POSTER PRESENTATION

A – Food compounds associated with nutritional and sensory quality

Studies on Enzymatic Crosslinking of Casein Micelles

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Abstract: The aim of our study was to gain insights into the reactions occurring in casein micelles during enzymatic modification with microbial transglutaminase (mTG). Therefore, UHT-treated milk was incubated with varying amounts of mTG and the caseins were analysed using different analytical methods. Regarding the casein species, it was observed that β-casein was crosslinked to a higher extent than the α-caseins. From this it can be suggested that β-casein is mainly located in the outer space of the micellar structure and therefore better accessible to mTG than α-caseins, which are located predominantly in the interior. Furthermore, it was demonstrated by gel-permeation chromatography and RP-HPLC that the caseins are fixed within the micellar structure, by what the ratio of extramicellar casein decreased. We conclude that an isopeptide network in the outer β-casein rich "shell" of the micelle is formed by mTG, which is responsible for the increased micellar stability.

Keywords: microbial trasglutaminase; casein micelle; crosslinking

INTRODUCTION

The enzyme microbial transglutaminase (mTG) [EC 2.3.2.13] catalyses an acyl transfer reaction between the γ-carboxyamide group of protein-bound glutamine and the ε-amino group of lysine residues. This leads to a formation of intra- and/or intermolecular crosslinks (isopeptides) resulting in the polymerisation of proteins, by what their functional properties are improved (Lorenzen 2002). Therefore, mTG is widely used in the food industry (Jaros *et al.* 2006), in particular in dairy technology, where the addition of mTG to milk results in a higher gel strength and decreased syneresis of the yogurt gel (Lauber *et al.* 2000; Lorenzen 2002). Regarding milk proteins, caseins can be easily crosslinked by mTG. In bovine milk, the caseins are aggregated as micelles, by what the enzymatic crosslinking reaction is influenced. The mechanism of intramicellar crosslinking is not yet clarified in detail. The objective of this work, therefore, was to gain insights into mTG-catalysed reactions occurring in casein micelles.

MATERIALS AND METHODS

UHT-treated skim milk was incubated with mTG (enzyme activity from 0 to 16 U/g casein) for 60 min at 40°C. Immediately after treatment, the enzyme was inactivated by heating up the samples to 85°C for 5 minutes. Sodium dodecyl

sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a modified method according to Schägger and von Jagow (1987). Protein bands were stained with Coomassie Brilliant blue G250. For quantification of the extent of casein oligomerisation, gel permeation chromatography (GPC) with UV detection at 280 nm using a column Superdex 200 10/300 GL (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) was performed according to Lauber *et al.* (2000). For gel-permeation chromatography of micellar and non-micellar casein, UHT-treated skim milk samples were mixed in a ratio of 1:20 (v/v) with distilled water and were subjected to gel-permeation chromatography using a column (6.6 mm × 1000 mm, Omnifit Ltd., Cambridge, England) filled with Sephacryl media S-500 HR (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) according to Holt (1998). The column was eluted at a flow rate of 1 ml/min with synthetic milk ultrafiltrate (SMUF, pH 6.8, Jenness & Koops 1962). UV-detection at 215 nm was performed. Quantification of individual caseins in UHT-treated skim milk samples was performed by RP-HPLC on a PLRP-S 300A column (Polymer Laboratories, Darmstadt, Germany) with UV detection at 220 nm according to IDF-Standard 178 (1996).

