Effect of β -lactoglobulin A and B whey protein variants on cheese yield potential of a model milk system

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ABSTRACT

Cheese yield mainly depends on the amount and proportion of milk constituents; however, genetic variants of the proteins present in milk may also have an important effect. The objective of this research was to study the effect of the variants A and B of β -lactoglobulin (LG) on cheese yield using a model system consisting of skim milk powder fortified with different levels of a mixture containing α -lactalbumin and β -LG genetic variants (A, B, or A-B) in a 1:2 ratio. Fortified milk samples were subjected to pasteurization at 65°C for 30 min. Miniature cheeses were made by acidifying (pH = 5.9) fortified milk and incubating with rennet for 1 h at 32°C. The clot formed was cut, centrifuged at $2,600 \times g$ for 30 min at 20°C and drained for determining cheese yield. Cheese-yielding capacity was expressed as actual yield (grams of cheese curd per 100 g of milk) and dry weight yield (grams of dried cheese curd per 100 g of milk). Free-zone capillary electrophoresis was used for determining β -LG A or B recovery in the curd during rennet-induced coagulation. The presence of β -LG variant B resulted in a significantly higher actual and dried weight cheese yield than when A or A-B were present at levels $\leq 0.675\%$ of whey protein (WP) addition. Results of free-zone capillary electrophoresis allowed us to infer that β -LG B associates with the casein micelles during renneting, as shown by an increase in the recovery of this variant in the curd when β -LG B was added up to a maximum at 0.45% (equivalent to 0.675% WP). In general, actual or dried weight cheese yield increased as WP addition was increased from 0.225 to 0.675%. However, when WP addition ranged from 0.675 to 0.90%, a drastic drop in cheese yield was observed. This behavior may be because an increase in the aggregation of casein micelles with a concomitant inclusion of whey protein in the gel occurs at low levels of WP addition, whereas once the association of WP with the casein micelles reach a saturation point at addition levels higher

than 0.675%, rearrangements of the gel network result in larger whey expulsion and syneresis. This knowledge is expected to be useful to maximize cheese yield and optimize processing conditions during cheese and cheese analogs manufacturing.

Key words: β -lactoglobulin whey protein variant, cheese yield, capillary electrophoresis

INTRODUCTION

Cheese yield is important for the profitability of a cheese plant, as it reflects the amount of cheese made from a given amount of milk. Therefore, much interest has been observed toward genetic variants of milk proteins, as they might influence cheese yield (Celik, 2003). Although several studies have reported on some association between genetic variants and cheese yield, most have failed to correct for factors such as milk composition, which has a profound effect on cheese yield (Choi and Ng-Kwai-Hang, 2002; Ng-Kwai-Hang and Grosclaude, 2002).

In general, milk protein (α -CN, β -CN, κ -CN, and β -LG) genetic variants influence cheese-yielding capacity, coagulation properties, and composition of milk, but this effect is mainly attributed to milk protein, milk fat, or milk solid content (Celik, 2003). Thus, it was reported that the β -LG B variant was associated with higher fat (Aleandri et al., 1990), protein (Ng-Kwai-Hang et al., 1984), CN (Ng-Kwai-Hang et al., 1986; Bonfatti et al., 2010), CN number (Hallén et al., 2008), total solids content (McLean et al., 1984), and increased cheese yield (Schaar et al., 1985; Marziali and Ng-Kwai-Hang, 1986; Aleandri et al., 1990; Choi, 1996). Furthermore, Tong et al. (1993) and Andrén (2007) suggested that milk containing β -LG B was more suited for cheese-making, as it produced a firmer curd than milk containing the A variant. On the contrary, others reported an association of β -LG A with shorter rennet clotting time and highest curd firmness (Marziali and Ng-Kwai-Hang, 1986; Choi, 1996; Glantz et al., 2011), whereas others could not find any relation with β -LG variants (Aaltonen and Antila, 1987; Pagnacco and Caroli, 1987; Lodes et al., 1996; Celik, 2003).

