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Effect of heating on the distribution of transforming growth factor-β2 in bovine milk

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ABSTRACT

The objective of this study was to characterize the impact of heat treatments on the distribution of transforming growth factor-beta (TGF-β2) between cream and skim milk and between the casein and whey fractions of skim milk. Skimming removed 45% and 62% of the TGF-β2 from raw and pasteurized milks and only 8% of the total TGF-β2 in skimmed pasteurized milk was found in whey, compared to 37% in whey from raw skimmed milk. The TGF-β2 content of whey decreased as the heat treatment of the milk increased in intensity (thermization > pasteurization > UHT sterilization). Using milk held for 1 or 2 min at temperatures ranging from 57 to 84 °C, it was shown that TGF-β2 in the whey portion decreases at temperatures above 66 °C and becomes undetectable at temperatures higher than 76 °C. Altogether, these data on the heat-induced changes in TGF-β2 content of cream, skim milk, casein and whey reveal a potentially negative impact of certain heat treatments in developing TGF-β2-enriched fractions from milk.

1. Introduction

Transforming growth factor-beta (TGF-β2) is one of the most abundant growth factors in bovine milk. Milk-derived TGF-β2 has been used in numerous nutraceutical formulations for therapeutic applications (Pouliot & Gauthier, 2006). In optimizing TGF-β2 extraction from cheese whey (Akbache, Lamiot, Moroni, Turgeon, Gauthier, & Pouliot, 2009), it has been observed that the thermal history of milk had a profound impact on the yield of active TGF-β2.

The TGF-β2 molecule is a homodimer composed of 68 amino acid residues with a molecular mass of 25 kDa (Daopin, Piez, Ogawa, & Davis, 1992). Four disulphide bridges stabilize each monomer and one disulphide bridge stabilizes the dimer. TGF-β1 is the only other member of the TGF-β family found in milk but occurs at much lower concentrations (Ginjala & Pakkanen, 1998; Montoni, Gauthier, Richard, Poubelle, Chouinard, & Pouliot, 2009). Rogers, Goddard, Regester, Ballard and Belford (1996) showed that TGF-β2 in bovine milk occurs as small latent complex with a molecular mass of 80 kDa and that not only acidification (pH 2) but also alkali (pH 11), boiling (2 min) and urea (8 M) treatments all increased the activity of TGF-β2 in a protein fraction obtained from cheese whey by cation-exchange chromatography, based on a growth inhibition bioassay.

Gauthier, Pouliot and Maubois (2006) pointed out that the published concentration values of TGF-β2 in milk varied between 13 and 71 ng/mL as a result of not only natural variations of milk composition and analytical methodologies but also differences between milk thermal histories. The TGF-β family is considered at least somewhat heat-resistant. Rogers et al. (1996) found TGF-β2 in pasteurized milk and in cheese whey at concentrations of 4.3 and 3.7 ng/mL, respectively, although the intensity of the pasteurization treatment was not specified. Pakkanen (1998) and Ginjala and Pakkanen (1998) showed that pasteurized milk contained TGF-β2 at a concentration close to that in the corresponding raw milk. Elfstrand, Lindmark-Månsson, Paulsson, Nyberg and Akesson (2002) obtained similar results with a fat-free colostrum fraction and low-temperature pasteurization (60 °C, 45 min). Ozawa et al. (2009) found significant but variable amounts (0.5–3.0 μg/L) of active TGF-β2 in six out of seven samples of commercial pasteurized milk and related the variability to differences in the pasteurization and milk processing methods. Peroni, Piacentini, Bodini, Pigozzi and Boner (2009) showed that commercial pasteurization significantly decreased the concentration of TGF-β1 in milk, while Akbache et al. (2009) observed that whey produced by microfiltration of raw milk was rich in TGF-β2 compared to Cheddar and mozzarella whey obtained using pasteurized milks. These observations raised questions about the effect of pasteurization not only on the TGF-β2 content of milk but also on the distribution of TGF-β2 between milk and whey. The hypothesis that this molecule would interact with casein micelles during milk pasteurization was proposed.

It is known that heating milk to temperatures above 70 °C denatures whey protein and induces its interaction with casein micelles (de Wit & Klarenbeek, 1984; Calvo, Law, & Leaver, 1995; Vasbinder, Alting, & de Kruif, 2003). It has been demonstrated that reactivity of the free thiol group of Cys-121 in β-Lactoglobulin (β-Lg)

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increases due to protein unfolding during heating and that this promotes SH/S-S intra- and intermolecular interactions (Hoffman, Sala, Olieman, & de Kruif, 1997; Sava, Van der Plancken, Claevs, & Hendrickx, 2005). In addition, Ye, Singh, Taylor and Anema (2004) have provided evidence of interactions between β-Lg, α-Lactalbumin (α-La) and milk fat globule membrane (MFGM) components upon heating of whole milk at temperatures lower (60–65 °C) than those required to denature whey protein.

Heat-induced interactions involving TGF-β2 are likely to occur since this molecule has a strong hydrophobic character, which favors its polymerization and non-specific interaction with other proteins. TGF-β2 also contains a free thiol group at Cys-77 (Daopin et al., 1992), making reactions with β-Lg, and hence α-La, casein micelles and MFGM components likely. It is therefore likely that TGF-β2 is distributed between the fat globule surface, casein micelles and the serum phase of milk and that this distribution is strongly dependent on milk thermal history.

