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ORIGINAL RESEARCH PAPER

EFFECT OF TRANSGLUTAMINASE TREATMENT ON SKIMMED YOGURT PROPERTIES

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The aim of the present study was to evaluate the effect of microbial transglutaminase on the stability and rheological properties of skimmed yogurt. The fermentation was carried out with *Streptococcus theromophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* after incubating the milk with various enzyme concentrations ranging from 0 to 0.04%, at different setting temperatures (30, 40 and 50°C), for 60, 90 and 120 min. The post-acidification process and the stability of the yogurt samples were influenced by the degree of polymerization of the milk proteins which depended on the conditions of the milk treated with microbial transglutaminase. The best results in terms of whey separation and rheological properties were obtained when preincubating the milk with 0.04% transglutaminase for 120 min setting at 40°C. The results indicate that transglutaminase may be successfully used for enhancing the functional properties of yogurt with low fat content.

Keywords: skimmed milk, transglutaminase, yogurt, rheological properties, syneresis

Introduction

Nowadays the public health and clinical concerns regarding overweight and obesity are increasing as the number of people suffering from these diseases is dramatically higher than few decades ago and it was shown that are directly related to important chronic illnesses such as hypertension, coronary artery disease, diabetes, arthritis and different types of cancer (McManus *et al*., 2001). The dietary intake is one of the important factors contributing significantly to the obesity epidemic. The best way to obtain a calorie balance is to monitor dietary intake and replace the high calories foods with nutrient-dense foods and beverages relatively low in calories (USDA, 2010). Part of the recommendations of most dietary guidelines for weight loss are related to the increased consumption of low-fat milk and milk products, such as milk, yogurt, cheese, or fortified soy beverages. The intake of fat-free milk products provides the same nutrients with less calories and decreased sodium, cholesterol, and saturated fatty acids contents (USDA, 2010).

As a consequence of consumer's demand, the low-fat food became a new trend. Concerning the skimmed yogurt, the acceptance is limited by the texture characteristics such as creaminess and viscosity, which are sensitive to the fat content (Ozer *et al*., 2007; Kähkönen, 2000). The texture of the yogurt can be traditionally improved by increasing the total solids content of the milk through the addition of different stabilizers such as pectin and gelatin (Farnsworth *et al*., 2006). Because this alternative for texture improvement is not allowed in many countries, in the last years several papers investigated different other methods for compensating the effect of fat removal from milk (Lorenzen *et al*., 2002; Faergemand *et al*., 1999). As shown previously by Ozer *et al*. (2007), one way to improve the physical and sensory properties of the skimmed set type yogurt is to incorporate low levels of transglutaminase before milk fermentation. Transglutaminase is an enzyme that catalyses the intra- and intermolecular crosslinking of peptide and proteins through acyl transfer reaction between g-carboxyamide groups of peptide-bound glutamine residues (acyl donor) and the primary amino groups of glutamine and lysine residues (Ozer *et al*., 2007). This enzymatic modification of protein structure appears a suitable tool for improving the functional properties of food proteins. Both caseins and heat denaturated whey protein are good substrates for transglutaminase (Schorsch *et al*., 2000; Lorenzen, 2000; Ozer *et al*., 2007).

The objective of the study was to investigate the effect of skimmed milk treatment with transglutaminase on the functional properties of yogurt.

Materials and methods

Materials

Pasteurized milk with 0.1% fat was used for preparing the yogurt. The milk was purchased from a local supermarket (Galati, Romania).

Microbial transglutaminase Activa TG-1 was purchased from Ajinomoto (Inc. Teanec, NJ, U.S.A.). The enzymatic product made up of 99% maltodextrin and 1% MTGase with a declared enzymatic activity of about 100 UE/g.

Enzymatic cross-linking of milk

Prior to acidification, the milk was treated with microbial transglutaminase MTGase. The milk was tempered to the cross-linking temperature (30, 40 or 50°C) in a water bath for 10 min prior to the addition of the enzyme. The enzyme concentrations used in the experiment were 0.02, 0.03 and 0.04 g MTGase/100 g milk and the samples were incubated for 60, 90 and 120 min. For each tested temperature the control samples was prepared without MTGase. The cross-linking reaction was stopped by thermally treating the milk samples at 90°C for 10 min; the temperature was increased at 1°C/ min. The thermally treated samples were afterwards cooled to 43°C using a mixture of water and ice.

