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Rheological, textural and microstructural features of probiotic whey cheeses

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ABSTRACT

Whey cheeses have been manufactured with probiotic bacteria – viz. *Bifidobacterium animalis* Bo and *Lactobacillus casei* LAFTI®L26, from combinations of bovine whey and milk, following protein denaturation at 90 °C; they were subsequently inoculated (at 10%) with those strains, and homogenized afterwards; additives such as salt and sugar were then incorporated; and the resulting solid matrices were stored at 7° C for up to 21 d. Oscillatory measurements and instrumental texture profile analyses were performed, and sensory analyses were carried out by a trained panel. Microstructural features were in addition ascertained by scanning electron microscopy.

L. casei exhibited a higher acidifying activity than *B. animalis*, which produced distinct textures; higher firmness and viscoelasticity were indeed found in matrices inoculated with the former. Incorporation of sugar and *L. casei* favoured consumer acceptability, relative to plain matrices. Microstructural differences were detected between matrices at different times of storage and formulated with distinct additives.

1. Introduction

Development of probiotic whey cheeses has been under investigation in recent years. Studies pertaining to viability and stability of the probiotic strains inoculated, throughout the whole shelf-life of the product (Madureira, Gião, et al., 2005), as well as to survival during simulated gastrointestinal transit (Madureira, Pereira, et al., 2005) have indeed been developed. To improve their final organoleptic features, incorporation of salty and sweet additives has also been considered – and their effects upon metabolism of the probiotic strains in such matrices were already ascertained by Madureira, Pereira, Gomes, Pintado, and Malcata (2007) and Madureira et al., unpublished.

All aforementioned features play a role upon the flavour perceived in the final matrices – which will constrain or enhance their appeal to the consumer. Besides flavour, texture also plays a crucial role, and strongly depends on the raw material and the processing techniques utilized; however, this issue has not been tackled till date.

On the other hand, sensory methods have been the primary means to assess textural characteristics of foods at large – yet the labour-intensive nature (and some degree of subjectiveness) of those analyses has inevitably led to development of instrumental methods, designed to measure specific food properties that might, at least, (in certain cases) correlate with sensory characters. Imitative tests – which attempt to mimic the forces and deformations associated with biting and mastication, are thus an option – and instrumental Texture Profile Analysis (TPA) fits well into this category.

For several matrices – especially spreadable food products, rheological assessment may be used as a basis to modify and control processing conditions, in attempts to manufacture products characterized by a higher quality (Ozer, Stenning, Grandison, & Robinson, 1999). In fact, small strain dynamic rheological tests are currently in use to ascertain both the elastic and the viscous nature of cheese. Since such methods are typically implemented within the linear viscoelastic region of the material, they are essentially non-destructive of the basic structure of the material tested– which constitutes an advantage *per se*. Recall that the loss (or viscous) modulus, G″, measures the amount of energy dissipated by the material (Lucey, Johnson, & Horne, 2003); in cheese, the viscous component is particularly related to the flow of the filler through the proteinaceous network, or to the existence of "dangling ends". The storage (or elastic) modulus, G′, in turn, is a measure of the amount of energy stored by the material; the elastic component of cheese is an indication of the elasticity of the protein network and its filler components (Lucey et al., 2003). The major advantage of

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small strain tests, applied within the linear viscoelastic region, is that the elastic and loss moduli become only a function of time – and not, in particular, a function of the magnitude of the stress or strain applied (Tunick, 2000).

Besides the effects arising from the whole technological process of whey cheese manufacture, the incorporation of (probiotic) bacterial strains and additives, e.g. salt and sugar, will likely produce changes in the texture and microstructure of the final product – and thus also upon measurable rheological parameters; note that the metabolic activity of said microorganisms during refrigerated storage will likely increase acidity, which is known to affect the aforementioned textural and rheological features. Hence, the aim of this research effort was to study the effect of incorporation of specific probiotic bacteria, together with selected additives, upon evolution of the textural properties of whey cheese throughout time – resorting to TPA and rheological behaviour, besides trying to go one step further in elucidating the observed changes via microstructural features and complementing with sensory assessment.

2. Materials and methods

2.1. Microorganism source

Bifidobacterium animalis strain Bo was previously isolated from fermented milks, and is currently marketed by CSK (Leeuwarden, The Netherlands) as frozen concentrate; *Lactobacillus casei* strain LAFTI®L26 was obtained as DELVO-PRO® freeze-dried, concentrated starter culture from DSM (Moorebank, Australia).

To prepare an inoculum suitable for incorporation in whey cheese, these bacteria were first grown in MRS broth (Merck, Darmstadt, Germany) to activate them, and thereafter cultured twice in skim milk – incubated for 48 and 24 h, in the case of *B. animalis* and *L. casei*, respectively, both at 37° C. The medium used to grow *B. animalis* was supplemented with 0.5 g l^{-1} cystein-HCl (Merck), to help lower the redox potential. Previous experience had indicated that this sequence of culturing steps assured proper activation, and that the cells were in their exponential phase when harvested just prior to experimentation – which provides the best conditions for viability.