The objective of this study was to characterize the influence of heat treatments on the distribution of TGF-β2 in bovine milk. The distribution of TGF-β2 between cream, skim milk and whey was first compared in raw and pasteurized milk. The effect of thermization (68 °C for 20 s), high-temperature short-time (HTST) pasteurization (72 °C for 20 s) and ultra-high temperature (UHT) sterilization (140 °C for 4 s) on the TGF-β2 content of whey was then compared. Finally, the changes in whey TGF-β2 content resulting from heating skim milk to temperatures between 57 and 84 °C were measured.

2. Material and methods

2.1. Milk processing conditions

2.1.1. Effect of pasteurization before or after skimming milk

Three lots (~100 L each) of raw milk were obtained in bulk from a local dairy plant (Natrel, Quebec City, QC, Canada). Each lot was split into two portions upon reception. One portion was pasteurized at 72 °C for 20 s using a tubular pasteurizer (Actini, model Mini-Actijoule, Evian-Les-Bains, France) and the other one was kept refrigerated. The raw and pasteurized milks were skimmed by centrifugation using a cream separator (DeLaval, model 619, Lund, Sweden) running at 6000 rpm. This centrifugation typically generated 45 L of skimmed milk and 5 L of cream. Skimmed milks were adjusted to pH 4.6 using 6N HCl to precipitate caseins and then centrifuged at 15,000*g* for 15 min at room temperature. The supernatants were then brought to pH 6.8 using 6N NaOH and kept for 8 h at 4 °C for sedimentation of the remaining casein fines. The casein pellets and supernatants were carefully collected and freeze-dried (Virtis, model FFD-42-WS Repp., Gardiner, NY, USA).

2.1.2. Effect of commercial heat treatments on skim milk

Raw milk (~100 L per lot) was obtained from the Université Laval dairy farm (Centre de recherche en sciences animales de Deschambault, CRSAD, Québec, Canada) once a week for three consecutive weeks. Each of these lots was treated as shown in Fig. 1. Upon reception, milk was warmed to 35 °C and skimmed using a cream separator (DeLaval). A Joule-effect pilot-scale indirect heating unit (Actini) was then used to perform the following treatments: thermization at 68 °C for 20 s, pasteurization at 72 °C for 20 s and UHT sterilization at 140 °C for 4 s. The system was equipped with a cooling unit that decreased the milk temperature to 15 °C within seconds immediately after heating. The milk was then processed as described above, beginning with the pH adjustment to 4.6. The pellets and supernatants were freeze-dried and stored before further analysis.

2.1.3. Effect of heating temperature between 57 and 84 °C

This series of experiments was performed using the lots used to study the effect of commercial heat treatments (Section 2.1.2). Aliquots of 40 mL of raw skimmed milk were placed in glass test tubes and covered with Parafilm™. The tubes were incubated at 57, 60, 63, 66, 69, 72, 75, 78, 81 or 84 °C for 1 or 2 min in an oil bath system with digital control. Individual test tubes were cooled rapidly in ice water upon removal from the bath and their contents were processed as in Section 2.1.1, beginning with the pH adjustment to 4.6. The pellets and supernatants were then freeze-dried and stored before analysis.

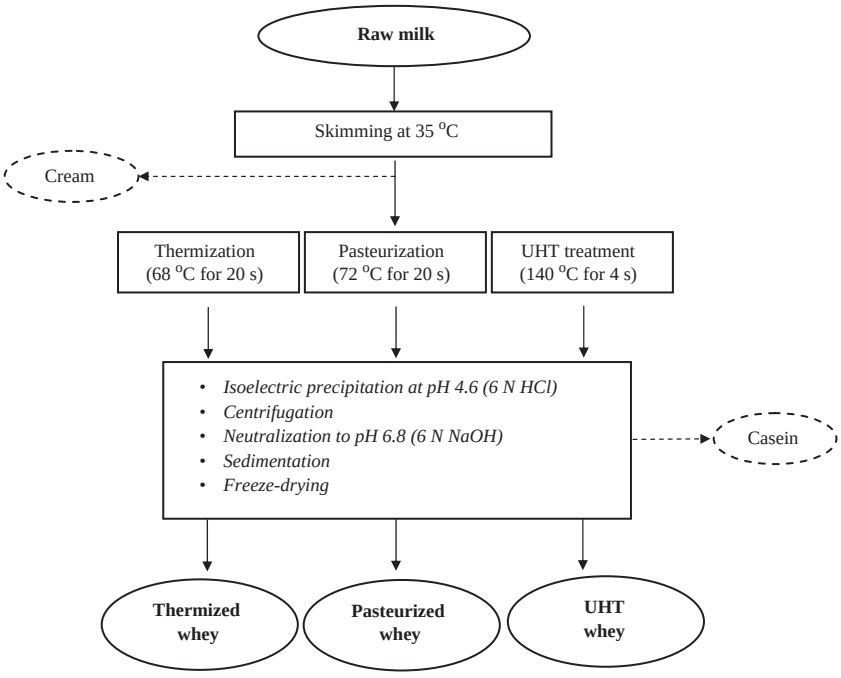

Fig. 1. Schematic diagram of the processing of raw milk into the analyzed samples.