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ORIGINAL PAPER

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THE POTENTIAL USE OF PROBIOTIC STRAINS *LACTOBACILLUS ACIDOPHILUS* NRRL B 4495, *BIFIDOBACTERIUM BIFIDUM* NRRL B41410 IN "LOR WHEY CHEESE" AND THE EFFECTS ON SENSORY PROPERTIES*

Reyhan Irkin[1]✉, Onur Yalcin[2]

[1]Food Engineering Department, Engineering Faculty, Balikesir University
TR10145, Balikesir, **Turkey**
[2]Biology Department, Art-Science Faculty, Balikesir University
TR10145, Balikesir, **Turkey**

ABSTRACT

Background. In recent years, probiotic bacteria have increasingly been incorporated into various foods as dietary adjuncts. The viability of the probiotic bacteria *Lactobacillus acidophilus* NRRL B 4495 and *Bifidobacterium bifidum* NRRL B41410 in salted (1% w/w) and unsalted lor whey cheese during storage (21 days) at a refrigerated temperature (4°C) was evaluated.
Material and methods. As well as the survival of the probiotic bacteria, total mesophilic bacteria, total lactic acid bacteria, *Pseudomonas* spp., yeast-mould counts and sensory characteristics were examined in the lor samples.
Results. The *Bf. bifidum* remained in large numbers, at 7.30 and 7.11 log cfu/g, and *Lb. acidophilus* also survived well, with counts of 7.60 and 7.47 log cfu/g, for unsalted and salted cheeses respectively. Salted lor cheeses with added *Lb. acidophilus* have the highest sensory scores in the groups.
Conclusion. "Lor" whey cheese showed good probiotic properties.

Keywords: functional cheese, cheese microbiology, lactic acid bacteria

INTRODUCTION

"Lor" cheese, produced from whey, is very important in the dairy industry in Turkey. Particularly popular with children, it has a soft texture, and a fat-free version is also available. Lor cheese contains essential amino acids but has a relatively short shelf life (Ciftcioglu et al., 2008; Irkin, 2011). Whey cheese has some advantages over other foods in terms of delivery of viable probiotics, because of its relatively high pH, fat content and mechanical consistency, coupled with a typically low oxygen level. Due to its unripened nature, whey cheese has to be kept refrigerated, and its typically short shelf life contributes to making

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✉reyhan@balikesir.edu.tr; phone +90 266 612 1194-5709, fax +90 266 612 1257

lor a particularly suitable carrier for probiotic bacteria (Plessas et al., 2012).

Functional food products are a new category of food products that are marketed as having health benefits. A food can be regarded as functional if it is satisfactorily demonstrated to beneficially affect one or more target functions in the body (Krystallis et al., 2008; Urala and Lahteenmaki, 2007).

The main science-based benefits related to probiotics are: antimicrobial activity, anticarcinogenic properties, beneficial effects on mineral metabolism (especially regarding bone stability), attenuation of symptoms of bowel disease, reduction of food allergy symptoms, and reduction of LDL-cholesterol levels (Granato et al., 2010; Ishibashi and Yamazaki, 2001; Kun et al., 2008; Nagpal et al., 2014).

Adequate numbers of viable cells, namely the 'therapeutic minimum', need to be consumed regularly for probiotics to have an effect on consumers (Hattingh et al., 2001).

Previous studies have demonstrated that the addition of probiotic bacteria to the various cheese varieties in recent years (Freitas et al., 2014; Tamime et al., 2005). However, there is a lack of information on lor with added probiotic bacteria, and under salted conditions.

Consequently, the objectives of this study were to:

1. Determine the growth of two probiotic strains (*Lactobacillus acidophilus* NRRL B 4495 and *Bifidobacterium bifidum* NRRL B41410) in salted and unsalted "lor" whey cheese and determine which probiotic strain exhibits a preference for lor cheese, for the development of a functional cheese.
2. Research the effects of salted or unsalted conditions on the growth of selected probiotic microorganisms, total mesophilic bacteria, total lactic acid bacteria, *Pseudomonas* spp., yeast-mould counts and sensory analysis over the 21 days of lor shelf life, at 4°C under vacuum-packed conditions.

