PAPER

EFFECTS OF DIFFERENT PREBIOTICS ON VIABILITY UNDER *IN VITRO* GASTROINTESTINAL CONDITIONS AND SENSORY PROPERTIES OF FERMENTED MILK

E. KOCER and G. UNAL*
Ege University Faculty of Agriculture, Department of Dairy Technology, 35100, Bornova, Izmir, Turkey
*Corresponding author: Tel.: +90 2323112732
E-mail address: gulfem.unal@ege.edu.tr

ABSTRACT

The effect of inulin, polydextrose and Hi-maize resistant starch on probiotic viability under simulated gastrointestinal conditions and sensory characteristics of ABT (*Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12, and *Streptococcus thermophilus*) fermented milk was investigated during 28 days. *B. animalis* presented higher survival rates under gastrointestinal stress than *L. acidophilus*. Although inulin addition enhanced viable counts of *L. acidophilus* more than those of other prebiotics during the gastric and enteric-1 phases, all samples showed similar *L. acidophilus* survival after the enteric-2 phase. The supplementation with Hi-maize indicated a protective effect on *B. animalis* tolerance to simulated gastrointestinal conditions on the 14th and 28th days. Inulin or Hi-maize did not affect the sensory properties of fermented milk whereas the product supplemented with polydextrose had the lowest scores specifically at the end of the storage period.

Keywords: *in vitro* gastrointestinal survival, fermented milk, prebiotic, probiotic

1. INTRODUCTION

Probiotics are live microorganisms, which when administered in adequate amount, confer a health effect on the host (FAO/WHO, 2002). To exert their functional properties, probiotics need to be delivered to the desired sites in an active and viable form. Probiotic viability should be at a minimum level during the shelf life, which can range from 10^6 to 10^8 cfu/mL, and must survive through the gastrointestinal (GI) tract by tolerating acid, bile, and GI tract enzymes (pepsin, lipase, pancreatin) and then adhere and colonize the intestinal epithelium (CASAROTTI *et al.*, 2015).

Most studies on probiotics have focused on lactic acid bacteria, especially the genus *Lactobacillus* and *Bifidobacterium*. *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* are the frequently used probiotics in the production of fermented milks. Using different probiotic combinations (RANADHEERA *et al.*, 2014), microencapsulation (DE ARAUJO ETCHEPARE *et al.*, 2016), supplementation with prebiotics (OLIVEIRA *et al.*, 2009; NOBAKHTI *et al.*, 2009; CASAROTTI and PENNA, 2015), and the use of different matrices (CASAROTTI *et al.*, 2015) have been proposed to increase probiotic survival in the GI tract and in the product until the time of consumption. Among these options, the addition of prebiotics has been preferred in many studies to increase probiotic viability and their resistance to GI conditions.

Prebiotics are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (GIBSON *et al.*, 2004). Different prebiotics (e.g., inulin, Hi-maize resistant starch, lactulose, polydextrose, β-glucan, lactilol, and maltodextrin) have been used as supplements in the manufacture of fermented dairy products to improve the growth and activities of selected *Lactobacillus* and *Bifidobacterium* strains (OLIVEIRA *et al.*, 2009; NOBAKHTI *et al.*, 2009; HEYDARI *et al.*, 2011) in related studies.

Inulin, a compound extracted from the chicory root, is a fructan and cannot be digested by α-amylase or other hydrolases in the upper section of the intestinal tract (OLIVEIRA *et al.*, 2009; GONZÁLEZ-HERRERA *et al.*, 2015). Aside from its prebiotic property, inulin also presents some technical characteristics, such as being a fat replacer, sugar replacer, and emulsion and foam stabilizer (GONZÁLEZ-HERRERA *et al.*, 2015). Polydextrose is a low molecular weight randomly bonded polysaccharide of glucose with an energy contribution of 1 kcal/g (DO CARMO *et al.*, 2016). This low-calorie content of polydextrose is a result of its poor digestibility in the small intestine and incomplete fermentation in the large intestine (OLIVEIRA *et al.*, 2009). However, resistant starch is a small starch fraction that has the ability to resist digestion and can be fermented by the beneficial microbiota in the colon (ZAMAN and SARBINI, 2016). All inulin, polydextrose and Hi-maize resistant starch have been already reported (GONZÁLEZ-HERRERA *et al.*, 2015; ZAMAN and SARBINI, 2016; DE ARAUJO ETCHEPARE *et al.*, 2016) as prebiotics and have been showed to enhance the viability of *L. acidophilus* and *B. animalis* in fermented dairy products (OLIVEIRA *et al.*, 2009; NOBAKHTI *et al.*, 2009; BEDANI *et al.*, 2013; PADILHA *et al.*, 2016). However, there is no knowledge about the effect of these prebiotics on probiotic *in vitro* gastrointestinal tolerance in ABT-cultured (*Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium animalis*) fermented milk.

The aim of this study was to investigate the influence of the addition of the inulin, polydextrose and Hi-maize resistant starch on the viability of starter culture bacteria, probiotic survival under *in vitro* simulated gastrointestinal conditions, and sensory characteristics in ABT-fermented milk throughout 28 days of storage at 4°C.

