Study on a Binder by Using Porcine Blood Plasma Transglutaminase, Thrombin and Fibrinogen

Chong-Ming Tsai[1], Tsai-Fuh Tseng[1,] *, Jeng-Huh Yang[1] and Ming-Tsao Chen[2]

[1]Department of Animal Science, National Chiayi University, Chiayi, 60077, Taiwan, ROC

[2]Department of Food Technology, Da-Yeh University, Chang-Hua 515, Taiwan, ROC

ABSTRACT : The purpose of this study was to prepare a binder containing porcine blood transglutaminase (TGase), thrombin and fibrinogen. Extracted TGase, thrombin and fibrinogen were used alone or mixed with different proportions of their volume (v/v/v) by nine combinations as follows were 0.5:1:15, 0.5:1:20, 0.5:1:25, 1:1:15, 1:1:20, 1:1:25, 1.5:1:15, 1.5:1:20 and 1.5:1:25, respectively. Five ml of each combination were mixed with 0.6 ml of 0.25 M calcium chloride before experiment. After storage at 4C for 0, 1, 2, 3, 4 and 5 weeks, enzyme activity, total plate count, pH value, and SDS-PAGE of TGase, thrombin and fibrinogen were tested and pH value, clotting time and gel strength of the nine combination binders were determined. The results showed that total plate count of thrombin and pH value of TGase were significantly higher ($p<0.05$) than in other treatments. SDS-PAGE results showed that purified TGase, thrombin and fibrinogen from porcine blood plasma compared with commercial products (Sigma) had the same band patterns and nine different combination binders had no significant effect. Enzymatic activity of TGase and thrombin decreased as storage time increased. Total plate count of TGase, thrombin and fibrinogen and clotting time of the binder increased as storage time increased. The higher amount of fibrinogen in combinations, the stronger the gel strength. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 1 : 137-143)***

Key Words : Porcine Blood Plasma, Transglutaminase, Thrombin, Fibrinogen and Binder

INTRODUCTION

Transglutaminase (TGase) exists extensively in tissue, cells or organs, such as hair capsule, blood plasma, epidermis cell, liver, tumor, placenta, plant and microbes (Kang and Cho, 1996; Harding and Rogers, 1972; Greenberg et al., 1982; Ando et al., 1989; Chen and Mehta, 1999). It may catalyst the transfer reaction of acyl group of inter- or intra-protein molecules occasionally (acyl- transfer reaction), promoting the formulating of covalent bond ε- (γ-glutamyl) lysyl cross-bridge (Folk, 1980; Motoki and Seguro, 1994). This can enhance the nutrition value of the food and modify the functionalities of proteins (Fargemand et al., 1998; Imm et al., 2000; Nonaka et al., 1994). Owing to the interactions among TGase, thrombin and fibrinogen in blood clotting phenomenon, and TGase induces to form cross-link matrix and gels with fibrinogen, and TGase promotes fibrinogen and collagen in meat to gel forming cross-link structure (Wijngaarrds and Parrdekooper, 1987). However, the activity of porcine plasma TGase was affected by different pH values, temperatures, metal ions and inhibitors. Tseng et al. (1999) pointed out that TGase had the highest relative activity at pH 7-8, temperature 35-45°C in the presence of Ca^{2+}. Therefore, this study attempted to prepare a kind of meat binder included TGase, thrombin and fibrinogen, by different combinations admixture of their volumes, to find what kind of ratio and storage time would be the best for binder preparation.

* Corresponding Author: Tsai-Fuh Tseng. Tel: +886-5-2717547, Fax: +886-5-2717527, E-mail: tseng@mail.ncyu.edu.tw

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MATERIAL AND METHOD

Preparation of the experimental material

Extraction of the porcine blood plasma TGase : Porcine blood was drawn from hybrid hogs (LYD), stunned and slaughtered in a slaughterhouse of Chiayi City. Anticoagulated by adding 3.2% sodium citrate solution to fresh pig blood in 1:9 (v/v) proportion to mix well. Be placed immediately in ice slash, brought back to laboratory in 30 minutes. Sample were centrifuged by a refrigeration centrifuge (HITACHI, himac SCR 20B) at 4,000 rpm for 15 min at 4°C. The supernatant, which was blood plasma, poured out for TGase extraction.