Preparation of Yogurt

The MTGase treated milk tempered at 40°C was inoculated with a mixed culture of lactic acid bacteria YF-L811-Yo-Flex (*Streptococus theromophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (1:1) provided by Christian Hansen, Denmark). The samples were afterwards poured into 250 mL plastic containers, and incubated at 43°C. The size of the inoculum used for yogurt preparation was according to the producer's recommendations.

The milk acidification was monitored during the entire incubation period, and the fermentation was considered completed when the pH of 4.4 was reached for the control samples. The samples were then cooled and stored at 4°C for 21 days.

Determination of pH and Acidity

The pH was measured using a Hanna digital pH-meter by immersing the sensor on the yogurt samples.

The acidity of the yogurt samples was determined by titration with 0.1 *N* NaOH using phenolophthalein as indicator and was expressed as Thorner degree.

Syneresis

The centrifugation procedure was used to determine the yogurt syneresis after 24 h of storage at 4°C. Approximately 20 g of yogurt was transferred into a 50-mL glass tubes and was centrifuged at 3500 rpm for 15 min at 20°C. The syneresis was estimated as the percentage of the released whey over the initial gel weight and was an average of three determinations:

Syneresis % = (weight of supernatant/weight of yogurt sample)·100.

Rheological Measurements

Rheological properties of the yogurt samples after 24 h of storage at 4°C were determined in duplicate by means of a RHEOTEST-2 type rotating viscosimeter (VEB-MEDINGEN, Germany), using the coaxial cylinder device S_3. All measurements were carried out on 50 g of sample that was previously prepared by gently stirring in identical conditions.

For each sample the shear stress (τ) was recorded for shear rates (γ) ranging from 0.1667 to 145.6 s^{-1}, for a working frequency of 50 Hz. The apparent viscosity (η) was calculated as:

$$\eta = \frac{\tau}{\gamma} \tag{1}$$

Statistical analysis

Three experimental batches were performed for each test and the results are reported as mean values. Typical standard deviations are less than 5%. Statistical analysis of the results was performed by means of Microsoft Excel.

Results and discussion

Before fermentation with lactic acid bacteria, the 0.1% fat milk was incubated with MTGase up to 2 hours at different temperatures ranging from 30 to 50°C. The influence of MTGase treatment on the quality of the final product was estimated by monitoring the acidification process and determining the water holding capacity and rheological properties of the final product.

Evolution of yogurt acidity and pH

The fermentation of the milk samples was carried out at 40°C, representing a compromise between optimum temperature for streptococci and lactobacilli. The fermentation temperature is very important for obtaining high quality final products: higher temperatures would favor the growth of lactobacilli, and the development of higher acidity and sour yogurts with poor flavor, whereas lower temperatures would favor the growth of streptococci and the achievement of flavored yogurt well with low acidity and no specific taste (Banu, 2000).

The lactic acid produced as a consequence of the metabolic activity of lactic acid bacteria causes the increase of milk acidity and the reduction of the pH. Therefore the intensity of the fermentation can be estimated based on the dynamic of the pH which might significantly influence the sensory characteristics of the final product as well as microorganisms' viability.

In order to highlight the influence of the milk treatment with MTGase on the lactic acid bacteria metabolism, the evolution of pH was monitored during the entire fermentation period for all tested samples. The lactic fermentation started immediately after the addition of the starter culture and was stopped when the control samples reached a pH of 4.6. For the entire fermentation period, the pH of the MTGase treated samples was slightly higher compared to the control sample (Figure 1).

