Short Communication: Influence of Transglutaminase on the Heat Stability of Milk

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ABSTRACT

Skim milk powders were prepared from control and transglutaminase-treated skimmed milk. The heat stability of reconstituted transglutaminase-treated skimmed milk (9.0% total solids) was markedly increased in the pH region of minimum stability (pH 6.8 to 7.1) compared with control milk, while the heat stability of reconstituted concentrated transglutaminase-treated skimmed milk (22.5% total solids) increased progressively as a function of pH relative to control milk. The effect of transglutaminase treatment on the heat stability of skimmed milk may have commercial applications, but extensive research is necessary to gain a better understanding of the mechanism by which transglutaminase improves heat stability.

(Key words: transglutaminase, heat stability, milk)

Abbreviation key: \(\beta\)-lg = \(\beta\)-lactoglobulin, HCT = heat coagulation time, TGase = transglutaminase, TS = total solids.

INTRODUCTION

The enzyme transglutaminase (TGase; EC 2.3.2.13) modifies proteins by means of amine incorporation and crosslinking, where the enzyme catalyzes an acyl-group transfer reaction between the \(\gamma\)-carboxyamide group of peptide-bound glutamine residues (acyl donor) and the primary amino group of a variety of amine compounds (acyl acceptors), including the \(\varepsilon\)-amino group of lysine residues in certain proteins, or through the deamidation of glutamine residues using water molecules as acyl acceptors (Figure 1) (Ikura et al., 1992; Motoki and Segura, 1998; Nielsen, 1995).

Crosslinking of food proteins by TGase modifies the hydration ability, the gelation, rheological and emulsifying properties, and heat stability of food proteins in model systems (Dickinson, 1997; Lorenzen and Schlimme, 1998; Motoki and Segura, 1998), but the rate of TGase crosslinking depends on the macromolecular structure of each protein substrate. The open conformation of the caseins makes them good substrates, but globular whey proteins are not susceptible to crosslinking by TGase in their native state and require modification before crosslinking (Han and Damodaran, 1996; Ikura et al., 1984; Nio et al., 1986; Nonaka et al., 1989; Traore and Meunier, 1992). Incubation of heated skimmed milk (92°C for 5 min) with TGase leads to a decrease in the rennetability of milk, which was attributed to ‘surface sealing’ of casein micelles crosslinked with whey proteins, especially \(\beta\)-lactoglobulin (\(\beta\)-lg) (Lorenzen, 2000).

The heat stability of milk refers to the resistance of milk to coagulation at sterilization temperatures (Singh and Creamer, 1992). The thermal stability of milk, especially concentrated milks, is affected by various compositional factors (pH, milk salts, and milk proteins) and processing treatments (preheating, concentration, and homogenization), which can cause coagulation during sterilization, as well as gelation and (or) sedimentation during storage (Singh and Creamer, 1992; Fox and McSweeney, 1998). Generally, the heat stability of milk can be classified as one of two types. Type A heat coagulation time (HCT)-pH profiles have a distinct maximum and minimum in the HCT-pH profile, while the heat stability of type B HCT-pH profiles increase as a func-
Raw milk was obtained from the experimental station of the Federal Dairy Research Centre (Kiel, Germany), warmed to 45°C, and separated with a disk centrifuge (Westfalia—separator, Oelde, Germany). Raw skim milk was preheated at 90°C for 20 s before incubation with a Ca²⁺-independent microbial TGase (Ajinomoto Europe Sales, Hamburg, Germany; declared activity was approximately 1000 units/g) at 6°C for 16 h at an enzyme to substrate ratio of 1/2000 (wt/wt). In some experiments, the enzyme was inactivated by heating at 80°C for 1 min before freeze drying (Leybold Haereus, Hanau, Germany) or spray drying (air inlet temperature: 180°C; air outlet temperature: 80°C; Niro Atomizer, Hudson, WI). Control milk powder was prepared following the procedure described above, except that enzyme was omitted.

