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Effect of the incorporation of salted additives on probiotic whey cheeses

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Abstract

The research effort described here has focused on incorporation of *Lactobacillus casei*, in whey protein matrices, in the presence of selected salty additives. Those matrices were produced via thermal processing of a combination of either ovine or bovine whey (or a mixture thereof) with ovine milk, and were inoculated (at 10%) with *L. casei* strain LAFTI®L26; salt, salt and herbs, or salt and xanthan were further added to such matrices, which were then homogenized and stored at 7 ºC for up to 21 d. In general, viable cell numbers maintained or even increased throughout the storage period, irrespective of the type of salty additive considered. Partial depletion of lactose was detected, and concomitant production of lactic acid throughout the 21 d-period of storage; lower lactic acid concentrations were found in matrices containing salty additives. In matrices with xanthan (SX), the probiotic strain exhibited the lowest metabolic activity. Matrices SX were less soft and firmer than the others, by the end of storage, and were similar to matrices with herbs (SH). The incorporation of salty additives affected bacterial metabolism, in terms of glycolysis and proteolysis, which in turn had a significant impact on the development of textural properties.

1. Introduction

There is an increasingly wider awareness that a sustained state of good health is directly associated with nutrition and eating habits. This realization has prompted a number of research and development efforts focused on functional

foods, so several products have accordingly reached the market stage—of which ca. 65% have been claimed to be probiotic foods. Such probiotic strains as those belonging to the *Lactobacillus, Bifidobacterium* and *Enterococcus* genera can indeed prevent health disorders, and even improve health conditions via adequate colonization of the lower intestine— thus restoring its original microflora, while providing an acidic environment that inhibits proliferation of pathogenic bacteria (Santosa, Farnworth, & Jones, 2006). Several attempts to incorporate the aforementioned beneficial bacteria in foods and therapeutic preparations were reviewed elsewhere (Agrawal, 2005); they prompted development of a few pro- biotic products on the commercial level, which are specifically targeted at human consumption.

Incorporation of probiotic bacteria has been successfully performed in whey cheese matrices as well (Madureira et al., 2005). Several strains of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus brevis* and *Bifidobacterium animalis* were indeed able to essentially maintain a high viability (with numbers above 10^7 CFU/g) for 28 d of storage under refrigeration. The experimental matrices were manufactured following a traditional recipe that has been for ages in Portugal to obtain *Requeijão*—which entails heat-precipitated proteins from whey. It is marketed as such, or following slight topping with salt. *Requeijão* contains moderate fat levels (in the range 8–14%, by mass) coupled with several proteins such as α- lactalbumin, β-lactoglobulin, lactoferrin, lactoperoxidase, serum albumin and glycomacropeptide; these proteins are acclaimed for their nutritional and health-related features. Hence, *Requeijão* may easily override more classical, low- added value uses of whey (Madureira, Pereira, Gomes, Pintado, & Malcata, 2007). Therefore, novel functional pro- ducts that combine existing nutritional richness with imported health promoting features are thus in order— especially if they are organoleptic appealing.

However, technological selection of probiotic strains for that purpose requires not only that they exhibit an intrinsic ability to maintain high viable populations (in the typical range 10^6–10^8 CFU/g), but also a capacity to withstand additives that convey desirable organoleptic features (Klaenhammer & Kullen, 1999); acceptability of the final product by the consumer will in fact hinge upon both these issues. On the other hand, whey matrices offer excellent conditions for survival and growth of probiotic bacteria—because of a high water activity, a pH above 5, a low salt content and absence of common preservatives. Furthermore, to their putative effect upon viability of probiotic strains, inclusion of additives in those matrices may also influence their texture. Examples of common additives deserving an in-depth study are salt and herbs (to improve taste), and xanthan (to improve texture owing to its stabilizer and binding properties).

In general, actively metabolizing microorganisms in dairy matrices play roles in lactose consumption and consequent organic acid synthesis; but also in proteolysis—i.e. protein hydrolysis, and peptide and amino acid release; and further in lipolysis—i.e. triglyceride hydrolysis and free fatty acid release. These bacterium-mediated activities contribute in different, but somehow complementary ways to the final organoleptic profiles of the dairy product at stake—either favorably, or via generation of off-flavors (Fox, Singh, & McSweeney, 1994). Incorporation of certain food additives may in turn modulate the metabolic pathways of dairy microorganisms—as is the case of several lactic acid bacteria, especially in what concerns lactic acid production and proteolysis; in some situations, undesirable tastes may be neutralized—or else such texture may be adequately modified, via addition of hydrocolloid gums. In all such cases, those additives will contribute to overall organoleptic improvements.

In view of the above considerations the aim of this research effort was to assess the influence of salty additives (viz. salt, garlic, aromatic herbs and xanthan) upon viability, as well as lactose- and protein-breaking down activities of *L. casei*, when incorporated in whey cheeses. The instrumental texture and sensory acceptance of those products were specifically addressed.

2. Materials and methods

2.1. Microorganism source

Lactobacillus casei LAFTI® L26 was obtained as a DELVO-PRO® freeze-dried, concentrated starter culture from DSM (Moor- ebank, Australia).