RESULTS AND DISCUSSION

Gel-permeation chromatography (GPC) on Superdex 200 10/300 GL (chromatograms not shown) showed that during incubation of milk with low mTG activities (4 U/g casein), the degree of casein polymerisation (dp) was 19.9%, consisting exclusively of dimers and trimers. With increasing mTG activity to 8 U/g casein, the dp increased to 28.1%, and oligomers as well as polymers were detectable in equal amounts, whereas using a higher mTG activity of 16 U/g casein resulted in a dp of 44.6% and a crosslinking of the caseins predominantly to polymers. Using SDS-PAGE (electropherograms not shown), it was observed that β-casein was crosslinked by mTG to a higher extent when compared to α-casein, which is in agreement with Sharma *et al.* (2001), who showed that in milk β-casein as well as κ-casein were most susceptible to crosslinking by mTG. For estimating the influence of mTG treatment on the ratio between intra- and extramicellar casein, GPC using high-porous Sephacryl material was performed, which allowed a simultaneous analysis of micellar and non-micellar casein in milk after mTG-treatment. From the chromatograms obtained (Figure 1), it was observed that the amount of extramicellar casein significantly decreased due to incubation with mTG, whereas the amount of intramicellar casein increased with increasing mTG activity. This indicates a covalent "fixation" of casein molecules within the micelle by mTG. To confirm this observation, UHT-treated skim milk samples with or without mTG-treatment were centrifuged for 10 min at 11 000 rpm in order to remove casein micelles. In the supernatants, the amount of extramicellar α- and β-casein was analysed via RP-HPLC (Figure 2). Compared to α-casein, a significantly more pronounced decrease of the amount of non-micellar β-casein concomitant with increasing mTG activity was observed. After treatment with high mTG activity (8 and 16 U/g casein), there was hardly any non-micellar β-casein detectable, whereas amount of α-casein decreased to values between 35% and 50% of the initial values obtained for a milk sample without mTG-treatment. These results strengthen the interpretation that predominantly β-casein molecules are fixed within the micellar structure by enzymatic crosslinking reactions. Since micellar β-casein is a better substrate for mTG compared to α-casein, we suggest that β-casein is located in the outer space of the micelle, whereas α-casein is predominantly positioned in the inner core. An

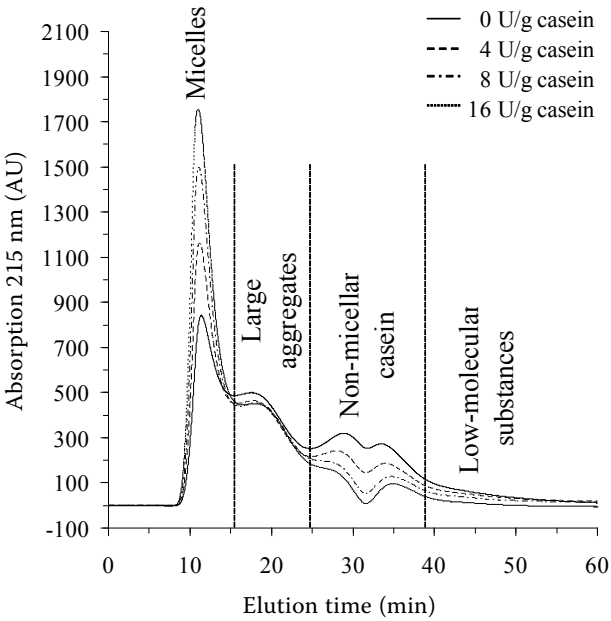

Figure 1. Gel-permeation chromatography of UHT-treated skim milk incubated with varying activities of mTG

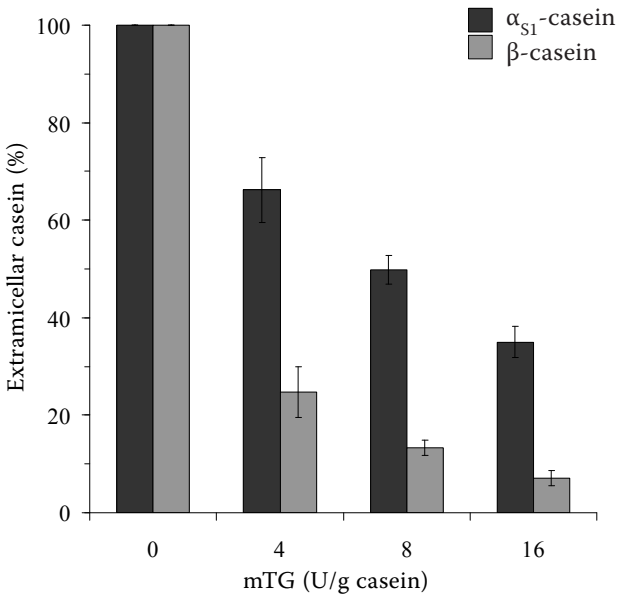

Figure 2. Amount of extramicellar α_{S1}- and β-casein of UHT-treated skim milk after incubation with varying activities of mTG

isopeptide network between β-casein molecules at the micelle surface is formed, leading to improved functional properties and enhanced stability of micellar structure toward destabilising reagent such as EDTA or ethanol (Partschefeld *et al.* 2007).

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