2.2. Whey cheese manufacture

Whey cheeses were experimentally manufactured from whey obtained as a by-product of low-fat cheese making – provided by Lacticínios Halos (Quinta da Tapada, Lousada, Portugal); upon arrival, this liquid feedstock was immediately refrigerated to 7 °C, and stored thereafter at that temperature for up to 2 d.

The manufacture protocol followed was described in detail by Madureira, Gião et al. (2005) and Madureira, Pereira et al. (2005): it departed from a mixture of whey and milk (at a volumetric rate of 10:1) as raw material, as discussed below. Said mixture was heated until ca. 95 °C; after precipitation, the resulting, floating curd was collected from the surface with a sterile skimmer, and poured into sterile plastic molds – which were covered and left to drain for 20 min at room temperature (ca. 20 °C), under a constant pressure of ca. 2 N.

Distinct formulations were associated with the experimental research batches run: three encompassed plain whey cheese (C; and Bo and L26, with microbial culture), two were added with salt (BoT and L26T), and the remaining two encompassed addition of sugar (BoS and L26S) – as described in Table 1. In order to simulate the major conditions affecting texture development of the inoculated whey cheeses, skim milk acidified with lactic acid (Sigma) was added at \10 ml/100 g to the control whey cheese matrices (C).

Table 1
Experimental conditions used for manufacture of whey cheese matrices.

Batch number	Matrix	Additive[a] (g 100^{-1} g)	Strain
1	C	–	–
	Bo	–	*B. animalis* Bo
	L26	–	*L. casei* LAFTI®L26
2	BoT	0.8	*B. animalis* Bo
	L26T	0.8	*L. casei* LAFTI®L26
3	BoS	5	*B. animalis* Bo
	L26S	5	*L. casei* LAFTI®L26

[a] The additive used in batches BoS and L26S was sugar, and in batches BoT and L26T was salt.

(Note that our major purpose was to ascertain the effect of additives upon probiotic whey cheeses, so a control with only such additives but no microbial culture was not germane.)

Whey cheeses Bo, BoT and BoS received a 10 ml/100 g inoculum of *B. animalis* strain Bo, whereas L26, L26T and L26S ones were inoculated with *L. casei* strain LAFTI®L26 – in both cases previously prepared in skim milk (Oxoid, Basingstoke, UK); such an inoculum level permitted initial viable numbers in the range 10^7–10^8 cfu/g to be attained. The inoculated curds, as well as the control curd (C, devoid of microbial culture) were stirred with an electric mixer (Kenwood, UK) for 5 min, equally distributed by sterile 50 ml-flasks (hence simulating closed packages), and stored at 7 °C for up to 28 d. Hygienic conditions were assured during the whole handling protocol, in order to prevent external contamination whatsoever.

2.3. Chemical analyses

The whey and milk employed in manufacture of the experimental whey cheeses were initially subjected to chemical analyses in triplicate – including pH, as well as fat, protein and lactose contents, using a LactoScope Filter C4 apparatus (Delta Instruments, The Netherlands).

Similar analyses were performed on the three types of whey cheese matrices (i.e. plain, salt-added and sugar-added) after manufacture. Acidification parameters such as pH and titratable acidity, as well as total solid and moisture contents were determined throughout storage time: the moisture content was determined following the international standard method (IDF, 1958); the bulk pH was measured with a penetration probe connected to a Microph 2001 apparatus (Crison, Barcelona, Spain); and the titratable acidity was determined according to the reference method (AOAC, 1980).

2.4. Microbiological analyses

Sampling of all whey cheese matrices took place at 0, 3, 7, 14 and 21 d, via collection of 8 g-aliquots. The post-manufacture putative contamination by aerobic mesophilic bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., *Enterococcus* spp., molds and yeasts was checked as previously reported by Madureira, Gião et al. (2005), Madureira, Pereira et al. (2005) and Madureira et al. (2008).

Enumeration of viable cells of *L. casei* was achieved on Rogosa agar (Merck), supplemented with 96 ml/100 ml acetic acid (Sigma) to attain a pH of 5.2, and incubated for 3 d at 37 °C; viable counts of *B. animalis* were obtained on MRS agar, supplemented with 0.5 g l^{-1} cystein-HCl (Merck), and incubated anaerobically for 2 d at 37 °C (Gas-Pak plus system, from Becton Dickinson, Maryland MA, US).