2. MATERIALS AND METHODS

2.1. Cultures and ingredients

The ABT-10 culture (Chr. Hansen A/S, Hørsholm, Denmark), composed of *Streptococcus thermophilus*, *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12, skim milk powder (SMP) (Pinar Dairy Products, Izmir, Turkey), inulin (Fibruline® Instant, Cosucra, Warcoing, Belgium), polydextrose (Litesse® IP Powder, Danisco, USA), and resistant starch (Hi-maize, Ingredion, Hamburg, Germany), were used in this study.
The ABT-10 culture (pack size of 200U) was poured into 1 L sterilized reconstituted milk at 40°C and mixed thoroughly, and then 1 L of each milk base was inoculated with 1 mL of the culture. The reconstituted milk was prepared from skim milk powder and has 130g/L of total solids. This procedure gave initial counts after milk inoculation of approximately 7 log cfu g^{-1} for *L. acidophilus* La-5 and *B. animalis* subsp. *lactis* Bb-12 and 8 log cfu g^{-1} for *S. thermophilus*.

2.2. Production of fermented milk

In the production of fermented milk, cow's milk containing 31 g/L fat and 29.2 g/L protein was supplied from Ege University, Agricultural Faculty (Izmir, Turkey). After standardizing it with skim milk powder to obtain 110 g/L of nonfat milk solids, the milk was divided into four lots. The control milk was not supplemented with prebiotics, whereas the other three groups were supplemented with 20 g/L inulin, polydextrose and resistant starch. After they were mixed properly, each milk base was heated to 90°C for 10 min by circulating it in a hot water bath and cooled to 42-43°C in an ice bath. At this point, they were inoculated with the ABT-10 culture. The mixtures were put into 100-mL plastic containers and incubated at 40°C until a pH of 4.75 was reached. After fermentation, the fermented milk samples were cooled and transferred to a refrigerator at 4°C, then stored at this temperature for 28 days during the analyses.

2.3. Determination of pH and microbiological analyses

The pH of the fermented milk was determined using a pH meter (model pH 211; Hanna Instruments, Woonsocket, RI).
The viability of bacteria in the ABT-10 culture was determined according to AKALIN and ÜNAL (2010). The counts of *S. thermophilus* were enumerated on M-17 agar (Merck, Darmstadt, Germany) after incubating the plates aerobically at 37°C for 48 h. *B. animalis* subsp. *lactis* Bb-12 was enumerated using MRS-NNLP (nalidixic acid, neomycin sulfate, lithium chloride, and paramomycin sulfate) agar. The inoculated plates were incubated anaerobically at 37°C for 72 h using an oxygen-free milieu and a CO_2 atmosphere in anaerobic jars (Merck, Darmstadt, Germany). The counts of *L. acidophilus* La-5 were enumerated on MRS-Sorbitol (Merck, Darmstadt, Germany) agar after incubating the plates anaerobically at 37°C for 48 h in anaerobic jars.

2.4. Survival of *L. acidophilus* and *B. animalis* subsp. *lactis* under simulated gastrointestinal conditions

The probiotic survival in the fermented milk samples subjected to gastric and enteric simulated conditions was evaluated after 1, 14 and 28 days of refrigerated storage according to the methods described by BEDANI *et al.* (2014) and CASAROTTI and PENNA (2015), but with some modifications. Each fermented milk sample was placed into

3 sterile flasks in order to perform the phases of simulated gastrointestinal conditions. 10 mL of sample, which was diluted in 0.5% NaCl, was used for the method. Prior to the gastric stage the sample is brought to pH 2.2-2.6 with 0.5 M HCl. Pepsin (from porcine gastric mucosa, Sigma-Aldrich) and lipase (Amano lipase G from *Penicillium camemberti*, Aldrich Chemical Company, St. Louis, MO, USA) solutions were added to the sample to reach a concentration of 3 g/L and 0.9 mg/L, respectively. The sample is placed for 2 h at 37°C in a shaking (150 rpm) waterbath (Mikrotest, MCS Series, Ankara, Turkey), leading to the simulated gastric phase. After then, the pH was adjusted to 4.3-5.2 with an alkaline solution (150 mL of 1 N NaOH and 14 g of PO4H2Na.2H2O and distilled water up to 1 L), which contained 10 g/L of bile (bovine bile, Sigma-Aldrich) and 1 g/L of pancreatin (from porcine pancreas, Sigma-Aldrich) in the final mixture. The sample was incubated again at 37°C in the water bath for 2 h for enteric phase 1. For the last stage, the pH level was adjusted to 7.0-7.3 using the same alkaline solution and the concentrations of bile and pancreatin were adjusted to 10 g/L and 1 g/L, respectively in the final mixture. The sample was then incubated at 37°C for the last 2 h for enteric phase 2. The viable counts of *L. acidophilus* and *B. animalis* were determined after each phase.

2.5. Sensory evaluation

A sensory evaluation of the samples was carried out according to the method modified from TURKISH YOGURT STANDARD (1989) and MARTÍN-DIANA *et al.* (2003). The panel group consisted of 8 experienced academicians from the Department of Dairy Technology (Ege University, Izmir, Turkey) who were familiar with attributes of fermented milk samples. Sensory evaluation consisting of appearance, aroma, taste, texture, and overall acceptability were based on 5-point hedonic scales (1: dislike extremely; 5: like extremely). Each sample was scored individually, and the samples were presented to the panelists inside individual plastic containers. Fermented milks, coded with 3 digits, were randomly presented to the panel group at each session. Panelists evaluated all of the samples after storage for 1, 14, and 28 d at 4°C.

2.6. Statistical analysis

The experiments, including fermented milk making, were performed in triplicate. Six values for each sample were averaged ($n = 6$). The results were analyzed using a one-way analysis of variance (ANOVA) and the general linear model (GLM) procedure of the SPSS software (version 11.05; SPSS Inc., Chicago, IL). The treatments were compared among each other in the same storage day, and the fermented milks of the same treatment were compared throughout the time in terms of *in vitro* simulated gastrointestinal tolerance. The means were compared using the Duncan multi-comparison test at the $p < 0.05$ level.