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Therefore, differences in the technological properties of milk due to certain genetic variants have to be confirmed by studies comparing isolated and pure forms of the individual milk proteins without the confounding effects of other milk components (Ng-Kwai-Hang, 1998). Thus, the aim of this work was to investigate the effect of mixing isolated and purified β -LG A or B with α -LA and CN on cheese yield and syneresis in a miniature cheese model system. In addition, including higher levels of whey protein (WP) content than that present in milk (approximately 0.30%), may help to gain some insight in the effect of WP addition in cheese analogs manufacturing.

MATERIALS AND METHODS

Materials and Reagents

Analytical grade reagents and standards of β -LG A and B variants and α -LA were from Sigma Chemical Co. (St. Louis, MO) and Chymosin was from Chr. Hansen A/S (Hørsholm, Denmark). Low-heat skim milk powder was used in all the experiments. Milli-Q water was used throughout unless otherwise stated. Purified β -LG and α -LA were prepared as described by the separation and precipitation method of Maillart and Ribadeau-Dumas (1988).

Samples

Mixed CN or whey renneting systems were prepared as described by Meza-Nieto et al. (2007). Briefly, milk powder solutions were made at 10% (wt/wt) and the necessary amount of WP were added so as to yield 0.225, 0.450, 0.675, 0.900, and 1.35% (wt/wt), β -LG concentrations of 0.15, 0.30, 0.45, 0.60, or 0.90% (wt/wt), and α -LA concentrations of 0.075, 0.150, 0.225, 0.300, or 0.450% (wt/wt; i.e., the α -LA to β -LG ratio was kept at 1:2) of A, B, or a mixture (1:1) of A and B variants. These will be designated as β -LG A, β -LG B, and β -LG A-B, respectively. Mixed systems (10 mL) were equilibrated (25°C, 1 h), pasteurized (65°C for 30 min) under continuous stirring in a thermostat-controlled water bath, cooled by immersion in ice water to below 10°C, and held at that level for 2 min.

Miniature Cheese Making Procedure

Miniature cheese model systems were prepared following the procedure reported by Melilli et al. (2002), with modifications. Samples (10 mL) were transferred to centrifuge tubes and pH was adjusted to 5.9 with acetic acid (9% vol/vol). Then, samples were heated until they reached 32°C and an aliquot of rennet (0.15 mL/L; equivalent to 90 international milk-clotting

units) was added and incubated for 1 h. At the end of incubation, all samples formed a firm coagulum. Then, tubes were centrifuged at 20°C for 30 min at $2,600 \times g$. After 30 min, the tubes were carefully removed from the centrifuge; each tube contained a curd pellet and a clear whey supernatant. Then, whey from each centrifuge tube was decanted from the pellet left in the bottom of the tube, collected, and weighed.

Cheese Yield

Two different methods were used to express cheese-yielding capacity for each batch of individual sample mixture used in cheese making: (1) actual yield, which is grams of cheese curd per 100 g of milk; and (2) dry weight yield, which is grams of dry cheese curd per 100g of milk (Melilli et al., 2002). Using a metal spatula, curds were quantitatively transferred into predried and cooled aluminum weighing dishes for the determination of actual yield. Two empty predried pans were weighed as blanks. For dry weight yield determination, pans with curds and blanks were dried at 100°C for 4 h in a forced-air drying oven (VWR, Sheldon Manufacturing Inc., Cornelius, OR). After 4 h, the pans were removed from the oven and cooled in a desiccator to room temperature and weighed.

Free-Zone Capillary Electrophoresis

β -Lactoglobulin A or B content in miniature cheeses were determined using the free-zone capillary electrophoresis method described by Olguin-Arredondo and Vallejo-Córdoba (1999). Briefly, separations were carried out using an HP 3D Capillary Electrophoresis System (Hewlett-Packard, Waldbronn, Germany) in an uncoated fused silica capillary (72 cm $\times$ 50 μ m i.d.) maintained at 40°C. Injection was carried out using N₂ pressure (50 mbar) for 10 s. The separation was carried out at 25 kV in 0.05 M of borate buffer containing Tween 20 (0.1%; Sigma Chemical Co.) at pH 8.0, and detection took place at 214 nm. Samples (25 mg) were prepared by resuspending freeze-dried miniature cheeses in distilled deionized water (0.5 mL). Then, 2 mL of borate buffer solution were added and samples were centrifuged at $3,000 \times g$ (15 min at 20°C). Aliquot samples of the supernatant were subjected to capillary electrophoresis.