MATERIALS AND METHODS

Lor production

Lor cheese was produced in TEK-SÜT Milk Products Company (Gonen, Balikesir). Whey from Kashar cheese production was heated to 50–55°C. Fat was separated from the whey and then heated to 80°C in a boiler tank with 2% (w/v) added salt. Lor cheese coagulum began to accumulate on the surface of the whey, and the temperature was increased to 90–95°C. Lor cheese coagulum was collected from the surface into cloths and drained for 12 h at 25°C. Then, the collected mass of lor was divided into six parts; bacterial cultures and/or salt were added and mixed into the different samples (Table 1). Each sample (200 g) was vacuum-packed and stored at 4°C for 21 days. The samples were subjected to analyses directly after production and after 1, 4, 7, 14 and 21 days of storage.

Preparation of probiotic cultures

Freeze-dried probiotic cultures of *Bifidobacterium bifidum* NRRL B41410 and *Lactobacillus acidophilus* NRRL B 4495 were obtained from the US Department of Agricultural Research Service, and the probiotic cultures were activated in Liver Infusion Broth (Difco, 226920) at 37°C for 3 days before use. Activated

Table 1. Lor sample groups in the research

Sample groups	Trial names
Whey cheese (control) unsalted	CW1
Whey cheese (control) 1% (w/w) salted	CW2
Whey cheese (*Lactobacillus acidophilus* NRRL B 4495 added) unsalted	LA1
Whey cheese (*Lactobacillus acidophilus* NRRL B 4495 added) 1% (w/w) salted	LA2
Whey cheese (*Bifidobacterium bifidum* NRRL B41410 added) unsalted	BF1
Whey cheese (*Bifidobacterium bifidum* NRRL B41410 added) 1% (w/w) salted	BF2

microorganism cultures (7.2–7.6 log cfu/ml viable cells) were then inoculated into sterilised skimmed-milk media, incubated for about 4 h at 37°C and then mixed into the lor samples (~10^8 kob/g lor).

Microbial analysis of lor samples

Ten grams of each lor sample were diluted with 90 ml of 0.1% sterile buffered peptone water and tenfold serial dilutions were then prepared in 9 ml of 0.1% sterile peptone water.

Counts of *Bf. bifidum* NRRL B41410 were enumerated on Reinforced Clostridial Agar (RCA) with 0.03 g/100 mL aniline blue and dicloxacillin (2 mg/mL, Sigma). Plates were incubated under anaerobic conditions at 37°C for 48 h (Kailasapathy et al., 2008).

Numbers of *Lb. acidophilus* NRRL B 4495 were determined on MRS (deMann, Rogosa and Sharpe, Merck 1.05463) with D-sorbitol (10 g/100 mL) media at 37°C for 72 h (Tharmaraj and Shah, 2003).

Total lactic acid bacteria counts were determined using double-layer de Man Rogosa Sharpe agar (MRS Merck 1.10660) under anaerobic conditions after incubation at 30°C for 72 h (Whitley et al., 2000).

Mesophilic microorganisms were determined on plate count agar (PCA, Merck 1.05463) using the pour plate method and incubated at 31°C for 72 h (Gonzales-Fandos et al., 2000).

Pseudomonas spp. were counted on Pseudomonas agar (PA, Merck 1.05284) with CFC supplement at 25°for 48 h (ISO 13720, 2000).

Yeasts and moulds were enumerated on yeast extract glucose chloramphenicol agar (YGC, Merck 1.037500500) plates using the surface plate method and incubated at 25°C for 5–7 days (Gonzales-Fandos et al., 2000).

All count data were written as logarithms (log cfu/g) prior to statistical analysis. The results were analysed statistically as described in the next section.

