

Evaluation of Transglutaminase from Pig Plasma on the Quality of Milk Curd

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ABSTRACT : The purpose of this study was to examine the effect of different levels of crude pig plasma transglutaminase (TGase) on the quality of milk curd. Different levels (0, 0.1, 0.2, 0.4, 0.8 and 1.0%) of crude pig plasma TGase was added to fresh milk, individually, and then incubated at 35°C. The time of milk curdling was recorded. Simultaneously, rheological properties, *L* value and microstructure of the samples were measured and observed. The results showed that the time of milk curdling decreased and the curd strength of milk curd increased with increasing of TGase ($p < 0.05$). The softness of milk curd had the highest value when 0.8% TGase was added ($p < 0.05$). However, *L* value of milk curds was not significantly different among all treatments. The microstructure of milk curd observed by scanning electron microscopy (SEM) became more dense with TGase level increased. (*Asian-Aust. J. Anim. Sci.* 2002, Vol 15, No. 1 : 106-110)

Key Words : Transglutaminase, Milk Curd, Microstructure

INTRODUCTION

Factor XIII is a transglutaminase (TGase, EC 2.3.2.13) that occurs as a zymogen in plasma, placenta and platelets. The reaction catalyzed by Ca^{2+} -dependent Factor XIIIa involves the formation of a ϵ -(γ -glutamyl)-lysyl bond between an acyl donor (glutamyl residue) and an acyl acceptor (lysyl residue). Thus, this enzyme catalyzes conversion of soluble proteins to insoluble high molecular polymers through formation of covalent cross-links. Therefore, the characteristics of TGase can be used to improve or modify the functional and rheological properties of food proteins (De Backer-Royer et al., 1992; Traore and Meunier, 1992).

Many food proteins are good substrates for TGase catalyzing cross-linking and among the milk proteins especially the caseins are excellent substrates for TGase (Aboumahmoud and Savello, 1990; Færgemand et al., 1997, 1998; Ikura et al., 1980; Nonaka et al., 1992; Nonaka et al., 1997; Sakamoto et al., 1994; Traore and Meunier, 1991, 1992). Milk protein gels are traditionally formed by treating casein with acid or proteolytic enzyme such as chymosin or by thermal denaturation of whey proteins (Dickinson and Yamamoto, 1996). These gel networks are stabilized mainly by weak non-covalent interactions. Development of new covalent bonds in milk protein gel may be different from milk protein gels by traditional methods in structure and enhanced functionality. For food purposes most chemical modifications would be undesired due to possible toxicity or unwanted side reactions (Færgemand and Qvist, 1997). An alternative way of making a milk protein gel can use

enzyme to catalyze cross-linking protein molecules to produce a network of covalent linkages. Since the different cross-links, such a protein network might be expected to have quite different rheological properties from a conventional milk protein gel (Dickinson and Yamamoto, 1996).

TGase from pig blood is one of methods to increase economical benefit and prevent environmental pollution caused by discarding the blood as waste. Since TGase has a functionality for polymeration of proteins. We expected to increase the utilization of pig plasma TGase, and partial replacement or substitution of rennin to maintain or improve the quality of milk curd in cheese manufacture or to develop new proteins for human consumption. The aim of this study was to evaluate milk curd made with the addition of crude pig plasma TGase.

MATERIALS AND METHODS

The preparation of crude pig plasma TGase

Crude pig plasma TGase was obtained by a modification of the method of Lorand and Gotoh (1970) and Jiang and Lee (1992) in which fresh pig plasma was subjected to fractionation using 40% ammonium sulfate. The precipitate protein was collected, freeze dried and stored at -20°C until needed (the specific activity was 1.32 units/mg).

The preparation of milk curd

The experimental treatment involved six groups, each group was replicated three times. The fresh milk obtained from experimental dairy farm of Chung-Hsing University was incubated in water bath at 35°C for 20 min prior to addition of TGase. TGase (contains 10 mM CaCl_2 , 10 mM cysteine) (Akamittath and Ball, Jr., 1992; Folk and Cole, 1966a) of 0, 0.1, 0.2, 0.4, 0.8 and 1.0% was added to each

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group and mixed for 30 seconds using a vortex mixer, respectively, and incubated in water bath at 35°C until milk curdling that was judged by fluidity of the fresh milk in a bottle placed at a slope angle of 45 degrees. The milk curd in good state was looked like the picture of figure 1 (the bottle was upside down).