According to Tseng (1999) method, 40% ammonia sulfate solution slowly added to 1 L blood plasma, then using refrigeration centrifuge (SIGMA, 3K30) to separate at 12,000 rpm for 30 min at 4°C. Discarded the supernatant and the precipitate was dissolved by 750 ml 0.05 M sodium citrate buffer (pH 6.8). The crude extracted TGase solution 522 ml (containing 72 g precipitate and 450 ml extraction solution; mean value of three replicates) was obtained from 1000 m1 of porcine whole blood, so the extraction rate(%) was equal to 52.2%, (522/1,000)×100%.

Extraction of thrombin : According to Divakaran (1982) method, 100 ml blood plasma was diluted to 1,000 ml by distilled water, adjusted pH to 5.3 by 2% acetic acid, centrifuged at 10,000 rpm for 20 min at 10°C, and the sediments were dissolved in 25 ml 0.85% saline, adjusted pH to 7 by 2% sodium carbonate, the resulting solution was thrombin. The crude thrombin solution 165.63 ml

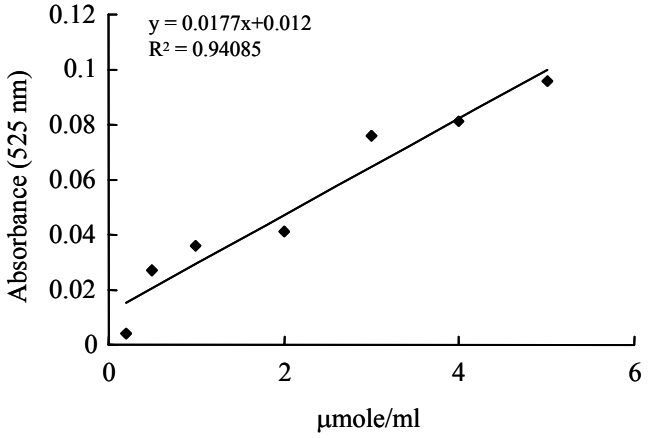

Figure 1. The standard curve of transglutaminase activity.

(containing 15.63 g precipitate and 150 ml extraction solution; mean value of three replicates) was obtained from 1,000 ml of porcine whole blood, so the extraction rate (%) was equal to 16.56%, (165.63/1,000)× 100%.

Extraction of fibrinogen : According to Ninomi et al. (1984) method, diluted blood plasma by distilled water at 1:1 volume ratio, adjusted pH to 5.5 with 1 N HCl or 1 N NaOH, set overnight at 4°C, then centrifuged at 6,000 rpm for 15 min at 10°C, the obtained sediments was dissolved in extraction solution (100 ml blood plasma added 10 ml extraction solution, 0.3 M NaCl and 50 mM Tris-HCl, pH 7.4). The crude fibrinogen solution 69.6 g (containing 9.6 g precipitate and 60 ml extraction solution; mean value of three replicates) was obtained from 1,000 ml of porcine whole blood, so the extraction rate was

(%) equal to 69.6%, (69.6/1,000)×100%.

Experimental treatment

Extracted TGase, thrombin and fibrinogen from fresh porcine blood plasma were mixed in different proportion of their volume (v/v/v) by nine combinations were 0.5:1:15, 0.5:1:20, 0.5:1:25, 1:1:15, 1:1:20, 1:1:25, 1.5:1:15, 1.5:1:20 and 1.5:1:25, respectively. Five ml of each combination were further mixed with 0.6 ml of 0.25 M calcium chloride before experiment.

Measurement and methods

TGase activity :

i) Standard curve of TGase activity : According to Tseng (1999) method, dissolved L-glutamic acid-γ- monohydroxamic acid into 1 M Tris-acetate buffer for preparing 0.2-5 μmole/ml concentration, added 1 ml of 15% trichloroacetic acid-5% $FeCl_3$ (dissolved in 0.1 N and 2.5 N HCl separately, then mixed with same volume), measured the absorbance at 525 nm wavelength. Producing 1 μmole hydroxamate every minute represents 1 unit of enzyme activity. The standard curve of TGase is shown as Figure 1.