3. RESULTS AND DISCUSSION

3.1. pH values and microbiological characteristics

The pH values of fermented milk samples during refrigerated storage are shown in Fig. 1. The values for all fermented milk types ranged from 4.73 to 4.37 during storage. Although some fluctuations are observed, all products presented significant pH reduction ($p < 0.05$) at the end of the storage term when compared to the beginning.

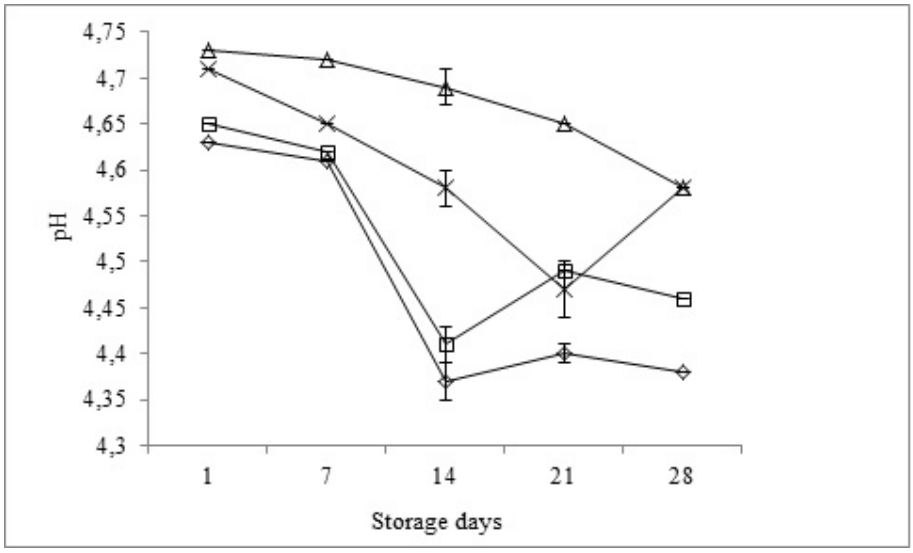


Figure 1. Changes in pH values in fermented milk control (FMC) without addition of prebiotic (◊), fermented milk with addition of 2% inulin (FMI) (□), fermented milk with addition of 2% polydextrose (FMP) (Δ), fermented milk with addition of 2% Hi-maize (FMH) (x).

The pH values of the control fermented milk were found to be lower than those of prebiotic added samples during 28 days, which can be attributed to the buffering capacity of ingredients used in the fortification of the fermented milk samples (HELLAND *et al.*, 2004). Similar results were obtained for the control product when compared to prebiotic added samples in other studies (NOBAKHTI *et al.*, 2009; BEDANI *et al.*, 2013). In contrast to our study, there were no significant differences between the pH values of the control products and prebiotic added products in some studies that could be attributed to the type of product or prebiotic used in these studies (OLIVEIRA *et al.*, 2009; HEYDARI *et al.*, 2011; SRISUVOR *et al.*, 2013).

The viability of *L. acidophilus*, *B. animalis* subsp. *lactis* and *S. thermophilus* during refrigerated storage lasting 28 days is presented in Table 1. The population of *S. thermophilus* remained above 8 log cfu/g throughout the storage period. However, the counts of probiotic bacteria (*L. acidophilus* and *B. animalis*) were maintained at the minimum effective dose for beneficial health effects, which has been suggested to be between 10^6-10^8 cfu/g, in all treatments during the storage time.

In general, the addition of inulin, polydextrose and Hi-maize provided a protective effect on the survival of probiotic bacteria by not allowing any decline in viability during the 28 days. Viable counts of *L. acidophilus* have been reported as lower in the Hi-maize added fermented milk drink than that of the control sample (NOBAKHTI *et al.*, 2009), which parallels our results. *L. acidophilus* has also been shown to not be stimulated by inulin in acidophilus-bifidus yoghurt by OZER *et al.* (2005) and in fermented soy product by BEDANI *et al.* (2013). Similar results were observed in some other studies (BURITI *et al.*, 2010; HEYDARI *et al.*, 2011). However, supplementation with polydextrose enhanced the survival rate of *L. acidophilus* more than supplementation with inulin throughout the 28 days of our study. ALLGEYER *et al.* (2010) obtained parallel results for yoghurt drinks containing both *L. acidophilus* La-5 and *B. animalis* Bb-12 during 30 days of storage.

The viable counts of *B. animalis* Bb-12 significantly decreased throughout the storage in all treatments except for the sample containing Hi-maize. The highest viability of Bb-12 was

detected in the control sample on 1st and 14th days, whereas fermented milk fortified with Hi-maize had the highest value at the end of the storage period ($p < 0.05$).

Table 1. Changes in the viable counts of *L. acidophilus*, *B. animalis*, and *S.thermophilus* during refrigerated storage of fermented milks (log cfu/mL).