2.5. Rheological analyses

Measurements of oscillatory and flow behaviour were performed in a controlled shear rate rheometer (Bohlin Advanced Instruments, Gemini, UK). The measuring geometry consisted of

a cone-plate characterized by 4°/40 mm, and a gap size of 2 mm. A plastic ring was placed on the lower plate, to guarantee no slipping of the sample at the boundary. Samples were tested immediately after manufacture at 7 °C, and all measurements were performed at 20 °C – with an equilibration period of 60 s before actual testing. Samples (ca. 2 g) from whey cheeses were placed in the lower plate, and then the upper plate was slowly lowered until the desired gap size was reached. To assure that the rheological measurements were carried out within the linear viscoelastic region, a strain sweep test was performed in advance.

The viscoelasticity of the samples was determined by small amplitude oscillatory shear; the frequency was set at 1 s^{-1}, and the percent strain was varied from 0.005 to 2. The strain eventually chosen was 0.3, which is well within the linear region; the frequency was then varied from 0.01 to 10 s^{-1}. Both G′ and G″ were calculated from the results of this test.

2.6. Textural analyses

Replicated samples of whey cheese – identical in weight and shape and taken at 0, 3, 7, 14 and 21 d of storage, were assayed via measurement of the force–time curve using a TA.XT apparatus (Stable Micro Systems, Surrey, UK). A 5 kg-load cell was selected and duly calibrated in advance with a 2 kg-weight. The probe used was P/30c (i.e. a 30′ conical device, made of perspex), and tests were performed (in triplicate) directly in the flasks, at three different locations on the sample.

A typical "mastication" testing profile was thus implemented, which involves two consecutive compressions at (controlled) room temperature (25 °C); the compression distance was 20 mm – to ensure that the sample did not fracture before the second compression; and the two consecutive compressions were performed automatically, at a test speed of 5 mm s^{-1}. The tests generated a plot of force vs. time – from which textural parameters were automatically calculated. Hence, it was possible to measure hardness, which can be defined as the maximum peak force during the first compression cycle (first bite) – and be viewed as the force necessary to compress the food between one's molar teeth; note that this concept has often been labelled as firmness (Uprit & Mishra, 2004).

2.7. Microstructural analyses

Samples by 0, 14 and 21 d of storage were prepared for scanning electron microscopy (SEM) using a JEOL-5600 Lv microscope (Japan), following the method of Kaláb and Modler (1985) – because the matrices were typically viscous and creamy, so they could not be cut as a hard cheese would. Agarose at 3 g/100 ml (Sigma) was dissolved by boiling in distilled water and stirring with a magnetic bar, and allowed then to cool to ca. 40 °C – and kept at this temperature afterwards. The sample to be examined by SEM was aspirated (through a 10 mm-long column) into a plastic Pasteur pipette (1 mm in diameter); the exterior of the pipette was wiped clean, and its tip was closed with a small droplet of agarose. Capsules were subjected to freezing at −30 °C for 3 d prior to analysis; capsules were cut as 1 mm-portions, and duly examined. The cold-stage (-20 °C) method was used to examine the samples. SEM was operated at the low vacuum mode, using a spot size of 36–37 and a potential of 20–22 kV. All analyses were performed at room temperature (20 °C).

2.8. Sensory analyses

An acceptance sensory panel assessed coded experimental whey matrices at random. That panel consisted of 15 members, specifically trained for dairy product sensory analyses, with ages ranging from 25 to 45 years old. Matrix pieces were placed into airtight plastic containers, and conditioned at room temperature for 15 min before testing (so as to guarantee that samples were tested while still fresh).

Duplicated samples were assessed at room temperature (20 °C) – after previous confirmation of microbiological safety, using a 5-point hedonic scale for appearance (1 = colorless; 5 = very dark), aroma (1 = undetectable; 5 = very intense), hardness (1 = very soft; 5 = very hard), taste (1 = undetectable; 5 = very intense) and acidity (1 = less acid; 5 = very acid); and a 7-point hedonic scale for overall acceptability (1 = least liked; 7 = most liked) (Lawless & Heymann, 1999). Crackers and water were accessible to all panellists, to clean the palate when appropriate.

2.9. Statistical analyses

Shapiro–Wilke normality tests were applied to the initial raw data encompassing viable cell count, pH, textural and rheological parameters, and most were found not to satisfy the homoschedasticity hypothesis. Since data transformation was not successful in all cases, a non-parametric test – e.g. Friedman's test, was eventually applied to the said data. This test is used to detect differences in treatments across multiple test attempts (it is also known as two-way analyses in ranks).

Statistical differences were unfolded between values, so the influence of storage time was assessed via a Wilcoxon test. This test involves comparison of differences between measurements, so it requires the data to be measured at an intermediate level (storage time). Differences between the seven types of whey cheese were analysed using Mann–Whitney tests, which assess whether two observations come from the same distribution.

One-way ANOVA was applied to the chemical composition, rheological and textural data pertaining to the whey cheeses. In the last case, means between matrices taken at the same storage time were compared to each other.

All tests were performed at a significance level of 0.05, and took advantage of SPSS (v. 17, Chicago IL, USA).