Physico-chemical analysis

The pH of the whey and lor samples was measured using a pH meter (Hanna HI221 Microprocessor, Hanna Instruments Inc., Woonsocket, Rhode-Island) which was previously calibrated with pH 7.0 and 4.0 standard buffers, on each sampling day. The moisture, fat and salt contents were determined according to AOAC (1995) procedures. The protein content of the samples was determined using Dumas Nitrogen Analyzor (Velp NDA 701) equipment. All analyses were carried out in duplicate at 20°C.

Sensory analysis

Analyses of the sensory characteristics of the samples were carried out according to IDF (1995) standards on each day of sampling. A panel, composed of 5 experienced members of our university community, was used to evaluate the whey cheeses for external appearance (colour), flavour, taste and texture with a point scale from 0 to 5 (0 − spoiled sample and unfit for human consumption; 5 − very good). LA1, LA2, BF1, BF2 whey cheese samples were compared with control lor cheese groups.

Statistical analysis

SPSS 19.0 software for windows (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analyses. A one-way analysis of variance (ANOVA) test was performed to determine mean differences between the lor groups. The level of significance between means was determined by the Tukey HSD and LSD tests.

RESULTS AND DISCUSSION

Composition and acidity of whey and lor samples

Proximate analysis of lor samples produced from whey showed average levels of dry matter to be 5.32 ±0.4%, protein 2.3 ±0.2%, and fat 1.1 ±0.5% (g/100 g moisture). Mean values of pH were 6.1 ±0.9.

Before the addition of the bacterial cultures, the composition of the lor cheeses was determined: average values of dry matter 28.94 ±2.1% (unsalted lor), 29.3 ±1.8% (salted lor), fat 5.5 ±1.3% (salted and unsalted lor samples), salt content 0.343 ±0.5 (unsalted lor), 1.1 ±0.8% (salted lor), and protein 11.56 ±0.8 (salted and unsalted lor; g/100 g moisture) were found.

The pH values of lor samples during the storage period are shown in Table 2. Acid production in control lor samples was lower than the other groups and there were significant differences ($p < 0.05$) in pH values between the control and other trials. Compared to the control groups, the addition of probiotic cultures influenced the pH of the samples. Among the samples, the highest drop in pH value was observed for BF1

Table 2. Changes of the pH and sensory scores of lor samples during cold storage ($n = 6$)

Day	CW1	CW2	LA1	LA2	BF1	BF2
	pH					
1	6.17 ±0.2^a	6.06 ±0.3^a	5.64 ±1.1^b	5.73 ±0.9^b	5.69 ±0.2^b	5.75 ±0.7^b
4	6.26 ±0.4^a	6.04 ±0.6^a	5.56 ±0.7^b	5.64 ±1.0^b	5.67 ±0.7^b	5.73 ±0.6^b
7	6.17 ±1.3^a	6.06 ±0.8^a	5.60 ±0.4^b	5.71 ±0.7^b	5.44 ±0.6^c	5.34 ±1.1^c
14	6.07 ±0.7^a	6.05 ±0.2^a	5.41 ±1.8^b	5.59 ±0.3^b	4.88 ±0.5^c	4.88 ±0.2^c
21	6.07 ±0.6^a	6.06 ±0.3^a	5.41 ±1.2^b	5.56 ±0.5^b	4.86 ±0.4^c	4.94 ±0.8^c
	Sensory evaluations (overall acceptability scores)					
1	5.00 ±0.0^a	5.00 ±0.0^a	4.26 ±0.9^b	5.00 ±0.0^a	3.40 ±0.3^c	4.03 ±0.6^b
4	4.46 ±0.8^a	4.63 ±0.5^a	4.06 ±0.2^b	5.00 ±0.0^c	3.30 ±0.2^d	3.76 ±0.4^d
7	4.26 ±0.7^a	4.83 ±0.6^b	4.36 ±0.4^b	5.00 ±0.0^b	3.96 ±0.3^a	4.10 ±0.2^a
14	1.00 ±0.0^a	1.00 ±0.0^a	3.73 ±0.1^b	5.00 ±0.0^c	3.20 ±0.2^d	3.30 ±0.5^d
21	1.00 ±0.0^a	1.00 ±0.0^a	3.30 ±0.8^b	4.33 ±0.4^c	2.96 ±0.5^d	3.26 ±0.3^b

