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Mini soft cheese as a simple model for biochemical studies on cheese-making and ripening

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Abstract

A new miniature cheese model obtained under controlled microbiological conditions was proposed, characterized and tested for reproducibility. Optimal heat treatment of cheesemilk was defined, as well as maximal ripening time. Miniature cheeses were obtained with batch pasteurized milk (65 °C, 30 min) and ripened at 5 °C. Lactic and nonlactic microbial populations were monitored by plate counts. Proteolysis was assessed by nitrogen fractions, electrophoresis and liquid chromatography, and a sniffing test was applied to evaluate aroma. Coliform bacteria decreased during ripening but moulds and yeasts increased up to 10^4 cfu/g after 60 d, which defined the end of ripening period. Starter population remained constant during all ripening (10^9 cfu/g), while nonstarter lactic acid bacteria increased from $\sim 10^2$ to 10^4 cfu/g. Soluble nitrogen levels at pH 4.6, in trichloracetic acid (0.73 mol/l) and in phosphotungtic acid (0.009 mol/l) were 151, 67, and 10 g/1000 g of the total nitrogen, respectively, after 60 d of ripening, which are usual values for soft cheeses. Proteolytic patterns as measured by electrophoresis were also similar to those of standard cheeses, as well as the aroma of the products. Peptide profiles revealed that the areas of most peaks increased with ripening time. The proposed model showed to be suitable for the production of mini cheese specimens for laboratory testing of cultures and enzymes in similar conditions to their real environment in the food matrix.

Keywords: Soft cheeses; Miniature cheese model; Cheese composition; Starter and nonstarter bacteria; Proteolysis

1. Introduction

Many aspects of the biochemical events involved in cheese production and ageing are not yet completely known. The impact of processing variables such as cheesemilk, temperature profile during cheese-making and starter or adjunct cultures on the quality of the resulting cheese are usually assessed by means of cheese-making experiments (Hunter, McNulty, & Banks, 1997). However, cheese-making trials are both expensive and time consuming. Reproducibility may also be difficult to obtain at pilot plant scale, and contamination with nonstarter microorganisms as well as sub-lethal phage infections can change ripening patterns leading to misinterpretation of the results (Chapot-Chartier, Deniel, Rousseau, Vassal, & Gripon, 1994; Martley & Crow, 1993). In this context, miniature cheese models have been proposed as an alternative to pilot plant experiments, as they can be prepared under controlled microbiological conditions and are more economical, reproducible and easier to obtain (Shakeel-Ur-Rehman, Fox, McSweeney, Madkor, & Farkye, 2001). Miniature cheese models have been reported for Cheddar cheese and 'Saint-Paulin' washed-curd cheese, but no soft cheese variety has been proposed yet (Hynes, Ogier, & Delacroix-Buchet, 2000; Shakeel-Ur-Rehman, McSweeney, & Fox, 1998).

Cremoso Argentino cheese consists of a relatively simple ecosystem which only includes a thermophilic starter of *Streptococcus thermophilus* strains, and contrary to many soft cheeses, does not comprise a surface flora

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(Choisy et al., 1997; Gripon, 1993; Zalazar, Meinardi, & Hynes, 1999). In addition, the cheese-making does not involve cooking or washing of the curd, or cheddaring and stretching steps, which makes it relatively easy to miniaturize and contributes to the reproducibility of the model (Hynes, Zalazar, & Delacroix Buchet, 1999; Zalazar et al., 1999).

Cremoso cheese is a protein matrix with high fat and moisture content: minimum value for fat content in the dry matter is 500 g/1000 g, according to Argentinean legislation (Código Alimentario Argentino). The product is also rich in calcium (8–12 g/1000 g), as coagulation is basically enzymatic, and contains relatively high initial content of galactose (8–10 g/1000 g) as most *S. thermophilus* strains are unable to metabolize this carbohydrate (Zalazar et al., 1999). Cremoso's texture is crispy and hard at the beginning of the ripening, but afterwards the cheese softens and can even liquefy if the ripening period is too long. Softening occurs throughout the whole body of the cheese, on the contrary than Camembert and other surface mould-ripened cheeses, which soften from the inside to the outer part, due to a pH gradient verified in the cheese body. Cremoso cheese softening has been traditionally related to proteolysis as α_{s1} casein was long considered as a structuring protein and it is extensively degraded during ripening (de Jong, 1976; Guinee, 2003; Hynes et al., 2001). However, other studies suggest that pH may have as much importance in cheese texture as proteolytic changes (Alonso, Candioti, & Hynes, 2005; Hynes, Delacroix-Buchet, Meinardi, & Zalazar, 1999; O'Mahony, Lucey, & McSweeney, 2005).