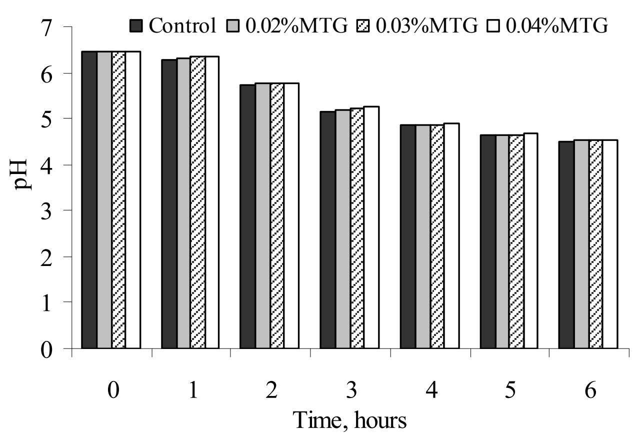

Figure 1. The evolution of the pH during yogurts fermentation at 43°C
Experimental conditions: setting temperature: 30°C; setting time: 90 min

In agreement with Lorenzen *et al*. (2002), our results suggest that MTGase interferes with the growth of the yoghurt starter culture, leading to longer fermentations. In addition, as a consequence of the reduced levels of available low molecular weight peptides necessary for bacterial growth, Lorenzen *et al*. (2002) indicate the reduction of the post-acidification during storage.

The titratable acidity of the yogurt samples was highly influenced by the MTGase treatment and setting temperature. Regardless of the setting time and MTGase concentration, the increase of the temperature from 30°C to 50°C during milk incubation with enzyme allowed obtaining yogurt samples with higher acidity (Figure 2). The setting time did not influence significantly the milk acidification process. The highest titratable acidity was obtained for samples preincubated for 90 minutes. After 24 hours of storage at 4°C, the highest titratable acidity was found in case of the maximum fermentative activity which was identified in the case of the samples treated with 0.03% MTGase at 50°C.

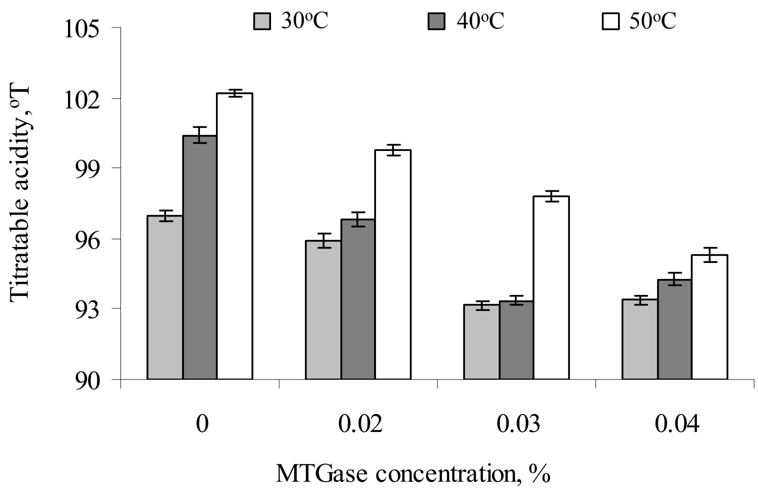

Figure 2. The influence of setting temperature on titratable acidity of yogurt samples with different concentrations of MTGase (setting time: 120 min)

The acidification process continues at a lower intensity during yogurt sample storage. The pH evolution at 4°C depended on the MTGase pretreatment conditions. Analyzing the results presented in Figure 3, one can see differences of 0.07-0.1 pH units in case of the milk samples treated with enzyme for 120 minutes before acidification, and lower variations of 0.04-0.07 pH units when treated with enzyme for 60 minutes. The increase of MTGase concentration induced a slower acidification of the yogurt samples, resulting in final products with higher pH.