Statistical Analysis

All experiments were performed in triplicate. ANOVA was applied to determine the effects of β -LG variants and WP added to milk on cheese-yielding capacity of model systems. Means were tested by Tukey's pairwise comparisons at a 95% confidence level.

RESULTS AND DISCUSSION

This study aimed to investigate cheese-yielding capacity of rennet-induced coagulation behavior of mixtures of skim milk powder with β -LG A or β -LG B WP variants in isolation or with a physical mixture of both (β -LG A-B) in the presence of α -LA at a fixed β -LG to α -LA ratio. Thus, it was of great interest to study the effect of β -LG A or B variants at different concentrations on cheese-yielding capacity in a miniature cheese model. Figure 1 shows actual cheese yield, which is grams of cheese curd per 100 g of milk, as WP addition is increased. The presence of variant B of β -LG showed a significantly higher actual cheese yield ($P \leq 0.05$) compared to when A or A-B variants were present when WP was up to 0.675%. Actual cheese yield was 1.3 or 2.3% higher when β -LG B was present compared to when variants A or AB were present at 0.675% of WP addition. Then, a drastic drop was observed at 0.9%, with a marginal recovery at 1.35% when β -LG A or B were present.

Conversely, dry weight yield was significantly ($P \leq 0.05$) higher when β -LG B was present compared to when A or AB were present at all levels of WP addition (Figure 2). Dry weight cheese yield was 0.32 or 0.42% higher when β -LG B was present compared to when variants A or AB were present at 0.675% of WP addition. Similarly, highest recoveries of total solids in cheese were observed for the presence of phenotype BB compared to when AB was present in milk (Choi and Ng-Kwai-Hang, 2002). Dry weight cheese yield

Figure 1. Actual cheese yield of miniature model cheese as a function of whey protein addition ($n = 3$). Model cheese containing β -LG B (white bars), β -LG A (gray bars), and β -LG A-B (black bars). Error bars represent the SD.

Figure 2. Dry weight cheese yield of miniature model cheese as a function of whey protein addition ($n = 3$). Model cheese containing β -LG B (white bars), β -LG A (gray bars), and β -LG A-B (black bars). Error bars represent the SD.

increased as WP increased up to 0.675%, dropped considerably at 0.90%, and recovered marginally at 1.35%.

Although the primary factor influencing cheese yield is given by milk composition, the kinetics during the coagulation of milk is also a determining factor (Vasbinder et al., 2003). To this end, the variation in actual and dry weight cheese yield with WP addition (Figures 1 and 2) revealed that 3 domains of behavior seem to operate in the miniature cheese model systems. At low levels of WP addition ($\leq 0.675\%$), an increase occurs in the aggregation of CN micelles with concomitant inclusion of WP in the gel, resulting in higher cheese yield (Figures 1 and 2). However, the association of WP with the CN micelles seems to reach a saturation point so that addition levels higher than 0.675% seem to interpose a steric hindrance, either in the primary stage of coagulation, preventing the action of rennet on CN micelles, or in the secondary phase during the aggregation of micelles. Thus, a drastic reduction in cheese yield at 0.9% WP was observed (Figures 1 and 2). Furthermore, with 1.35% of WP, cheese yield increased slightly when β -LG A or B were present (Figures 1 and 2), and this could be due to a destabilization of the CN micelles or self-association of CN due to exclusion effects between WP and CN micelles caused by an excess of WP.

Figure 3 shows the amount of β -LG A or B recovered in miniature cheeses as determined by capillary electrophoresis. An increase in the recovery of this variant in the curd when β -LG B was added up to a maximum at 0.45% (equivalent to 0.675% of WP addition) allowed us to infer that β -LG B associates with the CN micelles during renneting (Figure 3). Maximum β -LG B recovered in the curd at 0.675% WP

addition also corresponded to the highest cheese yield obtained in the miniature cheese model (Figures 1 and 2). This point may represent the most aggregation of CN micelles with concomitant inclusion of WP in the gel. Conversely, β -LG A did not seem to associate with the CN micelles during renneting, as recovered β -LG in the curd was significantly ($P \leq 0.05$) lower (Figure 3). Increased cheese yield when the variant B of β -LG was present could be partly due to the physicochemical differences between variants A and B. The lower charge density in β -LG B species than in β -LG A may account for the ease of the former to associate with the CN micelle surface, as the result of lower electrostatic repulsion expected. These results are consistent with the report that β -LG A does not associate with the CN micelles even before renneting regardless of heat treatment (65°C, 30 min; Meza-Nieto et al., 2007).