Means ±SD within each row not sharing the same lowercase letter are statistically different ($p < 0.05$).
CW1 – lor cheese (control) unsalted, CW2 – lor cheese (control) 1% (w/w) salted, LA1 – lor cheese (*Lactobacillus acidophilus* NRRL B 4495 added) unsalted, LA2 – lor cheese (*Lactobacillus acidophilus* NRRL B 4495 added) 1% (w/w) salted, BF1 – lor cheese (*Bifidobacterium bifidum* NRRL B41410 added) unsalted, BF2 – lor cheese (*Bifidobacterium bifidum* NRRL B41410 added) 1%.

and BF2. After 7 days, there were significant differences ($p < 0.05$) in pH values for all lor samples except CW2. However, there were not any significant differences between the salted and unsalted lor samples for each of the trial groups.

Viability of microorganisms in trial groups

The viability of *Lb. acidophilus* NRRL B 4495 in lor cheeses at 4°C during storage is shown in Figure 1a. The maximum cell population of *Lb. acidophilus* NRRL B 4495 occurred on day 14 as 7.86 log cfu/g and then decreased to 7.48 log cfu/g in salted lor samples. Subsequently, LA1 and LA2 samples showed similar trends, with all increasing in numbers and reaching a maximum on days 4 and 14 respectively and then declining by a small amount. No significant differences were detected between populations of *Lb. acidophilus* found in LA1 and LA2, except on the 4th day ($p > 0.05$).

Figure 1b shows the variations in *Bf. bifidum* numbers in the lor groups. The highest viable numbers were in the BF1 samples on day 14. No significant differences between the viable numbers of BF1 and BF2 samples were found ($p > 0.05$), except on day 4 and 14 of storage ($p < 0.05$).

On the first day, the total viable numbers of the samples CW1 and CW2 were shown to be statistically different from the other groups ($p < 0.05$; Fig. 1c). It was thought that total viable numbers were affected by probiotic cultures that were present in the samples BF and LA from the beginning. The samples BF2 exhibited a significantly greater reduction than the other groups after 4 days' storage ($p < 0.05$). On day 21, all the samples were < 5 log cfu/g, except CW1 and CW2. The highest total viable count was obtained from the CW1 lor sample, followed by CW2, BF1, LA1, LA2 and finally BF2, at the end of storage.

During storage, the average number of viable lactic acid bacteria increased rapidly and significantly ($p < 0.05$) in BF1 and BF2 samples (Fig. 1d). The highest lactic acid bacteria counts were determined for BF1 samples. Regarding total lactic acid bacteria,