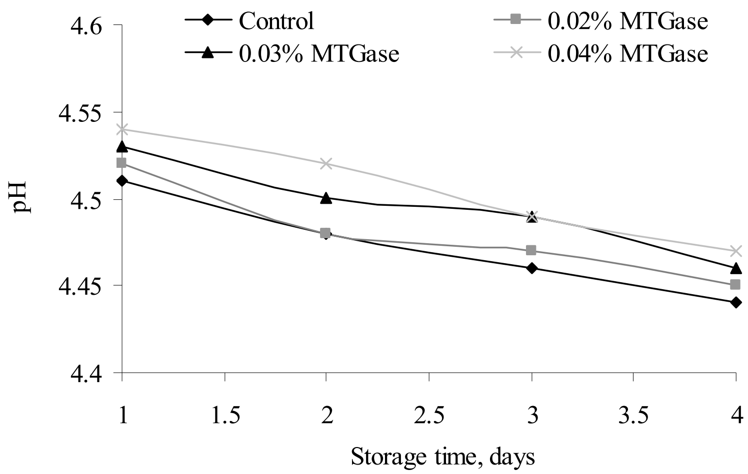

Figure 3. Variation of pH during yogurts storage at 4°C

Whey separation

The syneresis is a measure of the quantity of whey separated from the yogurt and is one of the most important factors influencing consumers' acceptance (Gürsoy *et al*., 2010).

The intensity of syneresis is higher in the case of yogurts with low levels of fat. The syneresis of the yogurt samples produced from skimmed milk treated with various concentrations of MTGase at 50°C is presented in Figure 4.

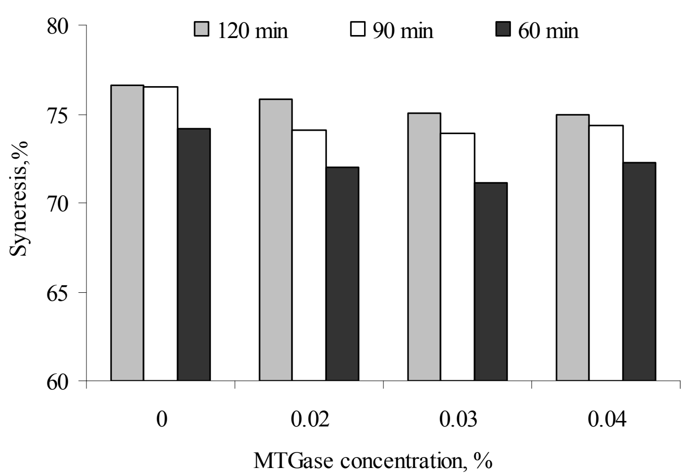

Figure 4. The influence of setting time on the syneresis of yogurt samples prepared with different MTGase concentrations (setting temperature of 50°C)

The syneresis varied significantly with the period of cross-linking reaction. Regardless of MTGase concentration and setting time, the water holding capacity decreases with the increase in the setting temperature increase. In the case of the setting temperature of 30°C and 40°C the best water holding capacity was obtained for a setting time of 90 min. When extending the cross-linking reaction time to 2 hours, no significant variations of syneresis with the MTGase concentration were obtained. For the setting temperature of 50°C, the lowest values of syneresis were obtained for the samples incubated with 0.02 and 0.03% MTGase for 60 minutes, followed by 90 minutes.

In agreement with Lorenzen (2002) and Lauber *et al*. (2000), our results indicate that the MTGase treatment of milk allowed improving the water holding capacity of the yogurt. The acidification process of the MTGase treated milk or casein micelles allows obtaining gels with increased firmness, lower permeability, finer protein networks and improved whey drainage (Færgemand and Qvist, 1997; Færgemand *et al*., 1999; Schorsch *et al*., 2000; Ozer *et al*., 2007).