In order to prepare an inoculum suitable for whey cheese matrices, an overnight inoculum of the bacteria was first made in MRS broth (Merck, Damstadt, Germany), and there- after cultured twice (at 5%) in skim milk (Oxford, Hampshire, UK)—and incubated, in both cases, at 37 °C for 24 h.

2.2. Whey cheese manufacture

Experimental production of whey cheeses used whey released, a by-product of manufacture of full-fat semi-soft cheese, from a mixture of 90% (by volume) ovine and 10% (by volume) bovine raw milks, which was added afterwards with raw ovine milk at 10% (by volume)—all of which were provided by Marofa (Figueira de Castelo Rodrigo, Portugal); upon arrival, both liquid feedstocks were immediately refrigerated to 7 °C, and stored thereafter at that temperature.

Four replicated batches of whey cheese were processed following the recipe

described elsewhere (Madureira et al., 2008), so as to generate as many final products. In each (duplicated) batch, the resulting curd was inoculated with the probiotic culture at 10% (by volume); such an inoculum allowed the desired initial level of 10^7 CFU/g of whey cheese to be attained. One batch was directly used as control (matrix C); the remaining three batches had added separately: 0.60% (by mass) salt—matrix S; 0.60% (by mass) salt, 0.05% (by mass) aromatic herbs (Margão, Vila Franca de Xira, Portugal) and 0.05% (by mass) garlic (Margão)—matrix SH; and 0.60% (by mass) salt and 0.35% (by mass) xanthan—matrix SX. These herbs were added to improve sensory features, whereas xanthan was aimed at improving texture (creaminess in particular).

All matrices were vigorously stirred for 5 min with an electric mixer (Kenwood Electronics, Hertfordshire, UK), with a whisk adapted to the rotating shaft: then they were equally distributed into sterile 100 ml-flasks which were immediately sealed (so as to simulate closed packages) and stored at 7 lC for up to 21 d. Aseptic conditions were assured throughout manipulation, in order to prevent environmental contamination.

2.3. *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 done previously by Madureira et al. (2005, 2008). For enumeration of the viable cell counts of *L. casei* plating was performed on Rogosa agar (Merck), supplemented with acetic acid (Sigma) at 96% (by volume) so as to achieve pH 5.2 (Rogosa, Mitchel, & Wiseman, 1951). All aforementioned media were plated using the Miles and Misra (1938) technique—except VRBGA and RBCA, which followed the pour and spread plate techniques, respectively (Busta, Peterson, Adams, & Johnson, 1984).

2.4. *Chemical analyses*

Whey and milk used in the manufacture of the experimental whey cheeses were initially submitted to physicochemical analyses in triplicate—which included pH, as well as total fat, protein and lactose contents, using a LactoScope Advanced FTIR (Delta Instruments, Drachten, The Netherlands).

Similar physicochemical analyses were also performed on the four types of whey cheese matrices (C, S, SH and SX); in addition, dry weight, moisture and acidity contents were assessed. The total protein content was determined via

Kjeldahl method (IDF, 1985). The fat content was determined using van Gulik's butyrometric determination (Portuguese Standard 2105, 1983). The dry weight was determined according to the standard international method (IDF, 1952). Measurement of bulk pH was made with a penetration probe, connected to a Microph 2001 (Crison, Barcelona, Spain). Finally, the titratable acidity was determined according to reference methods (AOAC, 1980).

2.5. *Lactic acid production assessment*

Replicated samples of whey cheese, taken at 0, 7 and 21 d of storage, were assayed for organic acids and sugars; these sampling times were chosen based on preliminary evidence on the expected time evolution of their concentrations. Quantification was by HPLC in a single run, based on calibration curves prepared in advance with appropriate chromatographic standards—using a LACHROM apparatus (Fullerton CA, USA), with an Aminex HPX-87X cation exchange column from BioRad (Richmond CA, USA); the flow rate was 0.5 ml/min; 0.005 N H_2SO_4 (Merck) was employed as eluant; and detection was by refractive index at 30 ºC for sugars, and UV absorbance at 400 nm for organic acids. Prior to analysis, all samples were pretreated as follows: 4 g of each sample was homogenized with 30 ml of 0.5 M perchloric acid (Merck), for 3 min in a Stomacher Lab Blender 400, allowed to stand for 2 h at a refrigerated temperature in a closed vessel, and then filtered through a 0.22 μm-membrane Syrfil filter (Nucleopore, Cambridge MA, USA). Samples were replicated to estimate experimental variability—which was expressed in the form of an average standard error, for each data set.

2.6. *Proteolysis assessment*

Replicated samples of whey cheese, taken at 0, 14 and 21 d of storage, were assayed for proteolysis (the sampling times were again chosen based on previous evidence of the likely progress of that phenomenon with time): water-soluble nitrogen (WSN), as well as nitrogen soluble in 12% (by mass) trichloroacetic acid (TCA-SN) and in 5% (by mass) phosphotungstic acid (PTA-SN) were determined via Kjeldhal, as described by Kuchroo and Fox (1982) and Stadhouders (1960)—except that a Stomacher Lab Blender was used for homogenization, and that the supernatant obtained was filtered through Nr. 42 filter paper. Such proteolysis indices as WSN-TN%, TCA-TN% and PTA-TN

% were then calculated as the ratios of WSN, TCA and PTA to total nitrogen (TN), respectively.