Table 1. The enzyme activity of thrombin*

Multiple of attenuated conc.	5	10	15	20	25	26**	27
Clotting time (5 min)	○	○	○	○	○	○	×

* Thrombin activity: Thrombin unit/ml = atttenuated concentration×5. Multiple of attenuated concentration: diluted concentration by saline used.

** Particularly atttenuated concentration of 1 ml thrombin could make 1 ml fibrin solution solidified within 5 min at 37°C. So the thrombin activity is 26×5 = 130 units.

○: Indicates that it can be solidified completely in 5 minutes.

×: Indicates that attenuated concentration, 27 times, could not be solidified at 5 min.

ii) The assaying of enzyme activity : The colorimetric hydroxamate assaying of the TGase activity was followed by Folk (1970) method, extracted TGase 100 μl was added with a reaction solution, which consisted of 700 μl 0.1 M tris-acetate buffer (pH 6.0), 50 μl 2 M hydroxylamine, 150 μl 0.1 M N-α-carbobenzoxy-gultaminyl-glycine (CBZ), And kept in a water bath (WISOOM BC2D 18 L) at 37°C for 10 min, then added 1 ml 15% trichloroacetic acid-5% $FeCl_3$ to cease the reaction. Sample were centrifuged (SIGMA, 3K30) at 6,000 rpm at 25°C for 5 min, the supernatants were poured out for absorbance (Spectrophotometer, Metertek SP-830) measurement by 525 nm wavelength. Obtained absorbance value(x) substitute in the equation of the standard curve of TGase enzyme activity (Figure 1) y = 0.0177x+0.012, then the unknown activity (y) of sample was calculated.

Thrombin activity : This method was followed by Anbe (1961) and modified as following :

i) Reagents preparation :

a. 1% fibrinogen solution : 1 g fibrinogen (Sigma) dissolved in 10 ml 0.5% Imidazole buffer (pH 7.1), then diluted to 100 ml by distilled water.

b. Reaction admixture liquid :
- 200 ml saline containing 15% Arabian gum
- 100 ml 0.5% Imidazole buffer (pH7.1)
- 100 ml saline containing 0.7% calcium chloride
- 200 ml 0.9% saline

ii) Procedure :

a. Crude thrombin subjected to check enzyme activity, sample was diluted accurately to one part of 5, 10, 15, 20, 25, 26, 27 portion times in tubes, respectively. Then put into those tubes in ice sink, in order to prevent its activity loses.

b. Test tubes put into a constant temperature sink at 37°C for a while, then added 1 ml 1% fibrinogen solution at first, then added 3 ml reaction admixture, then added 1 ml subjected thrombin sample, and then started to calculate the clotting time.

Figure 2. The appearance of transglutaminase, thrombin, fibrinogen and calcium chloride solution.

c. Put test tubes upside down, the time needed to keep its content did not flow or fall down at the end of clotting. The clotting time represented the activity of thrombin (Table 1), the greatest attenuated concentration of 1 ml thrombin could make 1 ml fibrin solution solidified within 5 min at 37°C. was 26 times, so the thrombin activity was 26×5 = 130 units. But above this dilution, such as 27 times, could not solidified at 5 min. 27 times, could not solidified within 5 min.