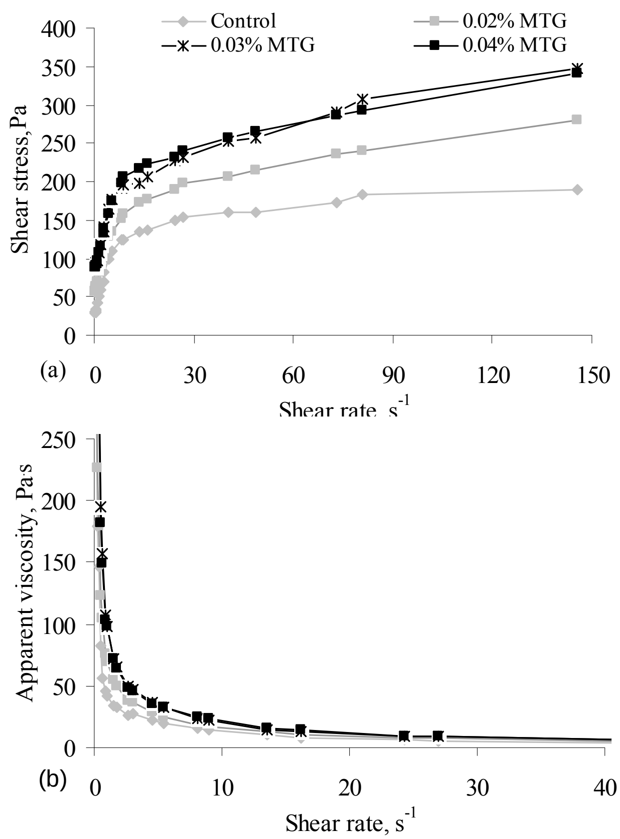

Figure 5. The influence of MTGase concentration on rheological behavior of yogurt samples (setting temperature: 40°C; setting time: 120 min). a) Shear stress vs shear rate rheogram; b) Apparent viscosity vs shear rate rheogram

The excessive whey separation may be a consequence of overcoming the fermentation or storage temperature, of inadequate cooling or of improper handling while in containers during storage and distribution (Lucey, 2004). The syneresis can be usually limited or eliminated by increasing the milk solids content. Anyway, certain food additives used for yogurt stabilization can affect the sensory properties of the final product. Our results indicate that milk proteins crosslinking with MTGase is a suitable alternative for the addition stabilizer in the skimmed yogurt.

Rheological measurements
The viscosity is an important parameter that can be successfully used for comparing the quality of the yogurt samples prepared in different conditions. In Figure 5 are presented the shear stress and apparent viscosity vs shear rate rheograms for the skimmed yogurt prepared using different enzyme concentrations. Regardless of the setting conditions, the MTGase treated samples have improved rheological properties compared to the control samples.

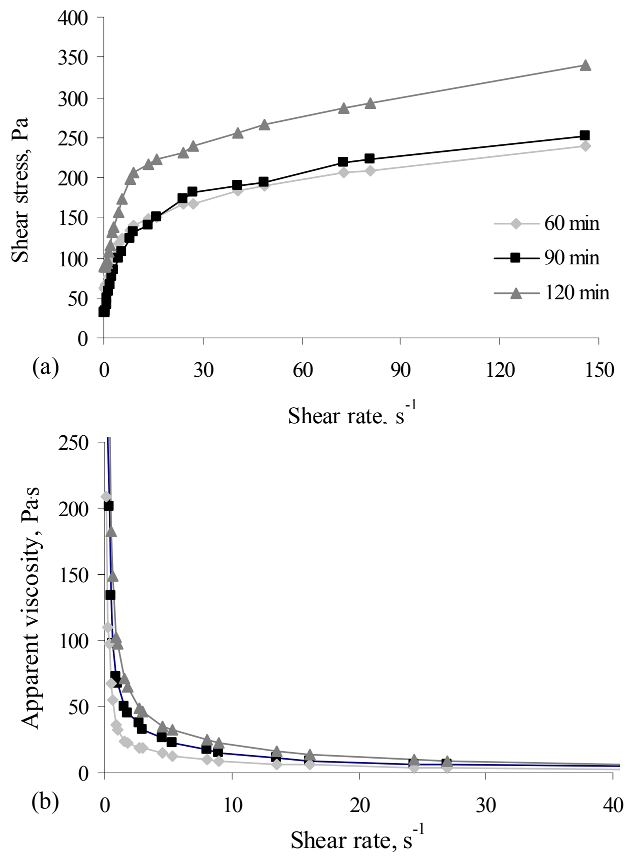

Figure 6. The influence of setting time on rheological behavior of yogurt samples (setting temperature: 40°C; enzyme concentration: 0.04 g MTGase/100 g milk). a) Shear stress vs shear rate rheogram; b) Apparent viscosity vs shear rate rheogram

The viscosity improvement was not only directly related to the enzyme concentration but also to the setting time. Analysing the results in Figure 6, one can see that for all shear rates, the apparent viscosity of the yogurt increases with cross-linking time from 60 to 120 minutes.