In this work, a new miniature cheese model based on Cremoso Argentino cheese and obtained under controlled microbiological conditions was proposed, characterized and tested for reproducibility. The model is aimed to study the impact of processing variables on biochemical events during soft cheeses cheese-making and ripening.

2. Material and methods

2.1. Cheese-making

Bulk raw milk was obtained from a nearby dairy plant. Cheeses were prepared with pasteurized milk, as cheese cannot be obtained with autoclaved or sterile milk (heat damage on milk proteins impairs coagulation).

Prior to cheese-making experiments, we assayed increasing slightly the pasteurization temperature, in order to identify the highest temperature that did not significantly decrease coagulation properties of the milk. This approach was aimed to diminish as much as possible the initial microbial charge of the cheesemilk, especially nonstarter lactic acid bacteria (NSLAB), which constitute an uncontrolled factor (Martley & Crow, 1993). Three heat treatments were assayed: the standard low-temperature long-time batch pasteurization (63 °C, 30 min), and two higher temperatures: 65 and 67 °C, both for 30 min. After pasteurization, milk was cooled up to 37 °C, and 30 ml were poured in 50 ml-centrifuge tubes, which were placed in a water-bath at the same temperature. Rennet was added: 700 μl of a solution of 1 g/l chymosin (9 g active chymosin/1000 g, Maxiren®, Gist-Brocades, Seclin, France) in acetic-acetate buffer (pH 5.50). Tubes were vortexed and quickly placed again in the water-bath. Coagulation time was determined subjectively by rocking the tubes gently and visually detecting the casein flocculation on their walls. After that, the tubes were kept in the water-bath for a period equivalent to the coagulation time, and then coagulum was cut with a stainless-steel spatula. The ability of the milk to coagulate was evaluated subjectively by observation of: cleanness of the cut, gel strength, consistency of the curd grain, fine particles occurrence and whey colour. This experiment was performed twice, with different milk on different days, and its results defined cheesemilk pasteurization for cheese-making trials.

Miniature Cremoso cheeses were manufactured in large-neck glass containers of 2.5 l of capacity, provided with glass covers and previously sterilized in an oven (140 °C, 2 h). The containers were filled with 2 l of bulk pasteurized milk, and placed in a water-bath at 37 °C. Calcium chloride (0.2 g/l) was added into the milk to compensate thermal damage caused by pasteurization. A lyophilized direct-to-vat starter culture composed of selected strains of *S. thermophilus* (Diagramma, Santa Fe, Argentina) was inoculated into the milk after being hydrated for 30 min at 37 °C in sterile reconstituted skim milk. Rennet was the same as described above; it was suspended into sterile water and added into the milk 15 min later than the starter culture (0.0225 g/l). The coagulation time was controlled as described; it generally was about 18–20 min. The coagulum was kept still for a similar period of time for gel strengthening, and then cut with miniature tools in large cubes (0.5 cm side). After healing of the curd for 3 min, the mixture whey–curd particles was gently stirred for 5 min. This operation was repeated thrice. Whey was drained and the curd was moulded; moulds were stored in an oven at 45 °C until pH of the cheeses was near 5.20–5.30. In the oven, cheeses were kept in a sterile stainless-steel box and inverted every 30 min. After that, they were salted in the same containers used as cheese vats, by pouring 1 l of sterile brine (200 g/l NaCl, pH 5.40) at 5 °C. After 10 min, the brine was discarded and the cheeses were placed in sterile boxes fitted with a grid to facilitate whey drainage. Boxes were stored overnight at 5 °C for salt and moisture balance in the cheeses. The next day, cheeses were dried with sterile tissue paper (sterilized in an oven at 120 °C for 4 h), vacuum packed and ripened at 5 °C, which is the common ripening temperature for Cremoso cheese.

All the operations that required the opening of the containers were performed after taking them out of the water-bath, in controlled microbiological conditions (adjacent to a flame). Miniature stainless-steel tools, boxes and grids were sterilized in an oven before use. Germicide UV light was directed to the work surface