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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 °C 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

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foods and therapeutic preparations were reviewed elsewhere (Agrawal, 2005); they prompted development of a few probiotic 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 products 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 (Moorebank, 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 thereafter 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 °C 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