2.7. Textural analyses

Replicated samples of whey cheese, taken at 0, 3, 7, 14 and 21 d of storage, were assayed via measurement of the force– time curve with a TA.XT apparatus (Stable Micro Systems, Surrey, UK). A 5 kg-load cell was calibrated with a 2 kg-weight. The probe used was *P*/30*c* (a 30⁰ conical device, made of perspex), and tests were performed directly in the flasks (in triplicate), at three different locations in the sample. The samples were identical in weight and shape. A typical "mastication test" testing profile was followed, which involves two consecutive compressions at controlled room temperature (25 ºC). The compression distance used was 20 mm, thus ensuring that the sample would not fracture before the second compression. The two consecutive compressions were performed automatically, at a test speed of 5 mm/s. This test made it possible to measure five attributes: hardness, gumminess, cohesiveness, adhesiveness and springiness. To measure softness a single cycle of compression was used—at a test speed of 2 mm/s and a compression distance of 20 mm.

2.8. Sensory analyses

An acceptance sensory panel assessed coded experimental whey matrices at random. The panel consisted of 15 members, specifically trained for dairy product organoleptic analyses, with ages ranging from 25 to 45 year-old. Whey cheese pieces were placed into air-tight plastic containers, and conditioned at room temperature for 15 min before evaluation (so as to guarantee that samples were consumed still fresh). Duplicated samples were evaluated at room temperature (20 ºC)—but only after previous confirmation of microbiological safety, by the consumer panel using a 9-point hedonic scale (in which 1 corresponds to "very bad", and 9 to "very good"). Between analyses, the panel took water and unflavored cookies, so as to eliminate the taste of the previous analysis. During classification, general remarks about aroma, consistency, flavor and acidity were also recorded (Lawless & Heymann, 1999).

2.9. Statistical analyses

Normality tests (Shapiro-Wilke) were applied to all raw experimental data—and most were found not to satisfy the homoschedasticity hypothesis. Since data transformation was not successful in all cases, non-parametric tests—e.g. Friedman's test, were eventually applied to such data. How- ever, statistical significant differences were detected between values; hence, the influence of

storage time was assessed via Wilcoxon tests. Differences between the four types of whey cheeses were assessed using Mann–Whitney tests. All tests were performed to a 5% significance level, using SPSS v. 20 (Chicago IL, USA).

Correlations between viable cell counts, physicochemical parameters, lactose and lactic acid contents, and texture were checked via Pearson's test, to a 1% significance level.

3. Results and discussion

3.1. Microbiological profiles

The viable cell numbers of *L. casei* in the four whey cheeses stored at 7 °C for 21 d are tabulated in Table 1. In all matrices, these numbers increased up to ca. 1.5 log cycles during the whole period. The numbers of viable cells were not affected by incorporation of additives, and no statistically significant differences were found between viability of the four matrices ($P > 0.05$); only the factor storage time influenced the viable cell numbers ($P < 0.05$), especially after 7 d of storage for matrices SH and SX. The other matrices C and S only showed statistical significant differences after 14 d of storage time. The aseptic conditions used during all experimental work resulted in no external contamination of the four whey cheeses, since manufacture and throughout the whole storage period (data not shown).

One of the major reasons behind selection of *L. casei* strain L26 for this work was its intrinsically good technological features—viz. good viability profile in solid whey matrices; this strain had been reported to increase its viable cell counts when inoculated in whey cheeses manufactured with bovine whey and milk (Madureira et al., 2005), and more recently in similar matrices further added with sweet additives (Madureira et al., 2008). Strains of *L. casei* were also shown to exhibit good viability profiles upon incorporation in such other related dairy products as fermented milk (Nighswonger, Brashears, & Gilliland, 1996), Cheddar cheese (Gardiner, Ross, Collins, & Fitzgerald, 1998; Ong, Henriksson, & Shah, 2006), Argentinean fresco cheese (Vinderola, Prosello, Molinari, Ghilberto, & Reinheimer, 2009), Brazilian Minas fresh cheese (Buriti, Rocha, Assis, & Saad, 2005) and Argentinean semi-hard cheeses (Bergamini, Hynes, & Zalazar, 2006).

3.2. Physicochemical profiles

The evolutions in pH and titratable acidity along storage time are represented in Table 1. All whey cheeses underwent a decrease of ca. 1 pH unit (from 5.5

to 4.5) along storage time. Acidity was relatively high, irrespective of the additive incorporated; hence, no significant differences were found in pH and titratable acidity among whey cheeses (P40.05), yet significant differences existed among storage times (Po0.05). The physiochemical parameters pertaining to the raw materials (whey and milk) used in the manufacture of whey cheeses, and to the final four matrices manufactured there- fore are represented in Table 2. All values encompassing milk and whey composition are in agreement with the literature (Morr, 1989). Chemical denaturation of whey proteins usually takes place in the pH range 5.5–6.0. The pH of whey and milk used for manufacture of the experimental whey cheeses was relatively low, as a consequence of the microbial-mediated acidification of milk during cheesemaking; addition of milk obviously increased the final pH of the mixture during manufacture.

Significant differences were found in total protein between SX whey cheese and the others, which can be attributed to the reduction of water content because of the presence of xanthan (P<0.05). The water content was also influenced by addition of salt and herbs (Table 3).