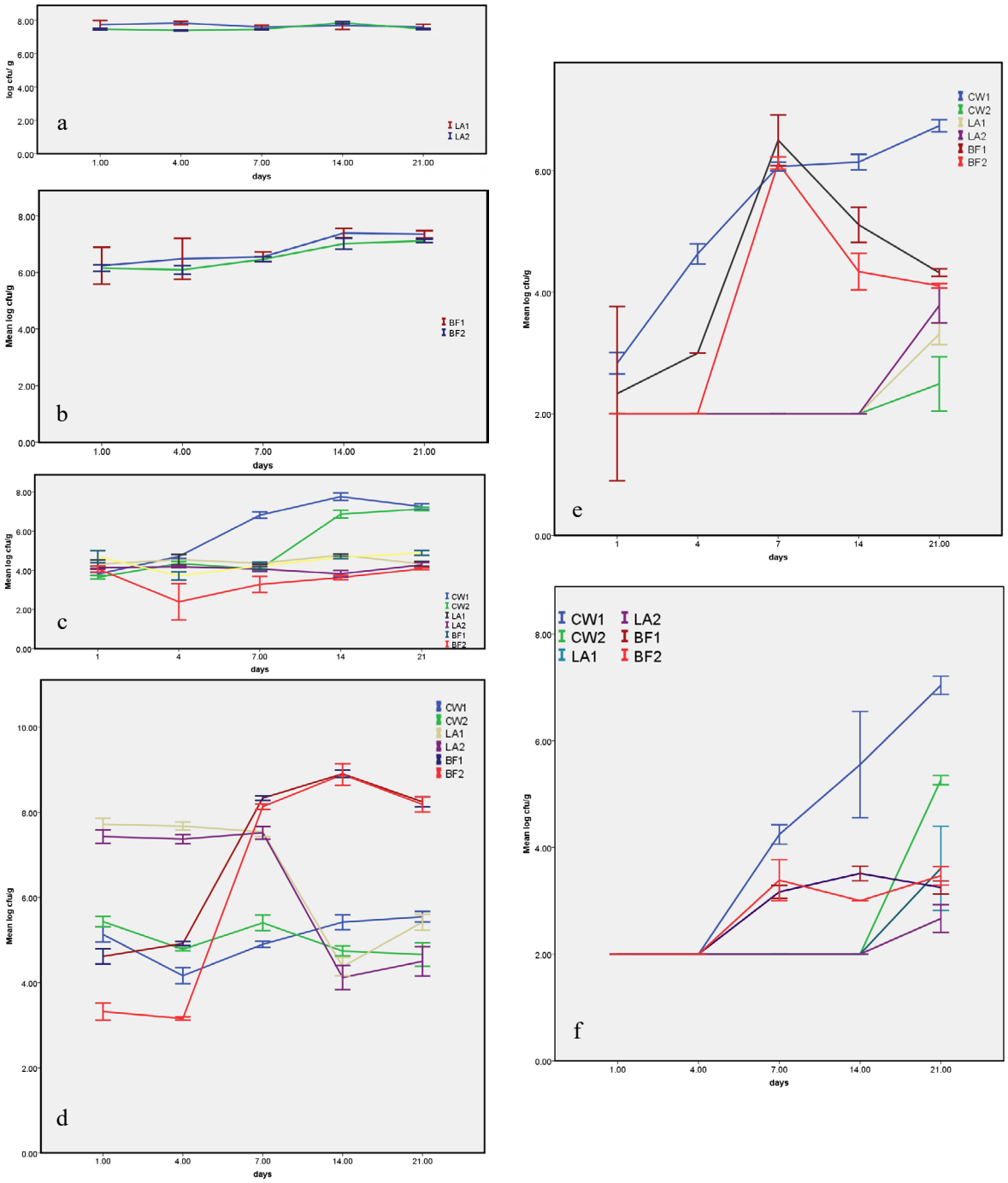

Fig. 1. Growth rates of *Lactobacillus acidophilus* NRRL B 4495 log cfu/ g (**a**), *Bifidobacterium bifidum* NRRL B41410 log cfu/g (**b**), total mesophilic bacteria log cfu/g (**c**), total lactic acid bacteria log cfu/g (**d**), *Pseudomonas* spp. log cfu/g (**e**), yeast numbers log cfu/g (**f**) in lor samples during storage period (21 days at 4°C). Error bars represent the standart deviation of means (n = 3). CW1 – lor cheese (control) unsalted, CW2 – lor cheese (control) 1% (w/w) salted, LA1 – lor cheese (*Lactobacillus acidophilus* NRRL B 4495 added) unsalted, LA2 – lor cheese (*Lactobacillus acidophilus* NRRL B 4495 added) 1% (w/w) salted, BF1 – lor cheese (*Bifidobacterium bifidum* NRRL B41410 added) unsalted, BF2 – lor cheese (*Bifidobacterium bifidum* NRRL B41410 added) 1%

these were present at averages of 5.13–5.54 log cfu/g (CW1), 5.43–4.66 log cfu/g (CW2), 7.72–5.42 log cfu/g (LA1), 7.43–4.50 log cfu/g (LA2), 4.61–8.24 log cfu/g (BF1), 3.26–8.18 log cfu/g (BF2) in the lor samples.