Determination of enzyme activity

TGase activity was measured using the procedure described by Folk and Cole (1966a; 1966b). A final volume of 1 ml of 0.2 M Tris -acetate buffer containing 0.1 M hydroxylamine, 5 mM CaCl₂, 10 mM glutathione, and 30 mM N-carbobenzyloxy-glutaminyI-glycine at pH 6.0 was used. The reactive mixture was incubated at 37°C for 10 min and then neutralized by adding an equal volume of 15% trichloroacetic acid-5% FeCl₃ solution. After centrifugation at 4,000×g for 15 minutes at room temperature, the supernatant was collected and the absorbance was measured at 525 nm. The calibration curve was prepared by using L-glutamic acid-γ-monohydroxamic acid as a standard. One unit of TGase activity was defined as the amount of enzyme that caused the formation of 1 μmole of hydroxamic acid within 1 minute at a reaction temperature of 37°C. Protein content was measured by the method of Bradford (1976) using bovine serum albumin as a standard.

Record the time of milk curdling

TGase was added to fresh milk of 35°C and mixed for 30 seconds using a vortex mixer, and incubated in water bath at 35°C until the fresh milk in the bottle at a slope angles of 45 degrees, it was judged as milk curdling when the milk was not fluid. The time of milk curdling was recorded. (Tseng et al., 2000)

Determination of rheological properties

Curd strength and softness of the milk curd were



Figure 1. Appearance of curd of milk in bottle which was upside down

measured using the procedure described by Færgemand and Qvist (1997) and Sakamoto et al. (1994). The curd strength and softness of the specimens were measured using a Fudoh Rheometer (NRM-2010J-CW, Japan) attached to a Rheo Plotter (FR-801, Japan). Measuring speed was 6 cm/min, spherical plunger diameter was 20 mm and sample height was 15 mm.

L value determination

The Hunter L value (lightness) of the curd appearance was determined by using a Handy colorimeter (NR-3000, Nippon Denshoku Ind. Co., Ltd.).

Scanning electron microscopy (SEM)

Samples were frozen at -70°C for one day then lyophilized (Kingmech, FD-6). The lyophilized samples were put onto a double-sided adhesive tape, mounted on aluminum stubs and gold coated and subjected by a scanning electron microscope (Topcon, ABT-150S) (Ashie et al., 1997; Færgemand and Qvist, 1997).

Statistical analysis

Statistical analyses were by applying Duncan's new multiple range test using the statistical analysis system (SAS, 1991).

RESULTS AND DISCUSSION

Effect of TGase on the time of milk curdling

The time of milk curdling was 180, 120, 90, 70 and 50 min for adding with 0.1, 0.2, 0.4, 0.8 and 1.0% of TGase, respectively (table 1). The results showed that the time of milk curdling decreased with increasing levels of TGase (p<0.05). This result was probably caused by the more TGase addition can catalyze higher the formation of ε-(γ-glutamyl) lysyl bonds among the milk proteins that resulted in forming milk curd rapidly (Færgemand et al., 1998; Ikura et al., 1980; Imm et al., 2000). Imm et al. (2000) reported that skim milk was incubated in water bath at 40°C for 3 hour to allow TGase-catalyzed cross-linking reaction. Moreover, Færgemand and Qvist (1997) also reported that reconstituted skim milk was incubated with microbial transglutaminase at an enzyme/milk protein ratio of 0.4% (w/w) at 41°C for 60 min to allow cross-linking of the milk proteins. Therefore, the time of milk curdling in this study

Table 1. Effect of the levels of TGase on the time of milk curdling

TGase(%)	0	0.1	0.2	0.4	0.8	1.0	Mean of standard error
Time (min)	-	180 ^a	120 ^b	90 ^c	70 ^{cd}	50 ^d	14

^{a,b,c,d} Means in the same row different letters are significantly different (p<0.05).

was in agreement with previously reported studies (Færgemand and Qvist, 1997; Imm et al., 2000).

Effect of TGase on the rheological properties of milk curd

The strength of milk curd increased with increasing levels of TGase, and the level above 0.8% of TGase was significantly higher than that of 0.1 and 0.2% of TGase added ($p < 0.05$) (figure 2). This result could be caused by the more TGase addition can catalyze higher ϵ -(γ -glutamyl) lysyl bonds among the milk proteins that resulted in a stronger curd strength of milk curd (Færgemand et al., 1998; Ikura et al., 1980; Imm et al., 2000). Moreover, Dickinson and Yamamoto (1996) also demonstrated the potential for enhancing the strength of transglutaminase-induced milk protein gels.

