Trying to Better Understand the Rheological Behavior of Acid Gels from Various Dairy-based Substrates Treated with Microbial Transglutaminase

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ABSTRACT

Much research deals with the application of microbial transglutaminase (mTGase) in the processing of foods. To evaluate the impact of mTGase on rheological properties and texture of acid-induced gels from different dairy-based cross-linked substrates, gelation was monitored with a strain-controlled rheometer. Gel firmness, measured in small and large deformation experiments, increased with increasing enzyme incubation time but, after exceeding a certain critical incubation time, decreased again. This decrease cannot be explained by the absolute amount of cross-linked proteins, expressed as the degree of oligomerisation. The substitution of thermal enzym inactivation by the application of N-Ethylmaleimide (NEM) did not affect this firmness decrease, indicating that heat-induced reactions between proteins are not responsible.

INTRODUCTION

Transglutaminase (EC 2.3.2.13) catalyses acyl transfer reactions between lysine and glutamine residues of various proteins, resulting in the formation of covalent cross-links. Milk proteins, especially the caseins, represent excellent substrates, and products such as yogurt have been well examined; it is reported that, when made from mTGase treated milk, yogurt exhibits enhanced firmness, a higher viscosity and reduced syneresis.[1] However, because of the complex nature of milk and, particularly, because of special features of the casein micelle, there is still a significant lack of knowledge on structure-function interactions caused by this enzymatic cross-linking. To eliminate the impact of heating on rheological properties of the gels enzyme inactivation may be carried out by adding NEM, which reacts with the sulfhydryl group of cysteine[64] of transglutaminase, thus causing the loss of its catalytic activity.[2]

For systematic modifications of the functionality of protein-based foodstuffs it is necessary to understand the impact of the action of mTGase on the molecular level. Therefore, the amount of cross-linked protein was determined and related to the changes in functional properties.

MATERIALS AND METHODS

Materials

2.7 % casein solutions (w/w) were prepared by dissolving the powder (Sigma-Aldrich Laborchemikalien, Seelze, Germany) in 0.1 mol/l phosphate buffer (pH 6.8); mixtures were continuously stirred overnight.

Raw milk was obtained from a local farmer (Pulsnitz, Germany) and defatted by centrifugation for 10 min at 5000 rpm at 6 °C (Heraeus BioFuge Stratos, Thermo Electron Corporation, Waltham, USA). After filtration, fat content[3] was < 0.5 g/kg and protein content[4] was 37 g/kg. Microbial transglutaminase Activa MP from *Streptoverticillium* ssp. (Ajinomoto Foods Deutschland GmbH, Hamburg, Germany),

measured activity: 100 U/g, was used for cross-linking.

Substrates were treated with 3 U mTGase/g protein at 40 °C, and the reaction was stopped either by heating (85 °C, 10 min) or by adding 0.1 % (w/w) NEM at predefined times.

Gel formation

Gelation profiles were monitored using a strain-controlled ARES RFS3 rheometer (TA Instruments, Alzenau, Germany) with a cup-and-bob geometry; temperature was controlled by a circulator and maintained at 30 °C. After thermostatting the substrate, acidification was induced by adding 4 % (w/w) D-gluconic-acid-δ-lactone (GDL). The mixture was transferred into the pre-thermostatted cup of the rheometer, and the measurements were started within 1 min after GDL addition. During gelation, a strain of 0.003 - low enough to ensure undisturbed gelation - was applied with an angular frequency of 1 rad/s. Storage modulus G' (Pa) as a measure for gel stiffness at small deformation, and tan δ were monitored during gelation. All experiments were performed in triplicate.

Large deformation tests

Samples were acidified in small glass beakers at 30 °C for 1 h and subsequently subjected to penetration tests with an RSA3 solids analyzer (TA Instruments), equipped with a 35 N transducer. Measurements were performed with a cylindrical plunger (d = 25 mm) at a velocity of 0.5 mm/s. Force/deformation data were monitored for six individual gels for each sample.

Protein oligomerisation

Gel permeation chromatography (GPC; Knauer GmbH, Berlin, Germany) was applied to determine the amount of cross-linked proteins. According to the method of Lauber et al.[5] samples were diluted in an elution buffer containing 1 % dithiothreitol (DTT) to achieve a protein content of 0.1 %. After cooling overnight, the samples were filtered through membrane filters (0.45 µm). Measurements were performed at room temperature using a Superdex 200 HR column (Pharmacia, Freiburg, Germany) with a UV detector at a flow rate of 0.5 ml/min. The buffer system contained 6 mol/l urea, 0.1 mol/l sodium phosphate, 0.1 mmol/l sodium chloride and 0.1 % 3-[(3-cholamidopropyl) dimethylammonium]-1-propanesulfonate and was adjusted to pH 6.8 with 6 mol/l HCl.

RESULTS AND DISCUSSION

Monitoring gel formation

Since pure casein solutions remain stable when heated to 85 °C, these were chosen as model substrates to check the suitability of NEM for inhibiting mTGase. Hence, prior to acidification, the enzyme reaction was either stopped by heating or by NEM. As can be seen from Figure 1, G'_{MAX} (maximum G' extracted from the gelation curves) is not affected by the mode of enzyme inactivation when comparing the data at each incubation time. Generally, cross-linking by mTGase action resulted in a large increase of G'_{MAX} up to an incubation time of approx. 3 hours.