Results also showed that the levels of *Pseudomonas* spp. in CW1 lor samples exceeded 6.74 log cfu/g over 21 days storage at 4°C, and this was significantly ($p < 0.05$) higher than the other groups (Fig. 1e). In LA1 and LA2 samples, the numbers remained constant at 2 log cfu/g until the 21st storage day, then increased to 3.31 log cfu/g and 3.78 log cfu/g respectively. LA1 and LA2 groups contained lower numbers of *Pseudomonas* spp. than the BF1 and BF2 sample groups.

In all of the samples, the quantity of yeast remained < 5 log cfu/g, except for the CW1 and CW2 samples; moulds were not observed in any of the lor samples ($p < 0.05$; Fig. 1f). The highest yeast numbers were in the samples CW1 (7.04 log cfu/g), followed by CW2 (5.26 log cfu/g), and the lowest were in LA2 (2.66 log cfu/g) and BF1 (3.25 log cfu/g) after 21 days' storage. There were significant differences in yeast numbers in CW1, BF1 and BF2 samples on the 7th day ($p < 0.05$).

Sensory properties of lor groups

The incorporation of probiotic bacteria into lor samples had no significant sensory effects on the 1st day ($p > 0.05$) but there were some significant differences between the groups on subsequent days ($p < 0.05$; Table 2). The worst scores for odour and taste were found in the CW1 and CW2 lor samples. The odour of CW1 and CW2 was very unpleasant, and the taste was unacceptable after 14 days of storage.

DISCUSSION

There were no significant differences between the fat, protein and dry matter levels of the different sample groups in our study. Similarly, Madureira et al. (2013) reported that there were no significant differences ($p > 0.05$) in the fat, protein and dry matter levels in cheeses to which *Lb. acidophilus* and *Bf. animalis* had been added in their research.

It has been stated that an important factor affecting the survival of probiotic bacterial strains in food is pH; viability is decreased at low pH values. Hence, whey cheese should be a good vehicle for probiotic strains because it generally has a pH in the 6.0–6.5 range, and never lower than 4.5, even after 28 days of storage (Madureira et al., 2005). In our study the lowest pH value, 4.86, was obtained in the BF1 group after 21 days of storage.

Madureira et al. (2013) reported pH values of 6.18–6.95 in control lor samples, 5.99–5.67 in groups with *Lb. acidophilus* added and 6.01–5.85 in lor groups with *Bf. animalis* added after 21 days of storage. Madureira et al. (2005) also found pH values of 6.22–5.51 for control lor samples, 6.24–6.95 for samples with added *Lb. acidophilus*, and 6.26–6.95 for samples with added *Bf. animalis* and 0.8% salt (w/v; salt/whey), after 28 days of storage.

Previous studies have reported that the most important factor for a decrease in bacterial viability is decreasing pH during storage, and the formation of organic acids (Kailasapathy et al., 2008; Shah, 2000). The pH in probiotic dairy products is generally 3.7–4.3, which is lower than the pH range of 4.8–5.6 for standard cheeses. But in a cheese matrix, probiotic bacteria can grow more easily, because the pH is closer to their optimal value (Plessas et al., 2012).

Many studies have shown that probiotics are highly viable in dairy-based products (Buriti et al., 2007; Kailasapathy et al., 2008; Phillips et al., 2006; Rodgers, 2008). Ganesan et al. (2014) reported that starter lactococci, nonstarter lactobacilli and probiotic bacteria are capable of surviving the Cheddar cheese making and ageing process. Miocinovic et al. (2014) found low-fat UF cheese represents a good vehicle for probiotic bacteria, which maintains satisfactory viability throughout the ripening process. The use of probiotic bacteria improves the sensory characteristics of cheeses. A high number of *Lb. acidophilus* LAFTI RL10, $>10^7$ cfu/g, was found throughout the ripening period; this is necessary for therapeutic effects.

Madureira et al. (2013) demonstrated that numbers of *Lb. acidophilus* were between 7.86–8.32 log cfu/g in whey cheeses during the 21 days of storage. Madureira et al. (2005) determined viable cell numbers of an *Lb. acidophilus* strain in a control probiotic whey cheese matrix increased by 2.0 log cycles within 28 days of storage; such a trend was not observed for the salt-added matrix ($P < 0.05$), but was found in the sugar-added matrix ($P > 0.05$).