The kinetics of gel formation in these rennet-induced gels with different levels of β -LG variants was addressed in a previous study by using small deformation-sensitive rheology (Meza-Nieto et al., 2007). The preferential effect of β -LG B over A on the mechanical strength of the gels was shown and may be due to the formation of cross-links and aggregates involving WP and rennet hydrolysis products or an increase in the size of the CN micelles caused by the grafting of β -LG B to its surface, or both (Meza-Nieto et al., 2007). Also, the critical gel time was slightly shorter for β -LG B than for β -LG A, and the variation in the critical gel time with the concentration of WP also revealed 3 domains of behavior (Meza-Nieto et al., 2007) that coincided

with the 3 domains observed for the cheese-yielding capacity reported in the present study.

Several studies have addressed the type of interactions between β -LG and CN micelles, and it was clearly established that β -LG and κ -CN form a heat-induced complex via thiol or disulfide exchange reaction and hydrophobic interactions in model systems as well as in milk (Reddy and Kinsella, 1990; Law et al., 1994; Corredig and Dalgleish, 1999). At low temperatures, of approximately 70°C, hydrophobic bonding is principally responsible for the complex formation between β -LG and κ -CN, and, at higher temperatures, the sulfhydryl-disulfide interchange reactions are involved (Jan and Swaisgood, 1990). As the model milk system in this study was subjected to low temperatures (65°C, 30 min) before renneting, it is most likely that the type of interactions between β -LG and CN micelles were of hydrophobic nature.

Figure 4 shows the amount of serum recovered from cheese-making as a function of WP addition; syneresis decreased with WP addition, achieving its lowest level at 0.675% when maximum cheese yield was also obtained. However, syneresis dramatically increased at 0.90% of WP addition, with a marginal drop at 1.35%. Again, this behavior may be because an increase in the aggregation of CN micelles with a concomitant inclusion of WP in the gel occurs at low levels of WP addition, whereas, once the association of WP with the CN micelles seem to reach a saturation point at addition levels higher than 0.675%, rearrangements of the gel network result in larger whey expulsion and syneresis. Therefore, the amount of serum recovered during cheese-making was related to WP addition level and to the presence of variants A or B or their combination. Cheese-making can be viewed as a dehydration process, and syneresis is the crucial method by which most of the moisture is lost from curd particles. In rennet-induced milk gels, the strong tendency of rennet gels to exhibit syneresis is related to extensive rearrangements of the network, which occur after gel formation (van Vliet and Walstra, 1985).

This study demonstrated that a higher cheese yield was obtained when β -LG variant B was present per se, and it was not necessarily due to the presence of this variant and its association to a higher content of CN (Ng-Kwai-Hang et al., 1986) and fat (Aleandri et al., 1990), as shown by previous studies using milk. By making miniature cheese model systems, these 2 milk composition variables were kept constant, so it was demonstrated that the differences in milk coagulation properties, in connection with β -LG, were due to the variants by themselves and not to the content of fat or CN in milk. Furthermore, the use of a model system allowed us to study the effect of β -LG variants present

Figure 3. β -Lactoglobulin A or B content in miniature model cheese as a function of whey protein addition [0.225, 0.450, 0.675, 0.900, and 1.35% (wt/wt) correspond to β -LG concentrations of 0.15, 0.30, 0.45, 0.60, and 0.90% (wt/wt); $n = 3$]. Model cheese containing β -LG B (white bars) and β -LG A (gray bars). Error bars represent the SD.

Figure 4. Syneresis of miniature model cheese as a function of whey protein addition ($n = 3$). Model cheese containing β -LG B (white bars), β -LG A (gray bars), and β -LG A-B (black bars). Error bars represent the SD.

in milk on cheese-yield capacity without the need for adjustment for milk composition.

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