Significant correlations were found between viable cell counts, pH and acidity: the highest correlation (r = 0.98; P<0.01), between viable cell counts and pH, was found in whey cheeses S, whereas the lowest (r = 0.91; P<0.01) was found in whey cheeses C.

3.3. *Influence of additives in lactic acid production*

The organic acids produced by *L. casei* in our whey cheeses, were lactic and acetic acids. In all experimental matrices, lactose was partially converted to lactic acid; in the case of acetic acid—which is normally a product of the degradation of pentoses, its content was low throughout storage time (data not shown).

The consumption of lactose, as well the concomitant production of lactic acid are represented in Fig. 1. *L. casei* is known to be a facultative heterofermentative, as it converts lactose as primary substrate into lactic acid, acetic acid and carbon dioxide. Lactose was not quantitatively converted, since several parameters may have influenced the metabolism of said strain, viz. storage temperature and time, and absence of oxygen—all of which play a role upon production of acetic acid (Martínez-Anaya, Llin, Macías, & Collar, 1994). At the time of production, the incorporation of additives produced significant statistical differences, i.e. differences between whey cheeses C and the others whey cheeses (P<0.05). In all whey cheeses, the concentration of lactic acid increased along with decrease in concentration of lactose during storage, as expected; significant differences were found throughout storage time, and between whey cheeses C and the others

(P<0.05). Consumption of lactose in whey cheeses C and SH was consistent with the highest production of lactic acid; by 0 d, lactose concentrations were ca. 20 mg/g, and these figures decreased ca. 6 mg/g by the end of storage (Fig. 1a). At the end storage (21 d), the lower conversion of lactose in lactic acid was found in whey cheeses added with salt and xanthan (SX) (Fig. 1b). Surprisingly, a poor correlation was found between lactose and lactic acid concentrations, throughout storage time (r =-0.60).

The aforementioned results show that salty additives have an influence upon the metabolic activity of *L. casei* when inoculated in whey cheese matrices. The incorporation of salt in the whey cheeses decreased the concentrations of lactic acid produced by *L. casei*, when compared with those obtained in whey cheeses added with sweet additives (Madureira et al., 2008). These phenomena can be explained by the effect of salt, and reduction of water activity, which was shown to negatively affect the metabolic activity of lactic acid bacteria (Troller & Stinson, 1998). Likewise, xanthan appeared to have a great impact on the glycolytic activity of such strain, since in these matrices the lowest conversion of lactose to lactic acid took place. This gum delayed in time the glycolytic events. This phenomena was already seen in other research studies (Soukoulis, Panagiotidis, Koureli, & Tzia, 2007), whereas yoghurt manufactured with xanthan presented higher fermentation times than the control matrices.

3.4. *Influence of additives in proteolysis*

Proteolytic indices during storage time are represented in Fig. 2. In general, the proteolytic activity of *L. casei* was throughout that period. Nevertheless, higher values of all nitrogen fractions (ca. 8%) were found in the case of whey cheeses with salt (S).

The ripening depth (TCA-SN%) evolved in a way similar to WSN (Fig. 2b), especially in the case of matrices SX (r = 0.99; Po0.01). Small peptides and even free amino acids were also formed; those were soluble in PTA, and led to high values of PTA-SN (see Fig. 2c). Statistical significant differences were found for WSN%, between matrices S and the others matrices at time 21 d (P<0.05). No significant differences were found between TCA-SN% associated with the four matrices (P<0.05). In the case of PTA%, matrices SX were statistically different (P<0.05), in which, proteolysis was less extensive than in the other whey cheeses. The values obtained are similar to those for Cheddar cheeses manufactured with milk inoculated with the same *L. casei* strain, but stored at a low refrigeration temperature (4 ºC) and for a ripening period of 8 wk (Ong et al., 2006).

The reasons for such low values may derive from the substrate proteins, which

are less available for hydrolysis owing to their denatured and aggregated form. Whey cheeses were as well manufactured without addition of rennet or coagulant; therefore, the extent of proteolysis (WSN%) observed (Fig. 2a) is likely a result of bacterial proteases and peptidases only. In addition, these strains are generally assumed to exhibit a relatively low level of caseinolytic activity, and a high level of peptidolytic activity (Fernández de Palencia, Peláez, & Martín-Hernández, 1997; Ztaliou, Tsakalidou, Tzanetakis, & Kalantzopoulos, 1996). The additives may also play a role: salt had no effect upon proteolysis (as expected); xanthan reduced water activity—and accordingly constrained release of water-soluble peptides, since matrices SX exhibited lower proteolysis levels than the others; and garlic and herbs may possess inhibitory activity upon bacterial peptidases and proteases.

3.5. *Textural analyses*

The evolution of textural parameters is represented in Fig. 3. These probiotic whey cheese matrices are spreadable, so no fracturability was detected whatsoever.

Whey cheeses C and S were the softer by the end of storage: until 7 d, they were always less soft than the SH and SX ones (Fig. 3a). Conversely, softness decreased as storage time elapsed, in the case of whey cheeses SH and SX; by the end of storage, these were 3-fold less soft than matrices C and S.