	Storage days		
Products	**1**	**14**	**28**
	L. acidophilus		
FMC	7.45 ± 0.03^{Ab}	6.91 ± 0.03^{Ac}	7.51 ± 0.02^{Aa}
FMI	7.38 ± 0.02^{Ba}	6.94 ± 0.03^{Ab}	6.96 ± 0.17^{Cb}
FMP	7.42 ± 0.02^{Aa}	6.82 ± 0.08^{ABb}	7.36 ± 0.07^{Ba}
FMH	6.84 ± 0.05^{Cb}	6.77 ± 0.18^{Bb}	7.47 ± 0.07^{ABa}
	B. animalis		
FMC	7.95 ± 0.01^{Aa}	7.80 ± 0.03^{Ab}	7.63 ± 0.07^{Bc}
FMI	7.41 ± 0.03^{Db}	7.61 ± 0.05^{Ca}	7.22 ± 0.07^{Dc}
FMP	7.85 ± 0.05^{Ba}	7.70 ± 0.05^{Bb}	7.42 ± 0.01^{Cc}
FMH	7.50 ± 0.04^{Cc}	7.61 ± 0.04^{Cb}	7.74 ± 0.09^{Aa}
	S. thermophilus		
FMC	8.82 ± 0.09^{Aa}	8.31 ± 0.07^{Cb}	8.43 ± 0.14^{Ab}
FMI	8.30 ± 0.03^{Bb}	8.48 ± 0.07^{Ba}	7.99 ± 0.13^{Cc}
FMP	8.40 ± 0.09^{Bb}	8.74 ± 0.07^{Aa}	8.15 ± 0.16^{BCc}
FMH	8.10 ± 0.11^{Cb}	8.09 ± 0.09^{Db}	8.32 ± 0.11^{ABa}

Values are means of triplicates. FMC: fermented milk control without addition of prebiotic; FMI: fermented milk with addition of 2% inulin; FMP: fermented milk with addition of 2% polydextrose; FMH: fermented milk with addition of 2% Hi-maize

[a-c]Means ± standard deviations in the same row with different superscript lowercase letters are significantly different ($P < 0.05$).

[A-D]Means ± standard deviations in the same column with different superscript uppercase letters are significantly different ($P < 0.05$).

NOBAKHTI *et al.* (2009) reported that the addition of Hi-maize significantly increased the bacteria level of *B. animalis* Bb-12 in the fermented milk drink immediately after fermentation. However, there were no significant differences ($p > 0.05$) in *B. animalis* Bb-12 counts between ABY-type probiotic yoghurt samples supplemented with 1.5% inulin and 1.5% Hi-maize during 21 days of storage in another study (HEYDARI *et al.*, 2011).
Even though some fluctuations were observed in the population of *S. thermophilus*, in general, the counts significantly reduced at the end of the storage term when compared to the 1st day. Similar fluctuations in viable counts of this microorganism were also reported in other studies (AKALIN and ÜNAL, 2010; BEDANI *et al.*, 2013; CASAROTTI and PENNA, 2015). The viable counts were mostly lower in supplemented fermented milk samples compared with those of the control fermented milk during storage in our study; thus, it is obvious that the addition of the prebiotic did not improve the viability of *S. thermophilus*. In contrast, inulin addition improved the survival of *S. thermophilus* in fermented soy ABT milk during 28 days of storage. This difference can be related to the high ability of this bacterium to metabolize soy oligosaccharides (DONKOR *et al.*, 2007).

3.2. The survival of *L. acidophilus* and *B. animalis* subsp. *Lactis* under simulated gastrointestinal conditions

The survival of *L. acidophilus* and *B. animalis* exposed to *in vitro* simulated gastrointestinal conditions is shown in Figs. 2 and 3, respectively. In general, there was a significant reduction ($p < 0.05$) in the population of both La-5 and Bb-12 during the simulation of the *in vitro* GI stress, which was also observed in other studies (CASAROTTI and PENNA, 2015; CASAROTTI *et al.*, 2015). *B. animalis* Bb-12 presented higher survival rates during the *in vitro* assay than *L. acidophilus*, especially on the 1st and 14th days, this was also observed in many studies (CASAROTTI and PENNA, 2015; CASAROTTI *et al.*, 2015). The resistance of both probiotic bacteria to simulated GI conditions significantly decreased during storage time for all treatments (data not shown). This behavior can be related to the sensitivity of bacteria in that cells were more stressed and damaged by the cold storage at the end of the storage period compared to the beginning (WANG *et al.*, 2009). VINDEROLA *et al.* (2011) also reported a significant reduction in probiotic resistance to gastric stress in fermented milk throughout 20 days of refrigerated storage. It has been also reported that the bile resistance of probiotic bacteria can be poor when used in the presence of each other compared with monoculture. This might probably be related to the potential antagonism between each other in bile salt stress (RANADHEETA *et al.*, 2014). A competition between bacteria probably occurs so that each bacterium can use essential nutrients for its growth and survival (SRISUVOR *et al.*, 2013).

The counts of *L. acidophilus* decreased by 2-3 log cycles after 2 h of the gastric phase. This shows that *L. acidophilus* is highly susceptible to simulated gastric juice containing HCl and pepsin, because the highest reduction in survival was observed during the gastric phase during all storage days. It can be related to the acid tolerance of lactic acid bacteria, which varies by species and strains, as well as exogenous conditions, growth medium, and incubation parameters (MADUREIRA *et al.*, 2011). No recovery of viability of this microorganism was detected after the pH level was increased in the enteric phases of the assay.

Although there were significant differences among fermented milk samples for the gastric phase, the viability of *L. acidophilus* was generally similar after 6 h of assay on the 1st, 14th, and 28th days. On day 1, the supplementation with polydextrose and Hi-maize protected the *L. acidophilus* cells in the presence of low pH (2.2-2.6); however, fermented milk fortified with Hi-maize had the highest counts. There were no significant differences among all treatments when pH was increased to 4.3-5.2 ($p > 0.05$) at the beginning of the storage. However, fortification with inulin caused an increase in the survival of *L. acidophilus* during gastric and enteric phase 1 on the 14th and 28th days compared to the fortification with polydextrose and Hi-maize. The protective effect of inulin can be attributed to the resistance of inulin to hydrolysis by the GI tract enzymes and to its high degree of polymerization (DP) when compared to short chain fructooligosaccharides.