It was stated that, during the ripening period, the viability of *Bifidobacteria* (*Bf. lactis* LAFTI R B94) was maintained at a relatively constant level. A significant reduction in their number was found at the end of the investigated ripening period (<10^7 cfu/g), probably due to their sensitivity to low pH values (Miocinovic et al., 2014).

Matias et al. (2014) developed a probiotic soy-based product similar to petit-suisse cheese. The viability of *Bf. animalis* Bb-12 always remained above 8 log cfu/g for all cheese trials during the 28 days of storage at 4°C. In another study, Madureira et al. (2013) reported that the viability of *Bf. animalis* numbers in whey cheese increased to 8.59 from 7.97 log cfu/g after 21 days of storage.

Ganesan et al. (2014) explained that survival of bifidobacterial species in even one cheese type may change. The variations in fat levels and physico-chemical conditions inside the cheese matrices can alter the viability of a given strain of bacteria.

Madureira et al. (2005) determined that the type of matrix (i.e. plain or supplemented with sugar or salt) is the most important factor affecting the viability profiles of bacterial strains; control and sugar-supplemented matrices in whey cheeses are better for growth (7.85–8.96 log cfu/g) than the salt-supplemented matrix (7.38–7.98 log cfu/g), for example in the case of the *Bf. animalis* strain.

The growth-inhibiting effects seen on probiotic culture-supplemented samples may be related to the formation of bacteriocins, lactic and acetic acids, hydrogen peroxide, ethanol, diacetyl, acetaldehyde and acetoin compounds (Fernandes et al., 2013; Matias et al., 2014; Ricciardi et al., 2014).

As a result, it can be said that consumption of a nominal serving (30 g) of lor cheese per day provides an intake of *Lb. acidophilus* or *Bf. bifidum* of 10^6–10^7 cfu/g, which is the optimum level recommended to provide therapeutic benefits (Phillips et al., 2006). Ganesan et al. (2014) explained that, when adjunct or probiotic bacteria are included in the cheese matrix, the balance of LAB populations can be altered because of the competition for nutritional components between the microorganisms.

Madureira et al. (2011) determined that the use of probiotic strains like *Bf. animalis* or *Lactobacillus casei* in whey cheeses can make them safer and extend the shelf life, due to the inhibition of *Listeria innocua*, *Salmonella Enteritidis*, *Staphylococcus aureus* and food spoilage microorganisms such as *Pseudomonas aeruginosa* and *Escherichia coli*.

Cichosz et al. (2014) found higher counts of mesophilic lactic acid bacteria in experimental ripened cheese than in control cheese, and explained that the addition of *L. rhamnosus* HN001 to experimental cheeses promoted the viability of starter cultures by inhibiting the growth of harmful microflora, such as the coliform group, yeast and mould, and increased the quantity of easily available substrates.

Minervini et al. (2012) explained that the addition of *Bifidobacteria* to Gouda and cottage cheeses has a negative effect on the flavour of cheeses, because the concentration of acetic acid was too high and proteolysis too extensive, which decreased consumer acceptability compared to traditional cheeses. In this research LA2, LA1 and BF2 samples had the highest total acceptability scores during storage.

CONCLUSION

In this work we have shown that lor whey cheese is a good vehicle for probiotic bacteria. Upon inoculation in whey cheeses, *Bf. bifidum* NRRL B41410 and *Lb. acidophilus* NRRL B 4495 maintained their viable cell numbers throughout 21 days of storage. No relevant physicochemical changes occurred in terms of fat, protein and moisture content during this period, except for a slight decrease in pH relative to the control. The sensory evaluation showed that the overall aroma of lor cheeses was improved markedly by the addition of the probiotic cultures *Lb. acidophilus* NRRL B 4495. The addition of salt to the lor samples caused growth restrictions for some microorganism groups and also affected the sensory results for lor cheeses.

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