Hardness evolved similarly in all matrices up to 7 d (see Fig. 3b); salt-containing matrices became harder than matrix C ($P<0.05$). Whey cheeses SH and SX exhibited higher acidification, which could positively influence whey protein aggregation as the isoelectric point of whey proteins is approached—so more compact, harder matrices would likely result. The former were also the softer at the time of manufacture, in contrast with matrices without additive (C) or with salt (S). As expected, softness correlated better with hardness ($r = 0.526$; $P<0.01$) than with the other textural parameters. The values of hardness obtained here were higher than those obtained for milk fresh cheeses inoculated with the same bacterial species and for refrigerated 28 d (Buriti et al., 2005). Whey cheeses with added xanthan were also the harder by the end of storage; this was a probable consequence of the use of this gum, since xanthan is a bacterial exopolysaccharide (commonly used as a stabilizer, emulsifier, thickener and binding agent, e.g. in the preparation of dairy products). The increase in hardness of other dairy products incorporated with xanthan, e.g. yoghurt, was shown previously (Soukoulis et al., 2007). This gum is also known to bring about increases in softness.

Whey cheeses S were always less adhesive than the others. Whey cheeses SH and SX became more adhesive along storage time, with some oscillations (Po0.05). Gummi- ness remained almost constant in matrices with salt and herbs (i.e. SH) (see Fig. 3d); this parameter increased in value for whey cheeses S and SX, especially toward the end of storage. All matrices were significantly different from each other (P<0.05). Adhesiveness and gumminess were inversely correlated to a high degree (r =-0.746; P<0.01), as expected; and were much higher for these matrices than for milk fresh cheese (Buriti et al., 2005). Moreover, addition of xanthan produced matrices characterized by higher adhesiveness and gumminess, owing to the natural properties of this gum that were mentioned before.

Springiness was similar for all matrices at the beginning of storage; however, by the end of the storage period, differences were found between the four matrices. Matrices S were found to be less elastic than the others, whereas the most elastic ones were matrices SH. Finally, cohesiveness decreased in all matrices during storage, but was always higher in matrices SH and SX. Cohesiveness correlated with springiness (r = 0.844, P<0.01) better than with the other textural parameters. Both properties were shown to be in agreement with those found for milk fresh cheese (Buriti et al., 2005).

The incorporation of additives and the moisture content were shown to be rather important for textural parameter evolution throughout storage time. *Requeijão* can be considered as a soft-cheese in its original form—as its moisture content lies in the range 48–80%. Softness of these matrices was certainly originated by the preliminary homogenization of both inoculum and additives. In fact, even without significant changes in moisture contents during storage (since they are kept in closed vessels), the initial moisture content is important for the eventual evolution of textural parameters: high levels of moisture weaken the protein network, thus turning matrices softer (Beal & Mittal, 2000).

Another important parameter that influences texture is proteolysis, especially in terms of hardness (Tunick, Malin, Smith, & Holsinger, 1995). Changes in calcium concentrations promoted by pH decrease are also responsible for fragile and fragmented matrices (Yasici & Dervisoglu, 2003). In matrices SX, a lower degree of proteolysis was indeed detected—and they were firmer than the others; a higher acidity was also noted by 21 d of storage. In fact, a high correlation resulted between pH, acidity and hardness (r=0.90; P<0.05). Matrices S and SH exhibited the higher proteolysis extent, and were in turn included in the group that proved less firm by the end of storage (Fig. 2a and b).

3.6. *Sensory analyses*

The results of the organoleptic assessment of the various whey cheese matrices are depicted in Table 4. The sensory analyses were performed at both times of storage 3 and 14 d. At these storage times, the higher acidification decrease occurs, as shown elsewhere (Madureira et al., 2005). So the impact of such acidification in the sensory acceptance, and the impact of the inclusion of additives in the whey cheeses was obtained at both times of storage. Matrices containing salt, garlic and herbs (SH) received the best scores by 3 d of storage. As storage time elapsed, all matrices tended to receive poorer and poorer scores, except the control matrices (C)—which remained essentially constant. The panel specifically suggested that all matrices were rather creamy. Therefore, the (unwanted) presence of granules— initially pointed out in traditional *Requeijão* by Pintado, Lopes da Silva, and Malcata (1996) were eventually eliminated via homogenization of the inocula and additives after their incorporation in the whey protein clot. Sensory scores of matrices S and SX by 3 d of storage were indeed lower; this realization can be associated with their higher moisture content, 73 and 68%, respectively. By 14 d, the scores received by those matrices decreased; despite their harder texture than by 3 d of storage, acidity increased and led to an overall decrease in organoleptic scoring.

Matrices S and SH underwent a statistically significant decrease by 14 d, relative to 3 d of storage—whereas matrices C and SX retained their initial scoring. Although incorporation of additives was also planned to improve organoleptic texture, the associated effects were not fully reproducible. This was especially noticed in matrices S and SH by 14 d: the panel described their taste as saltier than that of the others, although they had the same salt concentration—a higher acidity possibly enhanced saltiness perception (see Table 1). Furthermore, whey cheeses S and SH received the lower sensory scores because of a bitter flavor, probably related to the higher release of peptides and amino acids. Acidification was lower in matrices SX (see Fig. 3), and was certainly masked by the contribution of xanthan—so the salty taste was not potentiated by acidity. Nevertheless, the panel detected an unpleasant flour taste in matrices SX. On the other hand, and despite their low amounts, these compounds (peptides and amino acids) may be important as growth promoters of *L. casei*.