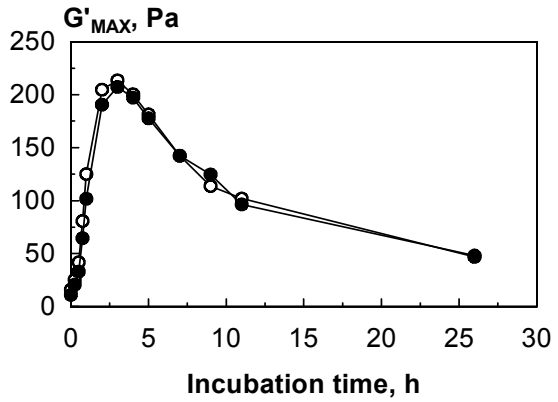

Fig 1. Maximum of the storage modulus G' of 2.7 % (w/w) casein solutions acidified with GDL as a function of enzyme incubation time. Open symbols, enzyme reaction stopped by heating (85 °C, 10 min); full symbols, enzyme reaction stopped by NEM (0.1 % w/w).

This enzyme-induced firmness increase of dairy-based acidified gels is widely reported in literature[6] and the main reason for the application of mTGase. However, as incubation time was prolonged, gel firmness showed a dramatic decrease.

When using raw milk as substrate of mTGase, this decrease in gel firmness already started when milk was incubated only for approximately 60 min (Table 1). A corresponding gelation behavior was found in an earlier study where softer gels were obtained from UHT milk after 60 min enzyme treatment.[7]

Table 1. Maximum of storage modulus and tan δ extracted from gelation curves of raw skim milk as a function of mTGase incubation time.

Time (min)	G'_{MAX}	tan δ
0	14	0.31
15	44	0.31
30	182	0.29
60	179	0.27
120	160	0.26
240	101	0.25

Tan δ showed a significant decrease with increasing enzyme incubation time, which was even more pronounced for acid gels from casein solution (0.37 and 0.25 for incubation time of 0 and 240 min, respectively). The slopes from the force/ disformation curves obtained in penetration tests showed an identical behavior, which means that gels became weaker after a critical time of incubation was exceeded (data not shown).

Gel rheology and protein cross-linking

The degree of cross-linking of the caseins, measured by GPC, is depicted in Figure 2. It is evident that the amount of monomers diminishes steadily with incubation time for the benefit of cross-linked aggregates. It is also obvious that the substrate shows an enormous impact on the performance of mTGase, resulting in quantitatively and qualitatively different mixtures of protein molecules. Because of the better accessibility of the monomers in pure casein solutions the rate of cross-linking is much higher, which is reflected by the more pronounced decrease of the number of monomers and the more pronounced increase of oligomers. Furthermore, the amount of dimers and trimers was much higher in casein solutions than in raw milk where the monomers are mainly available in complex casein micelles, which obviously constricts the action of the enzyme.

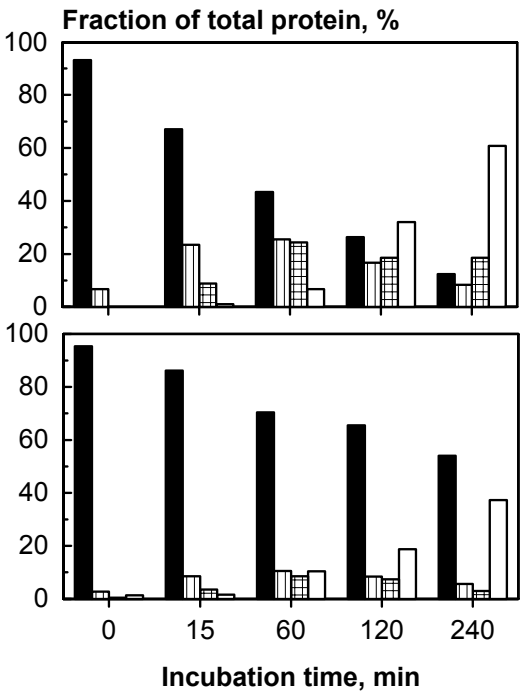

Fig 2. Cross-linked fractions of proteins as influenced by incubation time. Substrate: Upper graph, casein (2.7 %); lower graph, raw milk. Symbols; black, monomers; striped, dimers; chequered, trimers; white, oligomers.

To calculate the degree of the cross-linked proteins, the total peak area of the GPC chromatograms was divided by the peak area of dimer, trimer and oligomer fractions and the mTGase induced increase in oligomers (OD, %) was calculated by subtracting the OD of the reference sample at time 0. Generally, the degree of oligomerisation is much higher in enzymatically treated casein solution (approx. 81 % after 240 min) than in raw milk (approx. 41 % after 240 min). Interestingly, the critical time for cross-linking which led to gels with decreasing firmness is much shorter for the less cross-linked substrate. The corresponding ODs at this critical time were approximately 25 % and 70 % for raw milk and casein solution, respectively.

Although the basic composition of the caseins was almost similar, their aggregation states at neutral pH, at which the treatment with mTGase was performed, are different. In the model solutions, the caseins can be easily accessed by the enzyme. The formation of new covalent isopeptide bonds in milk, however, is restricted because of the presence of micellar casein; it is well-known that mTGase does not lead to intermicellar cross-linking.[8]

Upon acidification, charged groups of the caseins are neutralised, allowing the formation of a three-dimensional network.[9] A low amount of cross-linked molecules within the micelles supports the effective reorganisation of casein particles during acidification, which results in a higher gel firmness. In casein micelles, these necessary rearrangements are hindered beyond a relatively low critical level of mTGase-induced oligomerisation, leading to a loss of gel firmness. Since the mobility of single casein particles is already limited in a micellar structure, 25 % of oligomerisation seems to be enough to disturb optimum gel formation. The results emphasize the necessity to gain more knowledge about the structure-function relationship, allowing a careful tailoring of new products.

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