ($P < 0.01$) between different times of storage/Različita velika slova označavaju značajne razlike ($P < 0.01$) između različitih vremena skladištenja

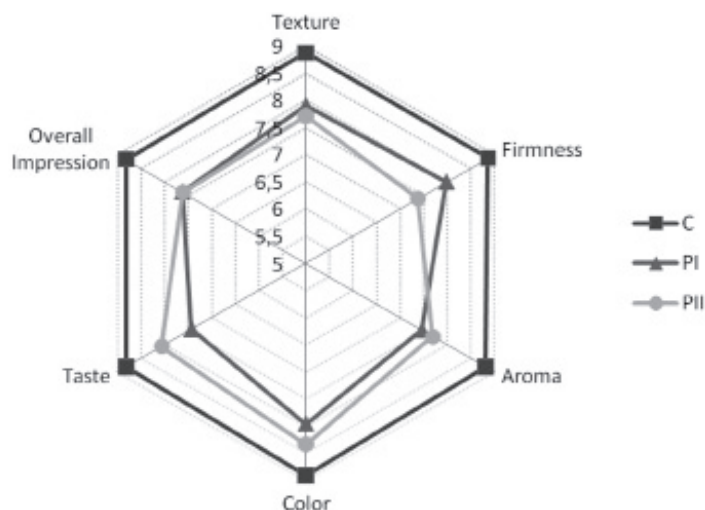


Fig. 3. Average sensory ratings of probiotic rice puddings (PI=LA-5 and PII=BB-12) throughout 21 days of storage in comparison with control (c)

Slika 3. Prosječne senzorske ocjene probiotičkog sutlijaša (PI=LA-5 i PII=BB-12) tijekom 21 dana čuvanja u usporedbi s kontrolnim uzorkom (c)

These micro-organisms compete for nutrients with pathogens and become the dominant flora through the produced inhibitory by-products. However, their growth is also related to pH decrease. When the growth and production of inhibitory substance of probiotic bacteria is reduced the antagonistic activity and thus the flora is altered, and the concentration of other micro-organism groups can increase.

Total *Coliform* group bacteria were not detected for PI, except in the case of PII these contaminants were detected at the end of 21 days of storage. This observation indicated that the use of *L. acidophilus* LA-5 is advantageous than *B. bifidum* BB-12 on hygienic quality of analyzed rice pudding samples. Similar results on the population of the contaminants in the chocolate mousse containing *L. paracasei* were reported by Aragon-Alegro et al. (2007).

The changes in pH values of rice pudding during storage for 21 days were given in Fig. 2a. The pH levels in probiotic rice pudding depended on the strain used. The pH values obtained in PI samples were significantly lower than those of control and PII samples, since *L. acidophilus* has higher acid formation ability and acid resistibility (Fig. 2a, b). In general, the three trials studied showed increasing values of acidity and decreasing values of pH.

The pH of the probiotic products is known to be influential on the growth and viability of probiotic culture, most notably Bifidobacteria (Hekmat and McMahan, 1992). The viscosity of the product under controlled conditions was measured as the Bostwick consistency, which is plotted in Fig. 2c. For C, the Bostwick consistency diminished with storage time, showing a significant increase in viscosity, however for PI and PII depending on the probiotic strains' activity consistency increased. The greater the Bostwick consistency values, the less viscous the product.

The judges found significant differences between control and probiotic rice puddings, however, the sensorial properties were not statistically different for PI and PII ($P < 0.01$) (Fig. 3). Control was for the most acceptable until day 14, scoring the maximum (like extremely) (data not shown). It obtained its lowest scores at the end of the period of 21 days, with a score corresponding to "like very much". Use of probiotic bacteria showed a score of "like very much" at the end of 7 days of storage, and decreased thereafter. PII was favoured for its taste, colour and aroma, however, due to occurrence of heterogenic texture and syneresis, PI was preferred for its textural attributes (Fig. 3, data not shown). No strange taste or aroma in the probiotic rice puddings was re-

ported. The panellists mentioned that the texture of the PI and PII were lower than C during the whole storage period, however, this difference would possibly not compromise the acceptability of probiotic rice puddings.

There are several studies indicating that probiotic micro-organisms affected the texture and flavour of the food product to which they were added (Aragon-Alegro et al., 2007; Cardarelli et al., 2008; Magariños et al., 2008). According to Helland et al. (2004) rice puddings containing *L. acidophilus* LA-5 and 1748, *B. animalis* BB-12, *L. rhamnosus* GG resulted in different taste profiles and overall acceptability.

Conclusion

In the present study, the survival of probiotic bacteria was studied in rice puddings for 21 days. Initial counts of the bacteria and their subsequent survival rates were determined. The inoculation of rice pudding with *L. acidophilus* LA-5 and *B. bifidum* BB-12 allowed survival rates of 89.44 and 87.40 % after being subjected to a storage period of 21 days. The results suggested that high levels of viable *L. acidophilus* LA-5 and *B. bifidum* BB-12 in rice pudding is a good source for probiotic bacteria delivery with appreciated sensory quality, leading to good perspectives for its future commercial production. Nevertheless *in vivo* studies are necessary to confirm its functionality, and further studies on use of some preservative or biopreservative that is not harmful for the viability of probiotic bacteria should be considered.

Preživljavanje bakterija *Lactobacillus acidophilus* LA-5 i *Bifidobacterium bifidum* BB-12 u sutlijašu

Sažetak

Svrha ovog rada bila je utvrditi preživljavanje probiotičkih mikroorganizama (*Lactobacillus acidophilus* LA-5 i *Bifidobacterium bifidum* BB-12) u sutlijašu, utjecaj tih bakterija na higijensku kvalitetu i potvrditi perspektivu proizvoda glede senzorskog prihvaćanja potrošača. U proizvodima je praćena

populacija mikroorganizama, pH, titracijska kiselost i konzistencija tijekom skladištenja na 4 ± 1 °C u trajanju do 21 dan. Također su testirana senzorska svojstva. Iako je preživljavanje probiotičkih bakterija smanjeno tijekom 21 dan skladištenja, broj preživjelih mikroorganizama bio je dovoljan da se dobije željeni terapijski učinak. Broj kvacusa, plijesni i *Staphylococcus* spp. smanjen je u uzorcima s dodatkom probiotičkih bakterija. Sutlijaš se može smatrati pogodnim probiotičkim proizvodom prihvatljivih senzorskih svojstava.

Cljučne riječi: sutlijaš, preživljavanje, *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* BB-12

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