3. Results and discussion

3.1. Chemical profile

Manufacture of the whey matrices resorted to both bovine milk and whey, which accounts for their low fat and protein contents; however, significant differences were found between the matrices in terms of a few chemical parameters, e.g. fat content ($P < 0.05$) (Table 2). Incorporation of additives influenced the chemical profile of the whey matrices chiefly at the time of manufacture. In what concerns total protein, the probiotic plain and its salty counterpart were found to exhibit higher values than the remaining matrices ($P < 0.05$); although very slight, these differences might have arisen from larger extents of syneresis, brought about by acidity and salt, respectively. Salt content was also higher in salted matrices – as expected, since salt was deliberately added to measurable concentrations ($P < 0.05$). Moisture remained in the range 70–74 g/100 g, and no significant changes were observed along storage time (data not shown). Small differences in pH were detected between control matrices and the others; note that addition of lactic acid to control matrices (C), to simulate the inoculum performance, contributed to minimize the magnitude of those differences ($P < 0.05$).

During refrigerated storage, the acidification parameters pertaining to all matrices inoculated with *L. casei* (i.e. L26, L26T and L26S) underwent a decrease of up to 1 pH unit, which was associated to growth increase throughout the same time frame – while

Table 2
Physicochemical composition (average ± standard deviation) of control and inoculated plain, salty and sweet whey cheese matrices by 0 d upon manufacture.

Matrix	Fat (g/100 g)	Total protein content (g/100 g)	Water content (g/100 g)	Salt content (g/100 g)	pH
C	11.00 ± 0.02[a]	12.01 ± 0.02[a]	74.20 ± 0.91[b]	0.3±0[a]	4.84 ± 0.01[c]
Bo/L26	11.16 ± 0.02[b]	12.24 ± 0.01[b]	73.57 ± 0.52[b]	0.3±0[a]	4.70 ± 0.02[a]
BoT/L26T	11.30 ± 0.02[c]	12.31 ± 0.02[b]	72.93 ± 0.19[ab]	0.9±0[b]	4.78 ± 0.02[b]
BoS/L26S	10.40 ± 0.02[d]	11.69 ± 0.03[c]	71.02 ± 1.29[a]	0.3±0[a]	4.71 ± 0.02[ab]

[a,b,c,d] Means pertaining to the same parameter (i.e. in the same column), with the same superscript, do not differ significantly ($P > 0.05$) from each other.

in matrices containing *B. animalis* Bo almost no decrease was detected, except for matrices added with sugar (i.e. BoS); in these, an important decrease was observed between 14 and 21 d of storage.

3.2. Microbiological profile

The viable cell numbers in whey cheese matrices inoculated with *L. casei* increased up to ca. 2 log cycles, during the 21 d-storage period at 7 °C (see Fig. 1); conversely, the initial viable cell numbers of *B. animalis* Bo remained as such in all whey cheese matrices, as storage time elapsed. The numbers of viable cells were not apparently affected by incorporation of additives ($P > 0.05$). However, storage time influenced the viable cell counts of either of the two bacterial species considered, at all storage times ($P < 0.05$). As found before (Madureira, Gião, et al. 2005; Madureira, Pereira, et al. 2005; Madureira et al., 2008), no external contamination was detected in any whey cheese throughout storage time, at least to the limit of detection of the enumeration technique chosen – i.e. 10^3 cfu g^{-1} in general, and 10^2 cfu g^{-1} for coliforms (data not shown).

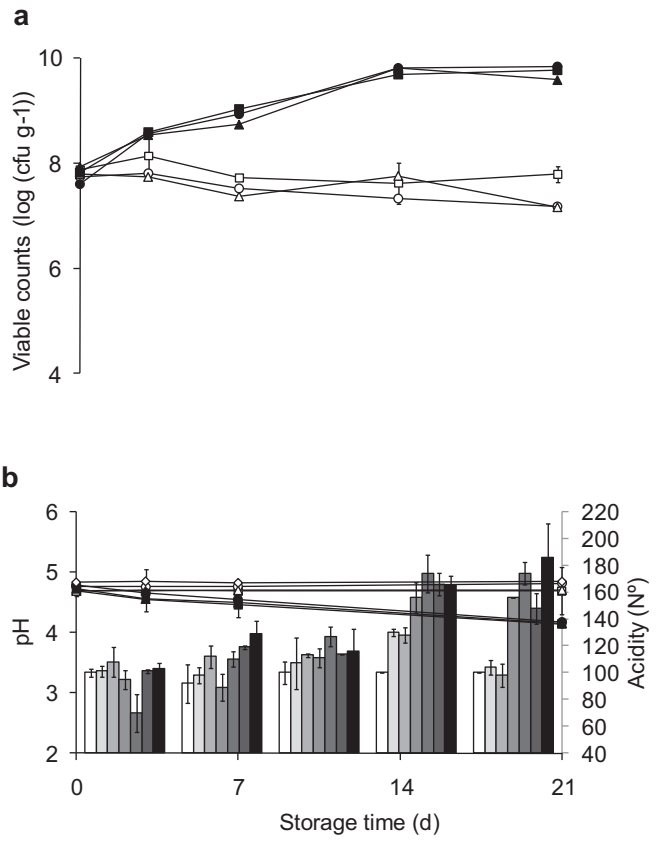

Fig. 1. Evolution of (a) viable counts (average ± standard deviation) and (b) acidification (pH and acidity) of matrices C (□), Bo (▭, □), BoS (▭, ○), BoT (▭, △), L26 (▭, ■), L26S (■, ●) and L26T (■, ▲), throughout storage at 7 °C up to 21 d.