The properties of TGase, Thrombin, and fibrinogen :

i) Total plate count : Method according to Huang and Wang (1997), ten grams ground sample in a sterilized bottle, then added sterilized distilled water 90 ml, shaken well in a shaker (FUNNEL SHAKER) at 150 rpm for 2 min, then made into one tenth attenuated liquid, then diluted into one part of 100, 1,000 and 10,000, if needed. Piping 1 ml of each diluted sample liquid into Petri dish, respectively. Then poured the plate count agar (PCA, Merck) 15-20 ml for each Petri dish, after the culture medium solidified, set upside down to incubate for 48±2 h at 37°C, measured its colony forming unit (log CFU/g), and calculated mean for 5 replicates.

ii) pH value : Before adding calcium chloride added, used pH meter (SUNTEX SP-701) to measure and calculated mean for 5 replicates.

iii) Solidify time : While calcium chloride added, then started accounting the time needed for sample to be completely solidified.

iv) Gel Strength : Used Rheometer (RHEOTECH FUDOH RHEOMETER RT-2002D, Japan), a food rheology assaying apparatus, measuring condition as follows: the height of tested gelling sample in a PVC cup (dia. 5 cm): 10 mm; power (force): 200 g; adapter: No.3, diameter 3 mm; pressed into the depth (preset): 5 mm; carrying speed: 30 cm/min; return number: once. The strength of gel was used as to indicate the power of that instillation with broken moment, the unit was g/cm^2.

v) Storage test : The enzyme activity of TGase and thrombin, stored at 4°C, was monitored for 5 weeks. pH value and total plate count of TGase, thrombin and fibrinogen were also determined in 5 weeks. Different admixture combination of TGase, thrombin and fibrinogen volume ratio were measured for their change in pH, solidifying time and gel strength in 5 weeks kept at 4°C. Five replicates for each tested item.

Protein electrophoresis : The extracted TGase, thrombin, and fibrinogen solution and nine admixture of their combinations, were analyzed by the method of SDS-PAGE (Laemmli, 1970) using 4% stacking gel and 12% separating gel.

Statistics analysis

Statistics analysis carried on with SAS software (SAS, 1996), used general linear models procedure to test the variance analysis, and tested the difference among means of treatments by Duncan's multiple range significant test.

RESULTS AND DISCUSSION

Enzyme activity, total plate count and pH value of TGase, thrombin and fibrinogen

The appearance of TGase, thrombin, fibrinogen and calcium chloride were shown in Figure 2. Their solutions color were yellowish, light blue, light blue, and transparent color, respectively.

The enzyme activity of TGase and thrombin, shown in Table 2, was 0.447 μmole/ml and 130 units, respectively. The TGase activity of this test was similar to the report of Liu (1999). The thrombin activity was similar to Huang (1987).

The total plate count of TGase, thrombin and fibrinogen was 2.48, 3.9 and 3.41 log CFU/g, respectively. The total plate count of thrombin was higher than TGase and fibrinogen. The total plate count may be affected by the environment (such as blood collecting tank and knife) of the slaughter house and staff slaughtering way. The total plate count could be affected possibly by collecting blood sample and extracting process through their effects on degree of

Table 2. Enzyme activity, total plate count, pH value of transglutaminase, thrombin and fibrinogen*

Item	TGase* (μMole/ ml)	Thrombin** (unit)	Fibrinogen	SEM**
Enzyme activity	0.447[1]	130[2]	-	
Total plate count (log CFU/g)	2.48^c	3.9^a	3.41^b	0.034
pH value	7.18^a	6.97^b	6.75^c	0.014

* Means within the same row without the same superscript are significantly different ($p<0.05$).