4. Conclusions

The viable cell numbers of *L. casei* increased in all inoculated whey cheese

matrices throughout storage time, irrespective of the additives used. All matrices underwent acidification during storage, mainly due to bacterial-mediated production of lactic acid. This production was influenced by incorporation of salty additives; lower levels of such acid were indeed found in matrices with salty additives (especially salt and xanthan) than those of control matrices (i.e. without additives).

The incorporation of additives, such as those used in matrices SH and SX, influenced the proteolytic activity of *L. casei*, which was low anyway—higher values were found in the control matrices (C) and in those with added salt (S). Matrices added with salt also exhibited higher proteolysis levels, which influenced such textural parameters as hard- ness and softness: matrices SX were less soft and firmer than the others, by the end of storage. Sensory assessment revealed acid notes; control matrices and those containing gum were clearly preferred by the panel by 14 d of storage. Furthermore, whey cheese matrices with herbs and with gum appeared similar in terms of texture. Incorporation of additives proved an alternative to change the overall organoleptic features, via masking (or at least delaying) acidification—and produced a series of events in terms of metabolic activities, that can favorably influence textural parameters.

Conflicts of interest statement

There are no conflicts of interest.

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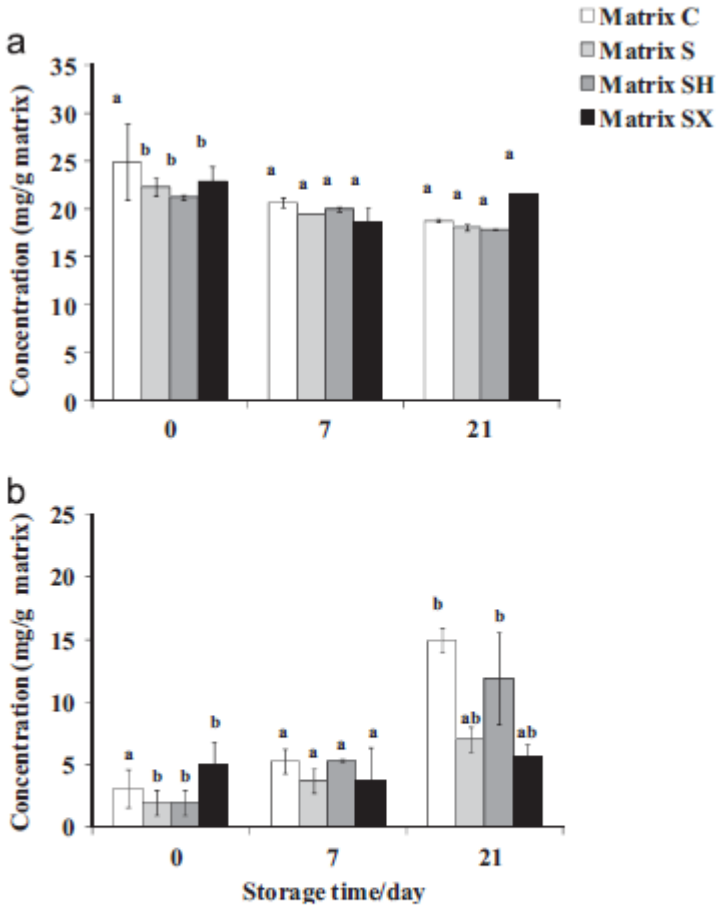

Fig. 1 – Evolution of (a) lactose and (b) lactic acid concentrations (average±standard deviation), of whey cheese matrices throughout storage at 7 °C. C—Control; S—with salt; SH—with salt and herbs; SX—with salt and xanthan. [a,b] Means of the same matrix type labeled with different superscripts differ significantly ($P < 0.05$).