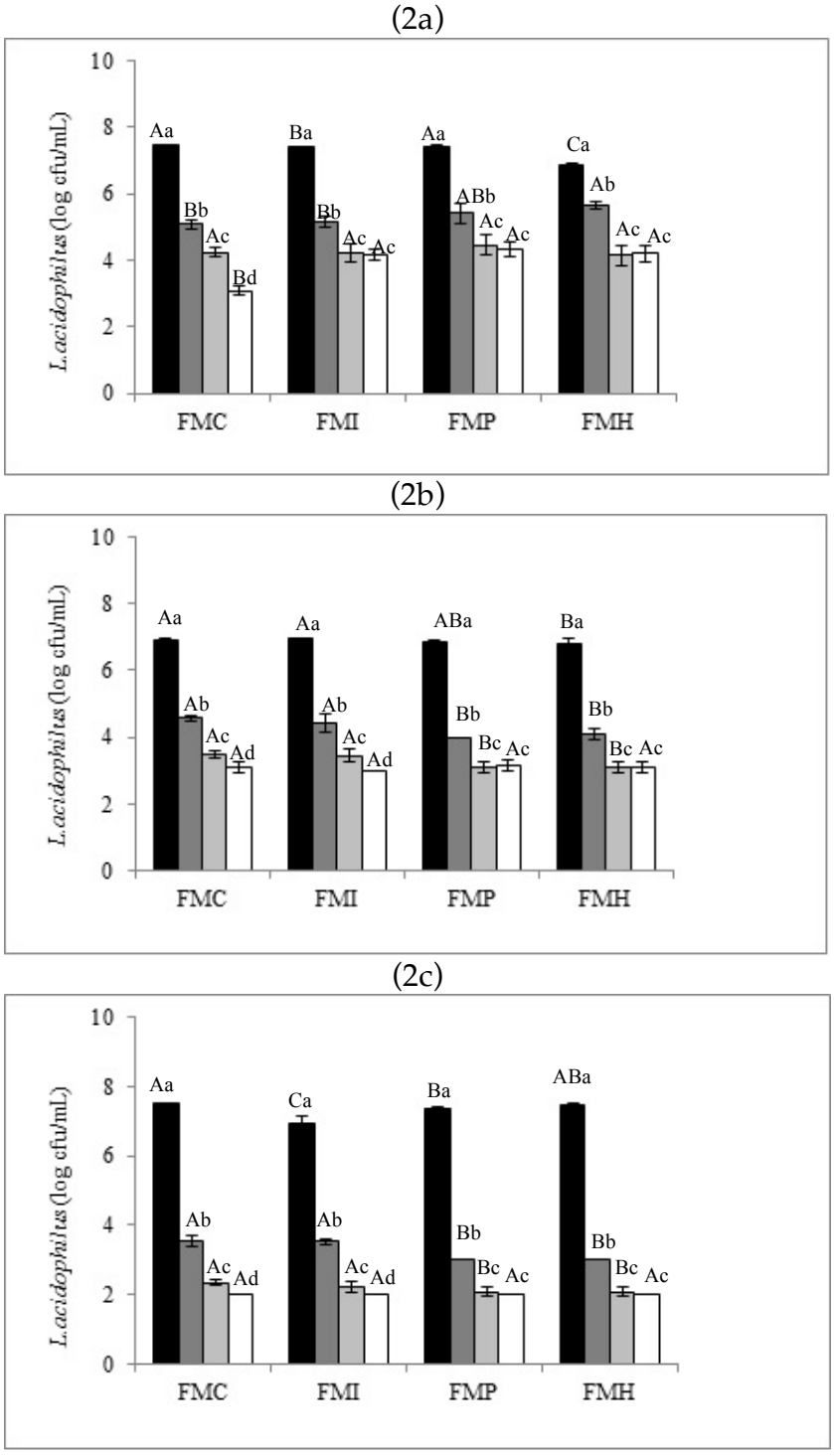


Figure 2. Survival of *L.acidophilus* La-5 (log cfu/mL) in fermented milk after 1, 14, and 28 days of storage (a, b, and c, respectively), before (black bar) and during exposure to *in vitro* simulated gastric conditions, for 2 h (dark gray bar) and enteric conditions, for 4 h (light gray bar) and 6 h (white bar). For the same storage period, $^{A-C}$Different superscript capital letters denote significant differences between formulations for the same sampling period of the *in vitro* assay ($p < 0.05$); $^{a-d}$Different superscript lowercase letters denote significant differences between different sampling periods of the *in vitro* assay for the same formulation ($p < 0.05$). FMC: fermented milk control, without addition of prebiotic; FMI: fermented milk with addition of 2% inulin; FMP: fermented milk with addition of 2% polydextrose; FMH: fermented milk with addition of 2% Hi-maize.