Viable cell counts and acidification profiles were typically similar to those obtained in matrices produced in previous research works (Madureira, Gião, et al. 2005; Madureira, Pereira et al. 2005). The acidification observed in such matrices is related to growth of bacterial strains, which in turn depends on incorporation of additives: higher acidification was observed in matrices characterized by higher viable cell counts and sugar addition levels, and thus higher lactic acid amounts resulted; this is also consistent with results reported elsewhere (Madureira et al., 2008).

3.3. Rheological and textural profiles

The dynamic rheology data produced with the Bohlin apparatus confirmed the viscoelastic nature of our experimental whey cheese matrices. The evolution in G′ and G″ throughout storage, pertaining to all matrices, is shown in Table 3. In general, the elastic modulus was higher than the viscous one, throughout the whole frequency range considered in the test (data not shown) – which unfolds a dominant contribution of the elastic component to the product viscoelasticity, as typically found in the case of solid viscoelastic materials (Kahyaoglu & Kaya, 2003). As expected, the values reported for whey cheeses are lower than those commonly found in milk cheeses (Muliawan & Hatzikiriakos, 2007). Similar spreadable cheeses – e.g. the light spreadable Philadelphia® cream cheese (from Kraft foods) that contains 14.8 g/100 fat, exhibited higher viscoelastic indices than our matrices (Kealy, 2006).

Whey cheeses which had been inoculated with *L. casei* (i.e. L26, L26T and L26S) were always more elastic and viscous than those with *B. animalis* (i.e. Bo, BoT and BoS). Furthermore, matrices containing extra sugar were more elastic and viscous than the remainder (i.e. BoS and L26S), whereas matrices inoculated with *L. casei* and containing additives (L26T and L26S) exhibited G′ and G″ values higher than the others – the higher rate of acidification probably accounts for this observation. Finally, as storage time elapsed, all matrices became more elastic.

The instrumentally measured hardness of whey cheeses containing sugar was higher than that exhibited by the other matrices (i.e. BoS and L26S). Matrices containing *L. casei* (i.e. L26, L26T and L26S) were also firmer; storage time contributed to make them firmer, except those added with salt and inoculated with *B. animalis* (i.e. BoT).

The source whey – in particular because of its fat content, moisture content and pH, is important upon the final textural properties of products similar to cream cheeses (Brighenti, Govindasamy-Lucey, Lim, Nelson, & Lucey, 2008), because the elastic proteinaceous net work entraps fat droplets inside it. Owing to the relatively low fat and protein contents, all such matrices possess in general weak internal bonds – so poor values result for their viscoelastic properties.

Salt-added matrices (i.e. BoT and L26T) showed much lower hardness than the sugar-added ones (i.e. BoS and L26S) by 21 d of storage. Salt (NaCl) influenced gelation time, but not gel strength of

Table 3
Evolution of rheological behaviour, expressed as storage (or elastic) modulus G′, loss (or viscous) modulus G″ and instrumental TPA hardness at 1.35 s^{-1}, throughout storage at 7 °C for up to 21 d.

Matrix	G′ (Pa s)					G″ (Pa s)					TPA (N)				
	0 d	3 d	7 d	14 d	21 d	0 d	3 d	7 d	14 d	21 d	0 d	3 d	7 d	14 d	21 d
C	780^{a}	1615^{a}	1565^{a}	2165^{a}	1550^{a}	267^{a}	400^{a}	375^{a}	587^{a}	459^{a}	0.367^{a}	0.211^{a}	0.305^{a}	0.241^{a}	1.70^{ab}
Bo	1615^{a}	2349^{a}	2165^{a}	2005^{a}	1460^{a}	400^{a}	1401^{b}	587^{ab}	589^{a}	365^{a}	0.391^{a}	0.557^{a}	0.343^{a}	0.283^{a}	0.187^{a}
BoS	1565^{a}	3173^{a}	1550^{a}	1493^{a}	2349^{a}	375^{a}	1000^{a}	459^{ab}	459^{a}	690^{a}	0.295^{a}	0.241^{a}	3.19^{a}	2.83^{bc}	0.163^{c}
BoT	2165^{a}	1156^{a}	2460^{a}	1314^{a}	2634^{a}	587^{a}	296^{a}	365^{a}	1937^{b}	627^{a}	0.883^{a}	2.07^{b}	2.44^{a}	5.64^{bc}	3.21^{a}
L26	1550^{a}	2122^{a}	2349^{a}	4663^{b}	2005^{a}	459^{a}	718^{a}	690^{ab}	1859^{b}	589^{a}	0.870^{a}	4.63^{bc}	4.45^{a}	5.87^{c}	5.92^{bc}
L26S	1460^{a}	2776^{a}	2634^{a}	1509^{a}	3027^{a}	365^{a}	942^{a}	627^{b}	491^{a}	889^{a}	0.722^{a}	3.06^{bc}	3.56^{a}	6.00^{b}	4.56^{abc}
L26T	2349^{a}	974^{a}	2005^{a}	2238^{a}	4263^{a}	690^{a}	331^{a}	589^{ab}	560^{a}	1253^{a}	0.596^{a}	3.94^{bc}	3.49^{a}	6.35^{bc}	6.49^{c}