Higher setting temperatures allowed obtaining yogurts with slightly increased rheological properties. There are not significant differences whitin samples incubated at 40°C şi 50°C in terms of yogurt viscosity. The lowest viscosity was obtained when the cross-linking reaction was carried out at 30°C (Figure 7). For a shear rate of 5.4 s^{-1} the apparent viscosity of the yogurt treated with enzyme at 30°C was ~ 1,48 times lower compared to the samples incubated at higher temperatures (40°C or 50°C).

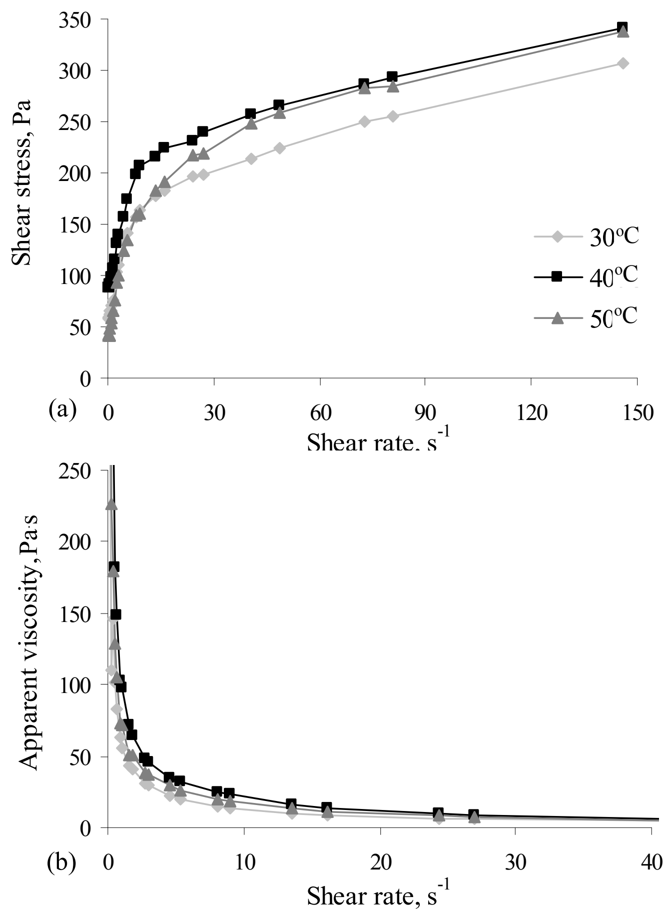

Figure 7. The influence of setting temperature on rheological behavior of yogurt samples (setting time: 120 min; enzyme concentration: 0.04 g MTGase/100 g milk). a) Shear stress vs shear rate rheogram; b) Apparent viscosity vs shear rate rheogram

The decrease of the apparent viscosity of all studied samples with the increase of the shear rate (Figure 5, 6 and 7) indicates the pseudo-plastic behaviour, with time-dependent structural viscosity. For shear rates over 35 s^{-1} the apparent viscosity reaches a plateau of constant values, meaning that the destruction and the reformation rate of the protein aggregates are comparable (Aprodu *et al*., 2011).

Conclusions

Enzymatic cross-linking reaction is well suited for obtaining set type yogurt without protein fortification. The transglutaminase catalyzed cross-linking reaction allowed obtaining skimmed yogurt with higher final pH and improved viscosity compared to the control sample. In the first 4 days of storage at 4°C, the pH of the MTGase treated samples decreased only by 0.01-0.05 units. Despite the low fat content (0.1%), in the case of the yogurt samples treated with MTGase, the syneresis phenomenon was significantly reduced. The best results in terms of rheological properties and whey retention capacity were obtained for the yogurt prepared from skimmed milk incubated with 0.04% MTGase for 120 minutes at 40°C.

Acknowledgment

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