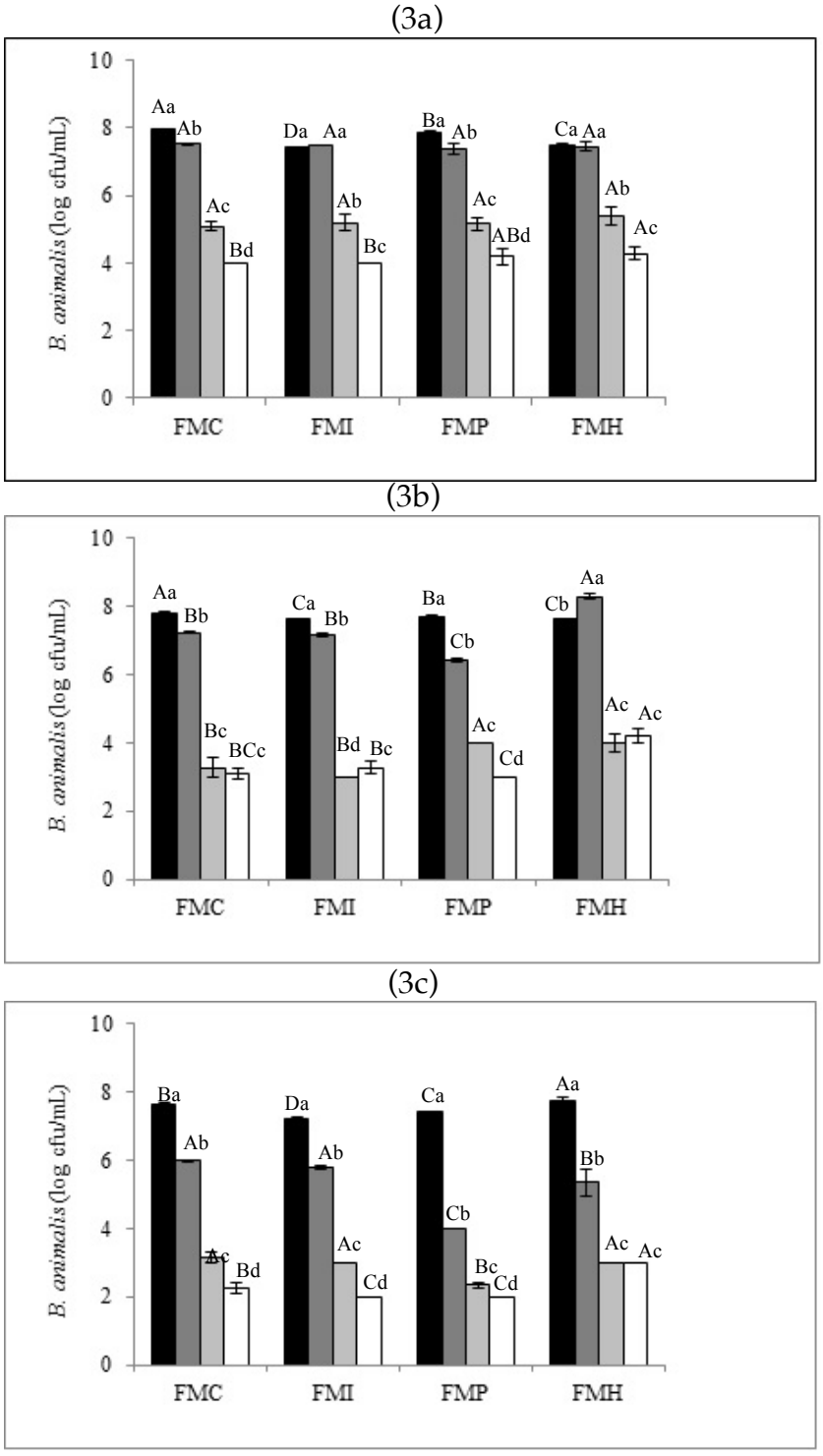


Figure 3. Survival of *B. animalis* Bb-12 (log cfu/mL) in fermented milk after 1, 14, and 28 days of storage (a, b, and c, respectively), before (black bar) and during exposure to *in vitro* simulated gastric conditions, for 2 h (dark gray bar) and enteric conditions, for 4 h (light gray bar) and 6 h (white bar). For the same storage period, $^{A-C}$Different superscript capital letters denote significant differences between formulations for the same sampling period of the *in vitro* assay ($p < 0.05$); $^{a-d}$Different superscript lowercase letters denote significant differences between different sampling periods of the *in vitro* assay for the same formulation ($p < 0.05$). FMC: fermented milk control, without addition of prebiotic; FMI: fermented milk with addition of 2% inulin; FMP: fermented milk with addition of 2% polydextrose; FMH: fermented milk with addition of 2% Hi-maize.

Inulin with high a DP has low solubility and an increased capacity to form a tridimensional network of microcrystals in the food matrix in which it is added (BURITI *et al.*, 2010). This structure containing small aggregates can act as a protective physical cover for bacterial cells against acid and bile (CASAROTTI *et al.*, 2015). On the other hand, HERNANDEZ-HERNANDEZ *et al.* (2012) reported that resistance to bile in *Lactobacillus* strains is dependent on carbon source. Hydrophobic index of bacteria, which is related to their adhesion capacity to intestinal cells, has been also reported to vary depending on the *Lactobacillus* strain by the same researchers. The addition of a mixture of inulin and fructooligosaccharide in the petit-suisse cheese containing the ABT-4 culture resulted in a protective effect for the probiotic survival during 6 h of *in vitro* simulated assay (PADILHA *et al.*, 2016). The authors emphasized that this protective effect of prebiotics might be specific for the food matrix. In this study, even though the prebiotics used had significantly different effects on the viability of *L. acidophilus* during *in vitro* simulated GI conditions among each other, they generally maintained the viable counts.

B. animalis Bb-12 was highly resistant to simulated gastric conditions in all fermented milks on the 1st and 14th days, whereas the viability decreased ($p < 0.05$) 1-2 log cycles after gastric phase at the end of the storage term. Although *B. animalis* showed higher survival rates in the presence of bile and pancreatin than *L. acidophilus*, significant reductions in the viability of *B. animalis* were observed during enteric phases of the assay.

