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Plasmin and coagulant activities in a minicurd model system: Study of technological parameters

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ABSTRACT

The effect of scalding temperature of the curd, the inclusion of a washing step, and the pH at whey drainage on plasmin and coagulant activities were assessed in a minicurd model of young hard cooked cheese. The variables were tested as follows: draining pH was assayed at 3 levels (4.6, 5.6, and 6.4), curd scalding temperature was tested at 50 and 56°C, and washing of the curd was examined at 2 levels (no washing step, and the replacement of the whey by water). Increase in pH at whey drainage and washing of the curd had a positive effect on plasmin activity, which was also evidenced by compatible changes in soluble peptide profiles. No effect of increased cooking temperature was found on plasmin activity. Plasminogen activation was not verified in any treatment. As for coagulant, lower pH values at whey drainage and a decrease in curd cooking temperature increased its activity; washing of the curd showed no influence on coagulant residual activity. These results were consistent with proteolysis described by peptide profiles, electrophoresis, and soluble nitrogen fractions.
Key words: plasmin, coagulant, minicurd model, hard cheese

INTRODUCTION

Proteolysis involves biochemical events that confer cheese unique characteristics of taste, aroma, and texture specific of each variety. The initial breakdown of caseins during cheese ripening is due to the residual coagulant and the indigenous enzyme plasmin, which results in the production of large and mid-sized peptides.

Plasmin (EC 3.4.21.7) is a serine proteinase derived from blood; its optimal pH and temperature are 7.5 and 37°C. In milk, it preferentially hydrolyzes β-casein into γ-caseins, but it can also hydrolyze α_{S2}-casein (Rampilli and Raja, 1998). Plasmin belongs to a complex system that includes the active enzyme, its inactive precursor plasminogen, plasminogen activators, and 2 inhibitors: the enzyme inhibitor and the plasminogen activators inhibitors. Whereas the proteinase inhibitors are located in the whey fraction of milk, plasmin is predominantly bound to the casein micelles (Grufferty and Fox, 1988). Thermal stability of plasmin is relatively high, as a temperature of 80°C for 10 min is required for full inactivation of the enzyme; on the contrary, inhibitors are heat labile (Somers and Kelly, 2002). Therefore, the contribution of plasmin to primary hydrolysis of caseins is thought to be more pronounced in cooked cheeses (Sousa et al., 2001; Somers and Kelly, 2002).

Previous investigations have addressed the effect of changing technological variables during different cheese making steps on the action of plasmin, with diverse results. As for pH, it has been studied that pH values lower than 4.8 at whey draining favored plasmin dissociation from caseins (Grufferty and Fox, 1988), and that high pH during ripening had great influence in plasmin activity (Voigt et al., 2011). The addition of exogenous plasmin, plasminogen, or plasminogen activators to milk before cheese making has also been applied, with concomitant increase in β-casein hydrolysis (Upadhyay et al., 2004; Milesi et al., 2008).

Plasmin activity and its influence on cheese proteolysis has been studied in Cheddar-type miniature model cheeses (Watkinson et al., 2001; Somers and Kelly, 2002; Upadhyay et al., 2004; Milesi et al., 2008), in nonripened curds (Choi et al., 2006), and in cheeses made with different technologies at pilot plant scale (Rampilli and Raja, 1998).

As for coagulant, it consists mainly of pure chymosin, which is an aspartic (acid) proteinase (E.C. 3.4.23.4) with a pH optimum of about 4.0 and a highly specific milk-clotting activity at the pH of milk (6.7). The residual amount of coagulant enzyme that remains in the curd after whey drainage is up to 15%, as the rest is lost in the whey (Sousa et al., 2001). The retention of coagulant activity in the curd depends on technologi-

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cal factors such as pH at draining, cooking temperature, and cheese moisture (Jacob et al., 2011). During cheese ripening, the proteolytic action of chymosin on α_{S1}-casein releases the peptides α_{S1} (f1–23) and α_{S1}-I (f24–199); the latter have been seen early in the ripening of soft cheese varieties as they retain large amounts of whey and curd is not scalded (Hynes et al., 2001; Bansal et al., 2007). Some uncertainty exists regarding the extent of inactivation of chymosin in cooked cheeses. It is known that lower amounts of rennet are retained in hard cheeses due to their lower moisture content, and to the denaturation caused by temperature. Despite these facts, inactivation of the coagulant in cooked cheeses has been reported to be partial or reversible (Hayes et al., 2002; Hynes et al., 2004; Costabel et al., 2015).