The softness of milk curd increased with increasing in TGase concentration from 0% to 0.8%, and the softness of milk curd had the highest value when 0.8% TGase was added ($p < 0.05$), but the level 1.0% was significantly decreased ($p < 0.05$) (figure 3). This result was probably due to high ϵ -(γ -glutamyl) lysine cross-links to be formed between milk proteins with 1.0% TGase and resulted in a higher curd strength (figure 2) and more rigid and brittle structure (figure 5F) (Færgemand et al., 1998; Ikura et al., 1980; Imm et al., 2000; Sakamoto et al., 1994; Seguro et al., 1995). Sakamoto et al. (1994) reported that at high enzyme concentration was not only soft but also fragile, and excessive formation of (ϵ -(γ -glutamyl)-lysyl) crosslinks would inhibit uniform development of thermally induced protein network. Imm et al. (2000) also indicated that TGase treatment improved not only gel hardness but also water holding capacity.

Effect of TGase on the L value of milk curd

The Hunter L value (lightness) of the milk curds was ranged from 83.64 to 86.22 (figure 4). There was no

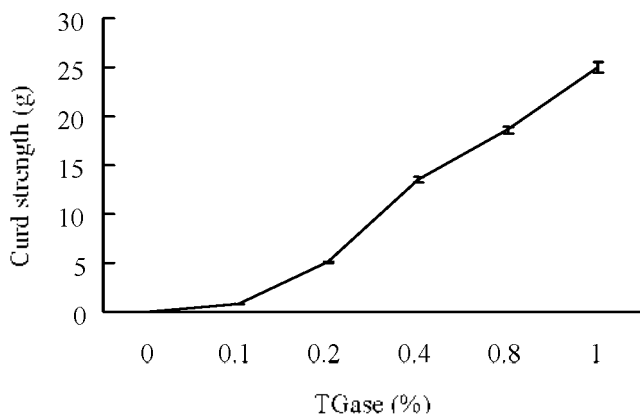


Figure 2. Effect of the levels of TGase on the curd strength of milk curd

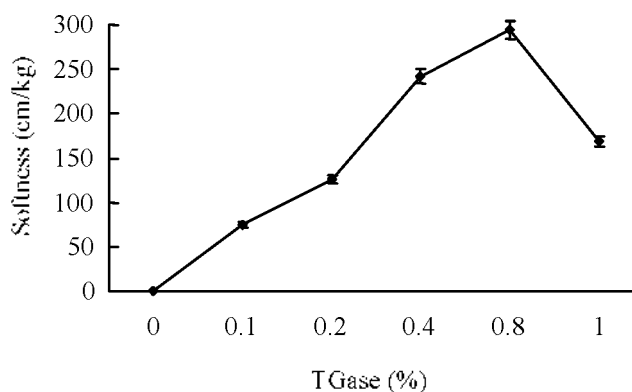


Figure 3. Effect of the levels of TGase on the softness of milk curd

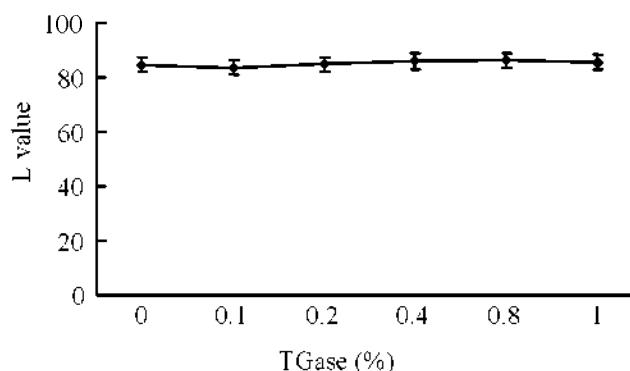


Figure 4. Effect of the levels of TGase on the L value of milk curd

significant difference among treatments. This meant that TGase treatment did not alter the lightness of the milk curds.