The higher survivability of *B. animalis* Bb-12 compared to that of *L. acidophilus* during *in vitro* simulated GI conditions has also been reported by other authors (BEDANI *et al.*, 2013; 2014; CASAROTTI and PENNA, 2015). CRITTENDEN *et al.* (2001) demonstrated that *B. animalis* Bb-12 was both acid and protease tolerant among commercial strains and able to survive well in an *in vitro* model. The ability of *Bifidobacterium* strains to improve their own tolerance to gastrointestinal environment has been revealed, which is a considerable factor in the performance of strains in the GI tract. Bifidobacteria can adapt their enzymatic systems to the different barriers found along GI tract. They have the ability to increase the activity of the membrane-bound F_0F_1-ATPase enzyme, which pumps protons from cytoplasm to the extracellular environment. When the cells are previously exposed to acidic conditions, the F_0F_1-ATPase enzyme is overproduced, and better control of the intracellular pH is observed (SANCHEZ *et al.*, 2013). This can be the reason for the higher tolerance of *B. animalis* Bb-12 to simulated gastrointestinal conditions in this study. As some strains of bifidobacteria may have acid stress throughout the gastric conditions (Huang *et al.*, 2014), the intrinsic tolerance of the strains has a decisive influence. The high resistance of *B.animalis* subsp. *lactis* to both oxygen and gastrointestinal stress was also verified by other researchers (PERRIN *et al.*, 2000; ANDRIANTSOANIRINA *et al.*, 2013; AMBALAM *et al.*, 2014). Bile and bile components have been reported to affect the adherence of *Bifidobacteria* in the gastrointestinal tract (KOCIUBINSKI *et al.*, 2002). The improved tolerance of *B. animalis* Bb-12 in this study can also be attributed to having bile salt hydrolase activity, which is active during its transit through the gastrointestinal tract (PICARD *et al.*, 2005). However, BEGLEY *et al.* (2005) reported that the tolerance of Gram-positive probiotic bacteria to bile is a strain-dependent characteristic that should not be generalized in terms of species.

Supplementation with Hi-maize caused a significant increase in the survival of Bb-12 during the assay; however, the most effective improvement of Hi-maize on Bb-12 survival was observed in enteric phase 2 during all storage days. This probably can be caused by the slow degradation of resistant starch in the first part of the GI tract and so, it can reach the distal part of the colon and show a prebiotic effect (ZAMAN and SARBINI, 2016). The authors also reported that the amylose to amylopectin ratio is an important property to determine the resistance of a starch and its enzymatic digestibility. The related mechanism has been explained as the interaction of amylose molecules with amylopectin which can

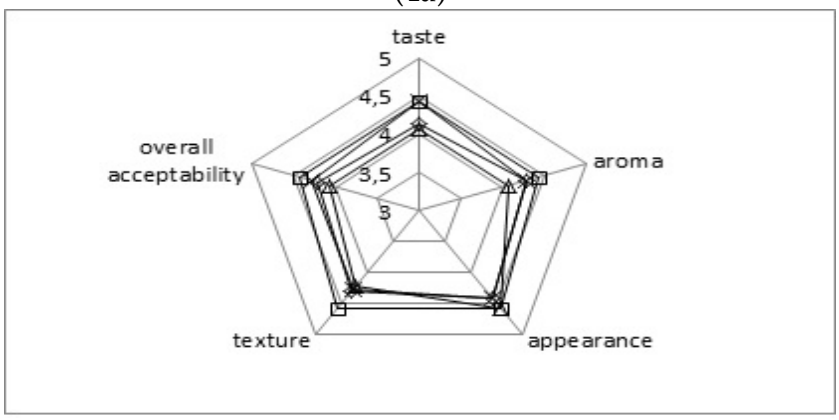
influence the accessibility of enzymes to hydrolyze starch molecules. In addition, NUGENT (2005) reported that Hi-maize, which belongs to Class 2 of resistant starches, comprises specially structured granules that prevent digestive enzymes from hydrolyzing them.

According to this study, supplementation with inulin may increase the viability of *L. acidophilus* La-5, whereas Hi-maize resistant starch can be preferred as a prebiotic ingredient to enhance the viable counts of *B. animalis* Bb-12 during the simulation of GI conditions. Therefore, choosing a suitable prebiotic for the manufacture of fermented dairy products can contribute to maintain the viability of probiotic bacteria in the gut. The *in vitro* analysis used in this study gives information about the survival rate of probiotic bacteria under gastrointestinal conditions but not about their probiotic activity. So, the probiotic activity of these bacteria should also been assessed by appropriate analyses. The species and strain specificity of probiotics to acid and bile tolerance should also not be forgotten (RANADHEERA *et al.*, 2014).

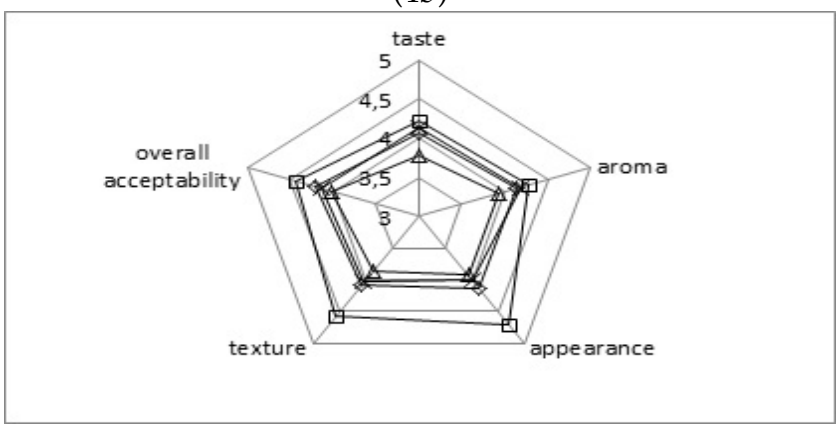
3.3. Sensory evaluation

The results of the sensory evaluation of the fermented milk samples are shown in Figs. 4a (d1), 4b (d14), and 4c (d28).