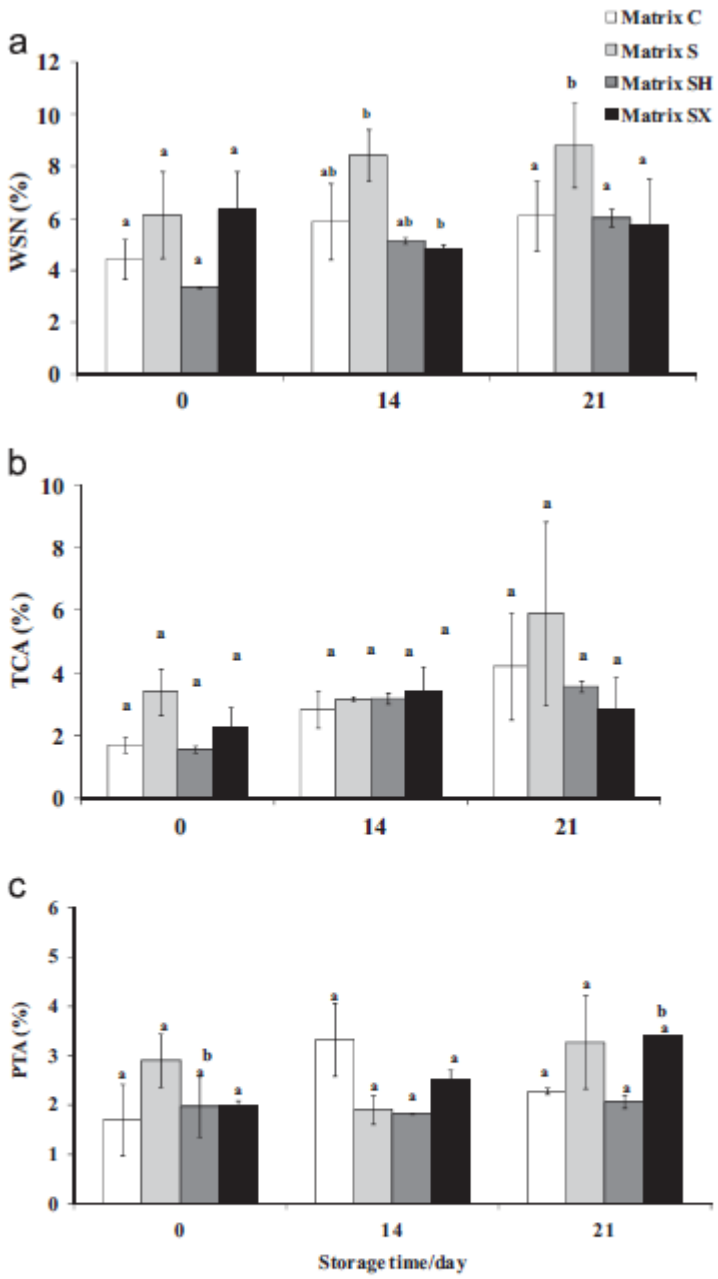

Fig. 2 – Evolution of proteolysis (average ± standard deviation), as (a) water soluble nitrogen; (b) TCA-soluble nitrogen, and (c) PTA-soluble nitrogen, of whey cheese matrices throughout storage at 7 °C. C—Control; S—with salt; SH—with salt and herbs; SX—with salt and xanthan. [a,b] Means of the same matrix type labeled with different superscripts differ significantly ($P < 0.05$).

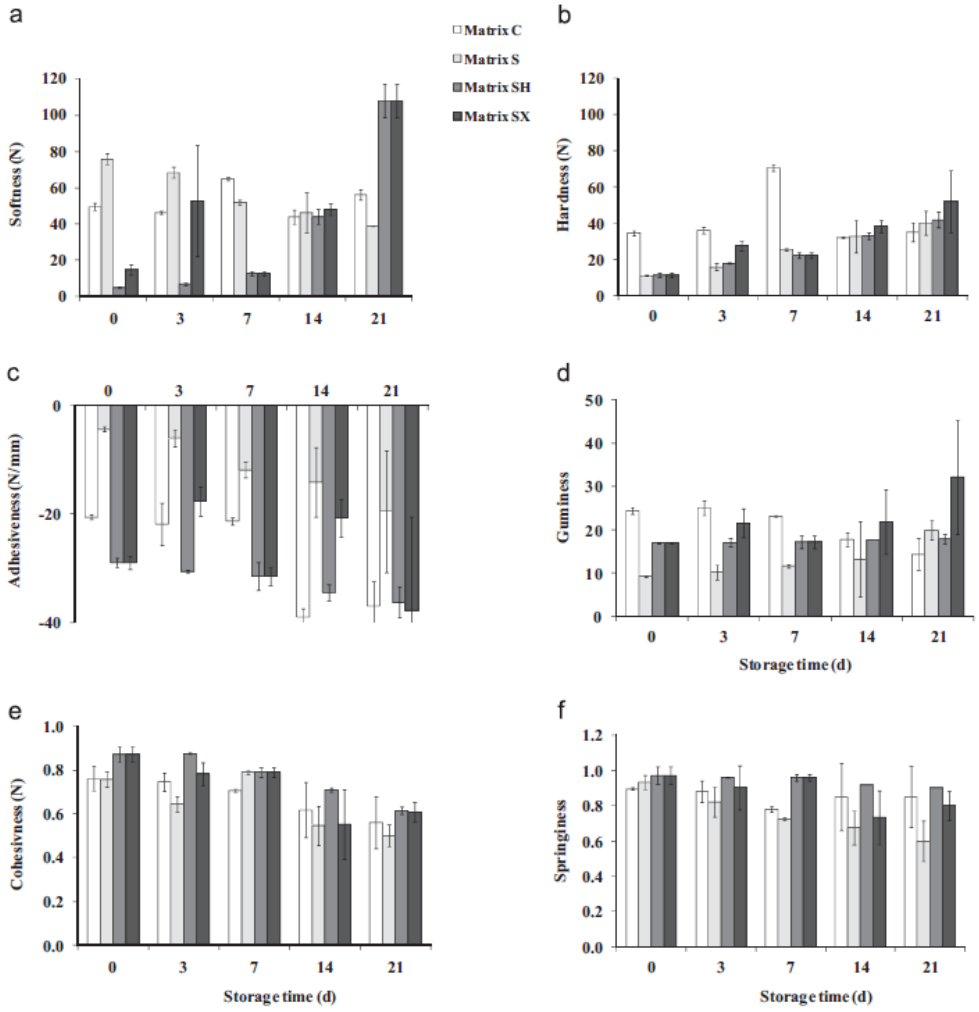

Fig. 3 – Evolution of instrumental texture parameters (average ± standard deviation) as (a) softness; (b) hardness; (c) adhesiveness; (d) gumminess; (e) cohesiveness; and (f) springiness, of whey cheese matrices throughout storage at 7 °C. C—Control; S—with salt; SH—with salt and herbs; SX—with salt and xanthan.