Effect of TGase on the microstructure of milk curd

The SEM photomicrographs showed that the control sample without adding TGase did not form gel network structure (figure 5A). In comparison, the milk curd with TGase treatment had a rather firmer than regular gel network structure, and denseness increased with the levels of TGase (figure 5B-5F). Færgemand and Qvist (1997) and Nio et al. (1986) reported that addition of TGase would form firmer gel networks through the formation of intermolecular ϵ -(γ -glutamyl)-lysyl cross-links. Chanyongvorakul et al. (1995) also indicated that by TGase-induced gelation, the association of protein molecules through isopeptide bonds might occur more regular structure than by thermal induced gels. Furthermore, formation of chemical bonds such as isopeptide bonds were irreversible and contributed to form a strong protein-protein interaction and stabilize the network structure. The results of this experiment were in agreement with previously reported studies (Chanyongvorakul et al., 1995; Nio et al., 1986).

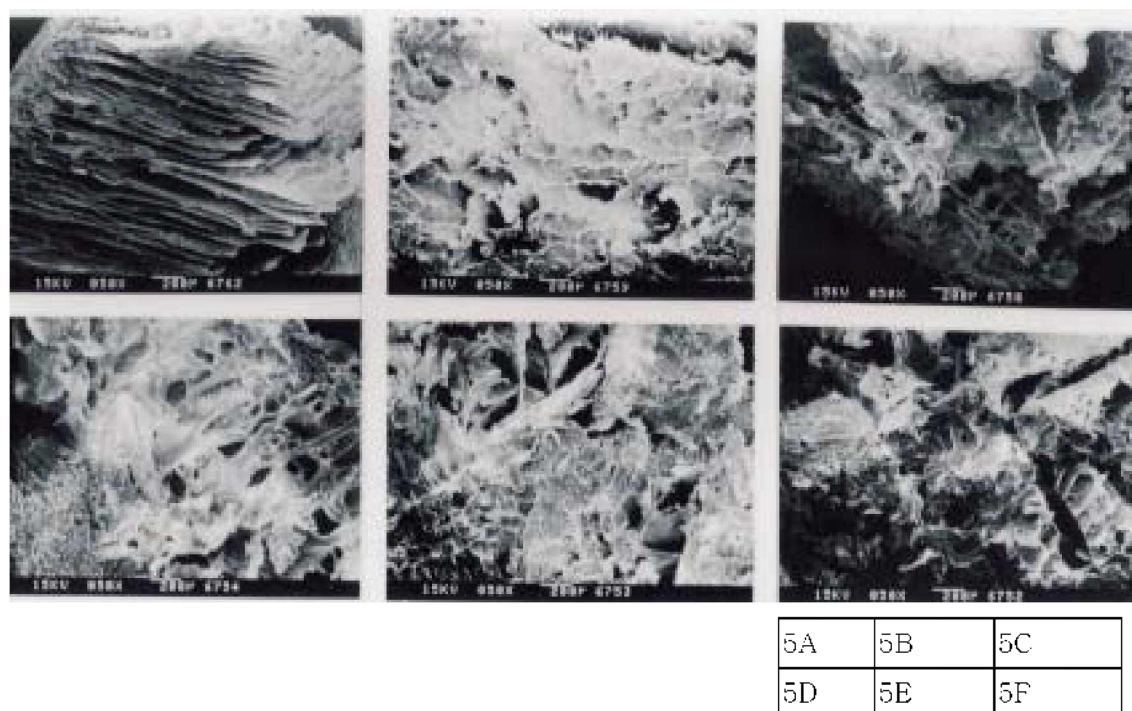


Figure 5. Effect of the levels of TGase on the microstructure of milk curd
 A, B, C, D, E and F were 0, 0.1, 0.2, 0.4, 0.8 and 1.0% TGase, respectively

Whereas, the milk curd with 1.0% TGase formed bigger and more complete gel clusters (figure 5F), and a broken curd was formed when omentum was found between gel clusters. This might be caused by the milk curd strength became rigid and brittle (figure 2) (Seguro et al., 1995), and the softness of milk curd could be explained by decreasing with 1.0% TGase in this experiment (figure 3).

CONCLUSION

To sum up, the time of milk curdling decreased and the curd strength of milk curd increased with increasing levels of TGase ($p < 0.05$). The softness of milk curd had the highest value when 0.8% TGase was added ($p < 0.05$). However, *L* value of milk curds was not different among all treatments. A rather firmer curd network microstructure of milk curd was observed by SEM when TGase level increased. Therefore, the use of TGase extracted from pig plasma in producing milk curd may be able to substitute or partially replace the rennin usage, maintaining the quality of milk curd.

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