Ripening of hard cooked cheeses, such as Argentinean Reggianito cheese, is a long process that demands from 6 mo to 2 yr. Little is known about nonmicrobiological enzymes such as plasmin and residual coagulant and their influence on cheese composition.

In a previous study, we validated a new cheese model for Reggianito and other hard cooked cheeses that employs frozen curd and whey and is simpler and less time consuming than standard cheese making (Vélez et al., 2015a); we applied it in the present investigation.

The objective of this work was to assess the effect of pH at whey drainage, curd scalding temperature, and curd washing on the action of plasmin and residual coagulant enzyme, in a minicurd model. Results were intended to gain scientific knowledge about the effect of technological changes on proteolysis mediated by plasmin and residual coagulant. Innovative cheese-making processes aimed at cheese ripening acceleration as well as control of primary proteolysis are possible examples of further applications.

MATERIALS AND METHODS

Experimental Design

The effect of 3 factors on plasmin and coagulant activities and proteolysis was assessed in separate experiments using a new minicurd model. The studied variables were pH at whey drainage, curd scalding temperature, and the inclusion of a curd washing step. The draining pH was assayed at 3 levels: 4.6, 5.6, and 6.4 at a cooking temperature fixed at 50°C. Curd scalding temperature was tested at 2 levels (50 and 56°C) maintaining a drain pH of 5.6. Finally, washing of the curd was tested at 2 levels (absence of washing step, and the replacement of all the whey by water) at a fixed pH of 5.6 and a curd scalding temperature of 50°C. The chosen ranges for the factors were selected to accentuate the effect of the enzymatic activities in the minicurd model. Besides, ripening time of minicurds was set to 7 d to use a rapid minicurd model to monitor proteolysis, based on a previous study in which the main effect was observed in the very first stage of ripening (Vélez et al., 2015b).

Minicurd Making

Minicurds were made according to the model validated by Vélez et al. (2015a). Briefly, raw materials were obtained from a conventional Reggianito Argentino cheese-making in pilot plant. Bulk raw milk (100 L) was obtained from a nearby dairy plant (Milkaut S.A., Santa Fe, Argentina), standardized at 2.8% of fat, and batch-pasteurized at 65°C for 30 min. Starter was a mix of *Lactobacillus helveticus* and *Lactobacillus bulgaricus* (Chr. Hansen Argentina, Quilmes, Argentina) added at a concentration of 10^6 cfu/mL of milk. Coagulant (Maxiren 150, 100% chymosin, Gist-Brocades, Seclin, France) was added at a final concentration of 0.012 g/L to coagulate the milk. After coagulation and cutting, curd particles and whey were taken from the vat, separated, and stored frozen (−18°C) until their use for minicurd making. On trial day, the mixture of whey-curd particles (~500 g) was reconstituted in the appropriate proportion (1:4) from the thawed whey and curds and incubated at 37°C until they reached the required pH (4.6, 5.6, or 6.4). After that, they were scalded to 45°C. For the curds in which a washing step was performed, the total volume of whey was replaced by distilled water (pH 5.5 at 45°C). After reaching 45°C, mixtures were scalded at 50 or 56°C according to the treatment. The curds were separated by centrifugation (Multi RF, Thermo Scientific, Waltham, MA) at 2,750 $\times$ *g* at 37°C for 20 min. Minicurds of approximately 25 g were obtained; they were refrigerated for 5 min and placed in 20% (wt/vol) brine at 12°C for 20 min. Three replicates trials were carried out, and in each one, 2 minicurds were made in parallel; one was sampled immediately and the other was vacuum packed in a plastic film and stored 7 d at 12°C.

Gross Composition

Minicurds were analyzed in duplicate, as follows: pH, measured by the American Public Health Association method (Bradley et al., 1992); sodium chloride by atomic absorption spectrophotometry (AOAC, 1990); proteins by the Kjeldahl method (IDF, 1993); and moisture content was determined by oven drying to a constant weight at 102 ± 1°C (IDF, 1982).