^{a,b,c} Means pertaining to the same storage time (i.e. in the same column), with the same superscript, do not differ significantly ($P > 0.05$) from each other.

the whey-protein polymers produced at several whey-protein ratios (Vardhanabhuti, Foegeding, Mcguffey, Daubert, & Swaisgood, 2001). Furthermore, salt incorporation did not bring about any significant effect, as generally happens in regular milk cheeses: salt is often used to decrease the water holding capacity, so that matrices become more compact and increase their viscoelastic features – as anticipated, higher moisture content produces less firm matrices (Uprit & Mishra, 2004). Matrices containing extra

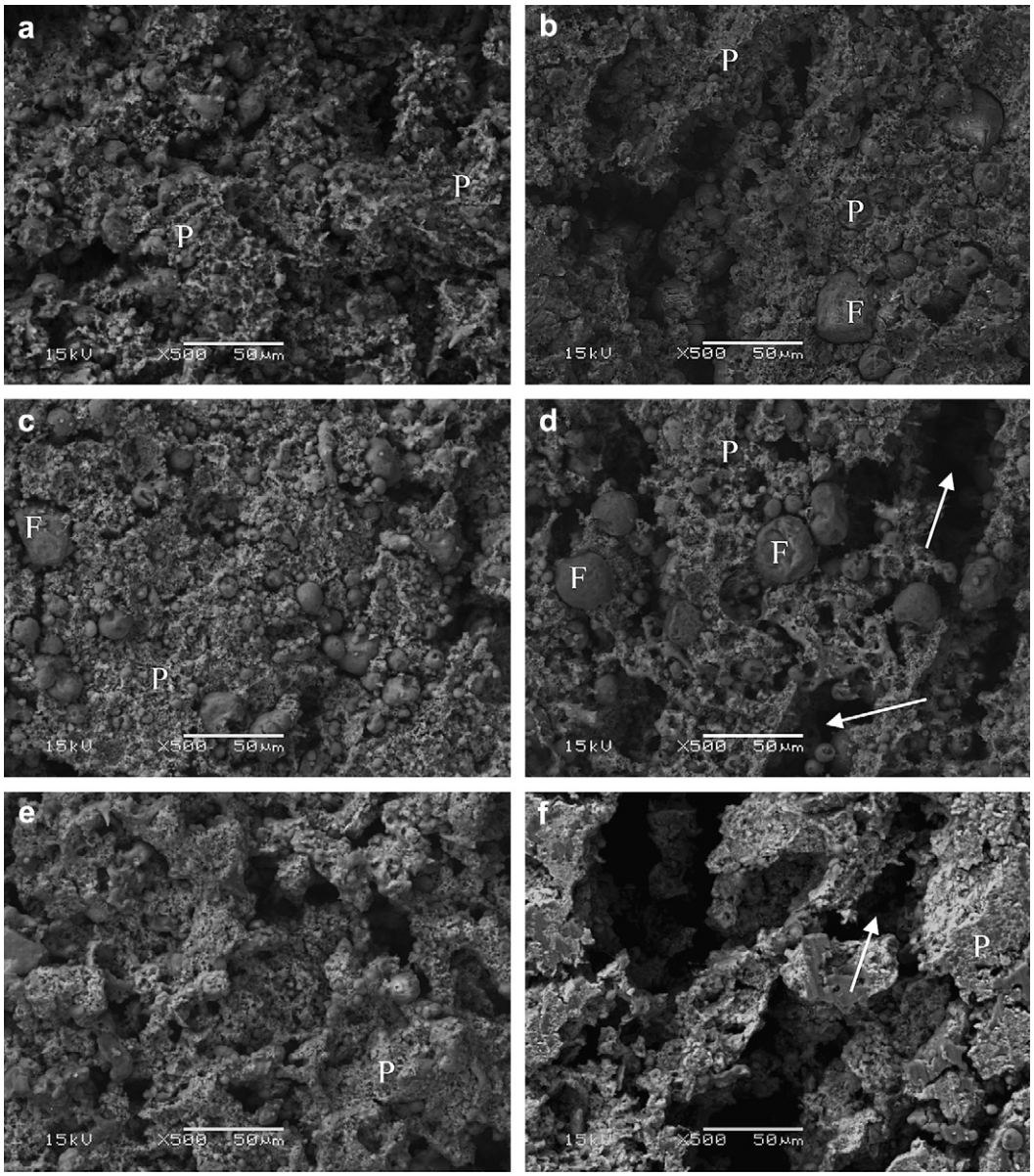

Fig. 2. Scanning electron micrographs (500×) of control matrices (C) by (a) 0 d and (b) 21 d of storage, as well as of salted matrices inoculated with *B. animalis* (BoT) by (c) 0 and (d) 21 d of storage, and sweet matrices inoculated with *L. casei* (L26S) by (e) 0 d and (f) 21 d of storage. Arrows point at dark voids (→), F denotes fat globules, and P denotes protein network.

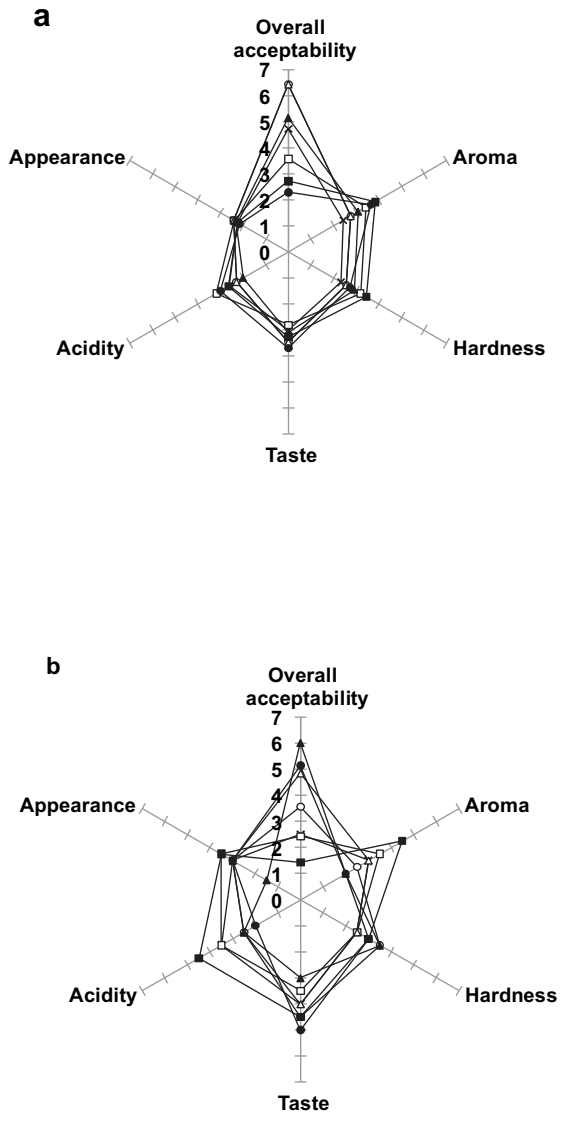

Fig. 3. Sensory evaluation of matrices C (×), Bo (□), BoT (○), BoS (△), L26 (■), L26T (●) and L26S (▲), throughout storage at 7 °C, by (a) 3 d and (b) 7 d of storage.

sugar and inoculated with *L. casei* (i.e. L26S) were firmer than the others, also because of their initial lower moisture content. Matrices characterized by a higher hardness (by the end of storage) were also more elastic (see Table 3); however, this trend did not hold with sugar-added matrices inoculated with *B. animalis* (i.e. BoS), so metabolism of the bacterial strain apparently plays an important role.

The hardness can also be correlated with acidification during storage – as generation of lactic acid led to more consistent matrices; hence, as emphasized before, matrices inoculated with *L. casei* (i.e. L26, L26T and L26S) became firmer than the others. Furthermore, matrices not inoculated were less firm throughout storage than those inoculated – which confirms the effect of the probiotic strain upon hardness, likely once again via their acid production. Cultures added to matrices digest residual lactose and produce lactic acid (Madureira et al., 2008), which aids in lowering pH and thus in creating an ideal environment for coagulation; pH affects reactivity of the binding sites on the casein molecules, and therefore influences the matrix structure (Rowney, Roupas, Hickey, & Everett, 1999).

3.4. Microstructural profile

Cold-stage SEM, following preliminary freeze-fracturing, has been used to examine the microstructure of cheese. No significant differences were detected between matrices inoculated with *L. casei* relative to those inoculated with *B. animalis*, but the opposite was observed between times of storage and additives used; hence, six typical examples of scanning electron micrographs (magnified 500×) of matrices labelled as control (i.e. C), added with sugar and inoculated with *B. animalis* (i.e. BoS), and added with salt and inoculated with *L. casei* (i.e. L26T), by 0 and 21 d of storage at 7 °C, are depicted in Fig. 2.