Table 1 – Evolution of viable counts (average ± standard deviation) and of physicochemical parameters (average ± standard deviation), of whey cheese matrices throughout storage at 7 °C.

Whey cheese	Storage time (day)	*L. casei* viable cell counts (Log CFU/g)	pH	Titratable acidity (°N)
C	0	8.14 ± 0.07^{a}	5.20 ± 0.11^{a}	68 ± 0.00^{a}
	3	8.09 ± 0.06^{a}	5.07 ± 0.04^{ab}	84.2 ± 0.3^{a}
	7	8.11 ± 0.08^{a}	4.89 ± 0.14^{ab}	164 ± 6.0^{b}
	14	9.19 ± 0.04^{b}	4.75 ± 0.01^{bc}	ND
	21	9.29 ± 0.02^{b}	4.43 ± 0.04^{c}	266 ± 3.0^{c}
S	0	8.00 ± 0.23^{a}	5.15 ± 0.02^{a}	62 ± 3.0^{a}
	3	8.08 ± 0.02^{a}	4.89 ± 0.01^{b}	86 ± 8.0^{a}
	7	8.76 ± 0.98^{a}	4.51 ± 0.07^{c}	164 ± 5.0^{b}
	14	8.73 ± 0.45^{a}	4.43 ± 0.02^{c}	186.0 ± 0.0^{bc}
	21	9.37 ± 0.01^{b}	4.18 ± 0.02^{d}	234 ± 3.0^{c}
SH	0	8.23 ± 0.01^{a}	5.05 ± 0.07^{a}	50.0 ± 3.0^{a}
	3	8.38 ± 0.11^{a}	4.87 ± 0.05^{ab}	90 ± 14.0^{ab}
	7	8.76 ± 0.00^{b}	4.75 ± 0.07^{abc}	164.0 ± 6.0^{bc}
	14	9.32 ± 0.05^{c}	4.61 ± 0.11^{bc}	204.0 ± 9.0^{c}
	21	9.18 ± 0.77^{c}	4.46 ± 0.05^{c}	260 ± 17^{c}
SX	0	8.21 ± 0.04^{a}	5.43 ± 0.20^{a}	78.0 ± 14.0^{a}
	3	8.18 ± 0.07^{a}	5.25 ± 0.07^{ab}	122.0 ± 3.0^{ab}
	7	8.84 ± 0.09^{b}	4.94 ± 0.06^{bc}	164.6 ± 7.0^{b}
	14	9.18 ± 0.04^{c}	4.68 ± 0.19^{c}	186.0 ± 7.0^{b}
	21	9.22 ± 0.14^{c}	4.27 ± 0.06^{d}	328.0 ± 6.0^{c}

C—Control; S—with salt; SH—with salt and herbs; SX—with salt and xanthan.

^{a,b,c,d} Means within the same column, labeled different subscripts, differ significantly ($P < 0.05$).

ND—not determined.

Table 2 – Physicochemical composition (average ± standard deviation), of whey and milk feedstocks.

Material	Component			
	Fat (%)*	Protein (%)*	Lactose (%)*	pH
Whey	0.78 ± 0.01	0.50 ± 0.01	4.90 ± 0.89	5.31 ± 0.02
Milk	5.18 ± 0.02	8.00 ± 0.03	4.30 ± 0.52	5.98 ± 0.03

* % (by mass).

Table 3 – Physicochemical composition (average ± standard deviation), of whey cheese matrices right after manufacture.

Whey cheese	Fat (%)*	Total protein (%)*	Water content (%)*	Lactose (mg/g)
C	13.90 ± 0.13^{b}	13.02 ± 1.73^{a}	66.33 ± 0.63^{a}	24.9 ± 3.9^{a}
S	12.31 ± 0.12^{b}	12.67 ± 3.35^{a}	72.6 ± 1.1^{b}	22.30 ± 0.93^{b}
SH	13.08 ± 0.53^{b}	15.27 ± 3.32^{a}	66.50 ± 0.58^{ab}	21.20 ± 0.25^{b}
SX	13.49 ± 0.10^{b}	10.49 ± 1.18^{b}	68.5 ± 2.1^{b}	22.9 ± 1.5^{b}

C—Control; S—with salt; SH—with salt and herbs; SX—with salt and xanthan.
^{a,b} Means within the same column labeled with different superscripts differ significantly ($P < 0.05$).
* % (by mass).

Table 4 – Sensory assessment (average ± standard deviation), of whey cheese matrices throughout storage at 7 °C.

Storage (time/d)	Whey cheese			
	C	S	SH	SX
3	4.70 ± 0.96	3.5 ± 1.3	5.2 ± 1.0	3.50 ± 0.95
14	4.50 ± 0.93	2.40 ± 0.82	2.20 ± 0.58	3.20 ± 0.88

C—Control; S—with salt; SH—with salt and herbs; SX—with salt and xanthan.