** SEM: Standard error of means. [1] μmole/ml; [2] Unit.

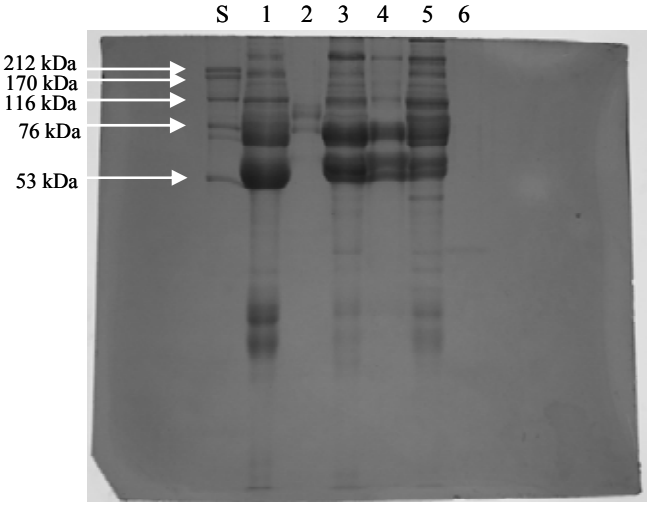

Figure 3. The SDS-PAGE of TGase, thrombin and fibrinogen compared with commercial product (Sigma). S: Standard. (Mysoin = 212 kDa, α-2-Macroglobulin = 170 kDa, β-galactosidase = 116 kDa, transferring = 76 kDa, L-glutamic dehydrogenase = 53 kDa). Lane 1: TGase, Lane 2: commercial product TGase, Lane 3: Fibrinogen, Lane 4: commercial product Fibrinogen, Lane 5: Thrombin, Lane 6: commercial product thrombin.

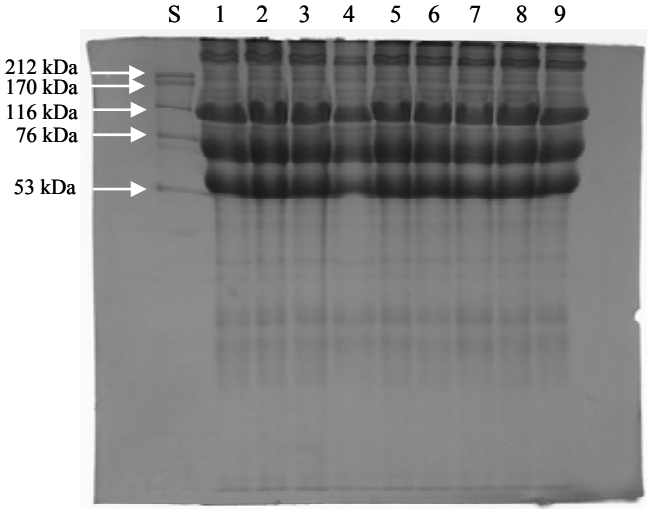

Figure 4. The SDS-PAGE of a binder prepared by TGase, thrombin and fibrinogen with various volume ratios. S: Standard. (Mysoin = 212 kDa, α-2-Macroglobulin = 170 kDa, β-galactosidase = 116 kDa, transferring = 76 kDa, L-glutamic dehydrogenase = 53 kDa). Lane1: (TG:T:F = 0.5:1:15), Lane2: (TG:T:F = 0.5:1:20), Lane3: (TG:T:F = 0.5:1:25), Lane4: (TG:T:F = 1:1:15), Lane5: (TG:T:F = 1:1:20), Lane6: (TG:T:F = 1:1:25), Lane7: (TG:T:F = 1.5:1:15), Lane8: (TG:T:F = 1.5:1:20), Lane9: (TG:T:F = 1.5:1:25), TG: Transglutaminase; T: Thrombin; F: fibrinogen.

contamination.

The pH value of TGase, thrombin and fibrinogen was 7.18, 6.97 and 6.75, respectively (Table 2). The pH value of the TGase was been obvious higher ($p<0.05$) than in thrombin and fibrinogen. The possibility of causing difference of pH value might due to the buffer solution quantity and extraction method.

Tseng (1999) pointed out the highest relative activity of TGase of porcine blood plasma was existing in pH 7-8, but Lee (1991) showed that pig blood plasma coagulating factor XIII (TGase) had the most suitable pH value in near 8.5. Lin (1995) pointed out the most stable pH of TGase was in 6.5, but in pH 5-7.5, the activity still preserved 87% of those in neutral range. Ando et al. (1989) indicated that microbial origin of TGase had the most suitable reaction pH in 6-7. From above studies, we could conclude that the suitable pH for TGase activity was in neutral ca. 7.0.

Machovich (1986) pointed out the isoelectric point of thrombin was at pH 7.2-7.6. From this study, the pH of extracted thrombin was 6.97, nearing to the isoelectric point. Tsai (1987) stated that the fibrinogen was sensitive to heat, would completely denature at 56°C in 30 min, would lose its solidifying characteristics at above pH 9.5, and its isoelectric point was at pH 6.5.