Skimmed milk or concentrated skimmed milk was prepared by dissolving skimmed milk powder in distilled water (20°C) to 9.0 or 22.5% total solids (TS), respectively. Reconstituted milk (9.0% TS) prepared from skimmed milk powder, which was unheated before drying (enzyme active), was stirred for 1.5 min at room temperature, to ensure complete dissolution of the powder, heated to 80°C, held for 1 min, and then cooled in an ice bath. All milks were stirred at room temperature for 1 h before analysis.

Samples of the reconstituted control and experimental skimmed milk or concentrated skimmed milk were adjusted to pH values in the range of 6.4 to 7.3, with 1.0 M NaOH or 1.0 M HCl. The heat stability of the reconstituted skimmed milks was determined in an oil bath at 140°C (for skimmed milk) or at 130°C (for concentrated skimmed milk) by the subjective method of Davies and White (1966), where ~1.5 ml of milk in a sealed glass tube is rocked at a definite rate until coagulated protein particles can be seen in the flowing milk. Traditionally, 140°C is the temperature chosen to determine the heat stability of skimmed milk, while a lower temperature is necessary to determine the heat stability of concentrated systems to high temperatures (140°C). Analyses were carried out in duplicate.

As far as we are aware, the effect of treating milk with TGase on its heat stability has not been reported. Preliminary results of such a study are reported in this communication.

Figure 2. Heat coagulation time-pH profile of reconstituted (9.0% total solids) control milk (—/) and milk treated with transglutaminase for 16 h at 6°C (—/). (a) Enzyme inactivated prior to drying; (b) enzyme inactivated after reconstitution of skimmed milk powders; ✱ original pH.
powders produced in May or November (Figure 3), which may be due to seasonality effects or to other factors.

The effect of TGase on the heat stability of concentrated skimmed milks (22.5% TS) is shown in Figure 4. As for unconcentrated milks, TGase increased the heat stability of concentrated milk, which increased progressively as a function of pH. Reconstituted spray-dried skimmed milk powders had greater heat stability than did reconstituted freeze-dried skimmed milk powders.

The current hypothesis for the maximum-minimum in the HCT-pH profile is that on heating at a temperature >90°C, κ-CN dissociates from the casein micelles; β-lg reduces the extent of dissociation of κ-CN at pH values below 6.7, but it promotes dissociation at pH values between 6.7 and 6.9 (Fox and McSweeney, 1998). Above pH 6.9, heat stability increases linearly as a function of pH. It would appear from the results of this study that treatment of skimmed milk with TGase prevented the dissociation of κ-CN from the casein micelles. Han and Damodaran (1996) showed that there is no evidence of crosslinking between caseins and whey proteins in their native state by TGase, but modification of the whey proteins by preheating skim milk before incubation with TGase may have allowed crosslink formation by TGase between the caseins and whey proteins. Crosslinking of β-lg and κ-CN may inhibit the dissociation of κ-CN from the casein micelles in the pH range of minimum stability, promoting improved heat stability.

Alternatively, incubation of skimmed milk with TGase may have crosslinked the whey proteins, reducing the effective concentration of “free” β-lg available, which can influence the dissociation of κ-CN at pH values of minimum stability or TGase may have crosslinked κ-CN moieties to other caseins, preventing their dissociation from casein micelles at minimum stability.

No work has been carried out on the mechanism by which crosslinking by TGase improves the heat stability of milk at pH values in the region of minimum stability; further research is warranted.

Stability of reconstituted milk powders in tea or coffee is a routine test in the selection of milk powders for use as tea or coffee whiteners, while UHT-sterilized milks and UHT-sterilized products containing milk are susceptible to coagulation during sterilization and gelation or sedimentation during storage; any positive effects on the stability of these products would be economically important. There are very few legally permitted food-grade additives available that can improve the heat stability of milk; the effect of TGase suggests that this enzyme may have potential commercial applications as a food-grade additive.

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