In general, our matrices appear to consist of a compact protein network (P), containing a small number of large and unevenly dispersed fat globules; this morphology accounts for the extensive elastic character detected in the rheological analyses. The semi-spherical morphology, and the substantial variation in size of these milk fat globules (F) can be observed by examining the void spaces (indicated by arrows) that they originally occupied.

Inoculated matrices (Fig. 2d–f) presented darker voids (see white arrows) by 21 than 0 d; these are expanded protein channels, probably filled with water/whey, since heat-treated whey proteins are insoluble. A similar conclusion can be drawn via comparison of salted matrices (i.e. BoT and L26T) with sweet matrices (i.e. BoS and L26S): darker voids appear in sweet than salty matrices (Fig. 2c and d).

In the micrographs taken, the variation in milk fat globule (F) size in control cheeses resembled that reported for raw milk (Buchheim & Dejmek, 1997). The microstructure of these matrices mimics that of low-fat milk cheeses, in which the number of fat globules is low and the protein matrix is more compact (Madadlou, Khosroshahi, & Mousavi, 2005) – as compared with full-fat cheeses, in which the open spaces are fully occupied by fat globules.

The whey proteins interfered with casein linking to each other, probably due to formation of disulfide bonds between β-lactoglobulin molecules and κ-casein-enrobed micelles – as well as to hydrogen bonding between whey proteins and water incorporated into the protein matrix. Especially in matrices by 0 d of storage (i.e. C and L26), one observes an essentially homogeneous whey-protein network (P), made up by round-shaped casein fibres that are shorter than those in 21 d-matrices (note that these matrices were subjected to homogenization). These dark voids (denoted as arrows) are certainly the result of lactic acid production, coupled with hydration of the protein network. Recall that lactic acid production enhances protein coagulation, turns matrices into harder entities and thus more susceptible to fracture, and aids in filling spaces with water or residual whey. Likewise, our matrices exhibited higher values for hardness, besides being more adhesive and characterized by a more intense openness than the others.

The void spaces shown as a part of the microstructural profile are sometimes rationalized by the hydration theory – which states that expression of whey is a result of *para*-casein fibre swelling during refrigerated storage (Auty, Twomey, Guinee, & Mulvihill, 2001). In our case, water/residual whey creeped back into the matrices themselves – since they were stored in closed vessels.

3.5. Sensory profile

The results of the organoleptic assessment of the various whey cheese matrices are represented as spider plots in Fig. 3. Each corner corresponds to one attribute, and each line to the scores using 7 levels of classification. Sensory attributes by 3 d of storage are represented in Fig. 3a, whereas those by 7 d are represented in Fig. 3b.

In terms of appearance, a similar classification was attributed to all matrices by 3 d ($P > 0.05$). By 7 d, matrices Bo and L26 – which have no additives and had been inoculated with *B. animalis* and *L. casei*, respectively, were classified better than the others, in terms of colour.

The matrices that received the best overall acceptability scores were those containing additives and inoculated with either microorganism, even by 7 d of storage; this clearly indicates that the incorporation of probiotics and additives improves the organoleptic features. By 3 d, the best scores pertained to the matrices inoculated with *B. animalis*, whereas the best scores by 7 d of storage pertained to those inoculated with *L. casei* and including additives (i.e. L26T and L26S). This can be related to the production of acetic acid by *B. animalis* which usually has a negative effect in terms of organoleptic features.

At both times of storage, matrix L26 received the poorest classification – likely because of aroma intensity, hardness and/or acidity. Conversely, matrices inoculated with *L. casei* and added with salt (i.e. L26T), which showed a low intensity in terms of taste and aroma, coupled with a poorly detectable acidity, were the most accepted; this indicates that the incorporation of sweet additives in *L. casei* inoculated whey matrices is crucial for development of acceptable organoleptic features (mainly aroma, taste and acidity).

Despite the poor correlation found between instrumental and sensory hardness in all matrices (0.51), those possessing the highest and lowest instrumental hardness were classified likewise by the sensory panel.

4. Conclusions

Plain whey cheeses undergo no significant changes in viscoelastic properties throughout refrigerated storage. The *Bifidobacterium* and *Lactobacillus* strains are characterized by distinct metabolic profiles, which affect the textural properties and thus the sensory scores of the products. Inclusion of additives, and inocula, or of inocula only produces distinct rheological features; in general, matrices with *L. casei* or with a probiotic strain, and added with sugar lead to higher values of viscoelastic parameters. Furthermore, matrices inoculated with *L. casei* show a better consistency, possibly derived from the higher extent of acidification. Sensory analyses rank best the sweet whey cheese matrices containing *L. casei* – specifically because of their most acceptable aroma, taste and acidity.

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