(4a)



(4b)



(4c)

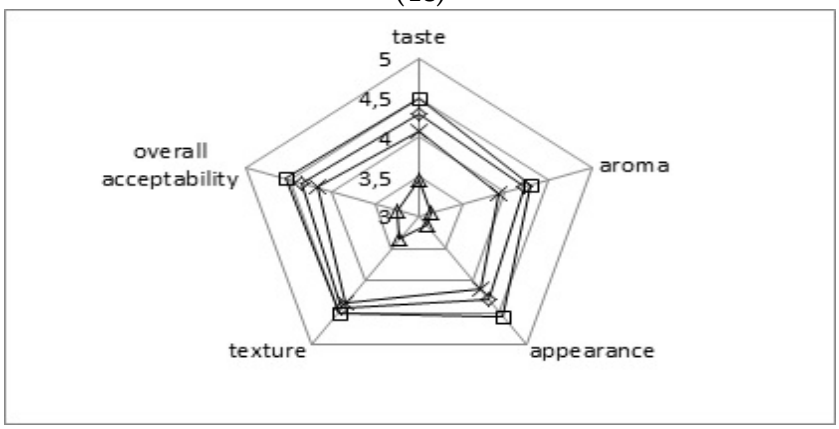


Figure 4. Sensory scores of (a) 1-d-old-fermented milks, (b) 14-d-old fermented milks, and (c) 28-d-old fermented milks; ◊ = fermented milk control without addition of prebiotic (FMC), □ = fermented milk with addition of 2% inulin (FMI), Δ = fermented milk with addition of 2% polydextrose (FMP), x = fermented milk with addition of 2% Hi-maize (FMH).

In general, the effect of storage time on the sensory characteristics of fermented milk samples was not found to be significant ($p > 0.05$). There were no significant differences ($p > 0.05$) among fermented milk samples in terms of taste, flavor, texture, and overall acceptability during the 14 days of storage. The addition of inulin or polydextrose at a ratio of 20 g/L also did not affect the flavor property of low-fat set yoghurts with probiotic-cultured banana purée (SRISUVOR *et al.*, 2013). The addition of inulin also did not negatively affect the sensory quality of sponge-cake products (ZBIKOWSKA *et al.*, 2017). Similarly, no significant inulin effect could be observed on the "firmness", measured as the force required to lift a spoonful of yoghurt in another study (GUGGISBERG *et al.*, 2009).

Fermented milk fortified with polydextrose had the lowest sensory scores among the samples at the end of the storage term ($p < 0.05$). The lowest appearance values were obtained again in the samples fortified with polydextrose throughout the storage period which can be attributed to the high solubility of polydextrose in water, thus resulting a non-viscous solution. Having a neutral taste without reflecting sweetness can also be a reason for indicating low scores (DO CARMO *et al.*, 2016). An artificially sweetened *misti dahi*, which is a popular fermented dairy product of eastern India, containing polydextrose was found to have lower flavor and lower overall acceptability scores when compared to the control sample and samples supplemented with other sweeteners (RAJU and PAL, 2011). ALLGEYER *et al.* (2010) also observed worse sensory attributes for a symbiotic yoghurt drink containing polydextrose compared with the control ($p < 0.05$).

The addition of inulin or Hi-maize did not negatively affect the sensory properties of the fermented milk samples throughout the 28 days. Similar results were also obtained in other studies (KIP *et al.*, 2006; RINALDONI *et al.*, 2012).

4. CONCLUSIONS

The importance of this study is that it provides knowledge about the efficacy of different prebiotics on the viability of probiotic bacteria in the fermented milk during *in vitro* simulated gastrointestinal conditions. According to the results obtained in the present

study, the supplementation with prebiotics showed a protective effect and did not allow a decline on the viability of *L. acidophilus* La-5 and *B. animalis* Bb-12 in ABT fermented milk, and the probiotics maintained the recommended level for the beneficial health properties, ranging from 6 to 8 log cfu/g during the 28 days of storage. *B. animalis* Bb-12 was more resistant to the simulated gastrointestinal conditions than *L. acidophilus* La-5 throughout the storage period. Although inulin added fermented milk had higher viable counts of *L. acidophilus* La-5 during the gastric and enteric-I phases, there were no significant differences among products at the end of the *in vitro* assay, except on the 1st day. However, it was obvious that the use of Hi-maize improved *B. animalis* Bb-12 resistance when subjected to simulated gastrointestinal conditions. The addition of inulin or Hi-maize to fermented milk had no influence on the sensory characteristics whereas the lowest scores were obtained for the sample supplemented with polydextrose especially on day 28. Therefore, the findings of this study suggest that an appropriate prebiotic can be suitable to ensure the viability of the probiotic strains during the manufacturing time and shelf-life of the product, and protect the probiotics during the passage through the gastrointestinal tract so that they can reach the colon. This can be a strategy for providing a broad range of health benefits of probiotic microorganisms to the host and for the development of future functional foods. In addition, further studies are necessary to follow which components of the prebiotics and/or technological processes might influence the probiotic tolerance to simulated gastrointestinal juices in fermented dairy products. The use of higher ratios of the prebiotics can be tested in the future studies and clinical trials should also be needed to support *in vitro* studies.

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