Yeh (2000) elucidated that pH value affected upon the enzyme activity, mainly lied in all the ionizing group in the active site of the enzyme, and such as a pH change, enzyme active site and substrate differ in ion distribution to allow to form a mutual bonding for catalysis action, so the pH value was rather important to the activity of enzyme.

Electrophoretic analysis of TGase, thrombin and fibrinogen

The electrophoretic photogram of TGase, thrombin and fibrinogen was similar to its commercial product (Sigma) (Figure 3), manifestated same protein bands, could be sure these extracted samples of TGase, thrombin and fibrinogen were just the targeted subjects. But the commercial thrombin showed only one band lesser than 53 kDa, as shown in lane 6 of Figure 3.

The admixture of TGase, thrombin and fibrinogen by different volume ratios for preparing a binder, showed by electrophoretic photogram no obviously different (Figure 4). This may be due to all of the components consisted of by TGase, thrombin and fibrinogen constitutive enzyme, so the components that displayed also would not have very big variety.

Tseng (1999) analyzed the molecular weight of extracted TGase from porcine blood plasma by SDS- PAGE, were about 75 kDa and 80 kDas. Ando et al. (1989) pointed out that the microbial TGase had molecular weight 40 kDa. Schwartz et al. (1973) and Chung et al. (1974) pointed out that TGase had 4 subunits (A_2B_2), its molecular weight is about 320 kDas, among them, subunit A was of catalytic activity, its molecular weight is about 75 kDa, and subunit B did not show the catalytic activity, its functions for maintaining their molecular structure and protecting active

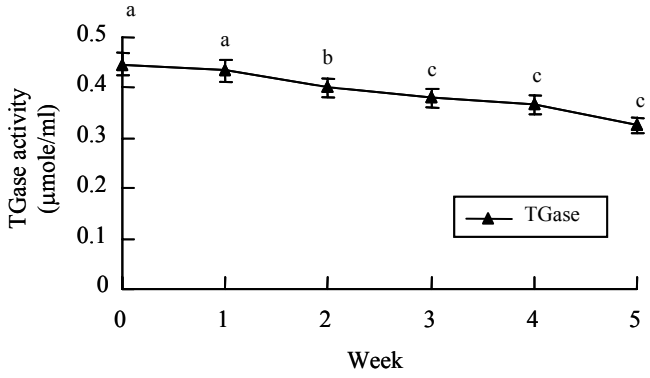

Figure 5. Change in activity of transglutaminase during storage at 4°C for 5 weeks.

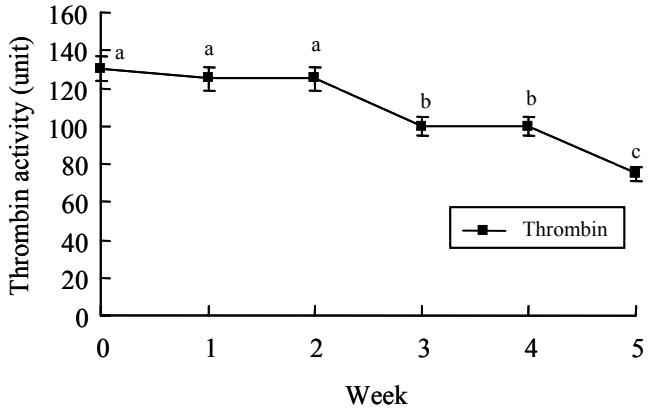

Figure 6. Change in activity of thrombin during storage at 4°C for 5 weeks.

site of subunit A, the molecular weight was about 80 kDa.

Herrick et al. (1999) pointed out that fibrinogen was of 340 kDa molecular weight, as a glycoprotein with a length of 460 Å, mainly synthesized by liver cells. Binnie and Lord (1993) also pointed out that fibrinogen molecule is a dimmer, its monomer consisted of Aα, Bβ and γ, three polypeptide chains cross-bridged by 29 sulfur bonds, had molecular weight for 64-67 kDa, 56-57 kDa and 47-48 kDa, respectively.

Changes of enzyme activity, pH value and total plate count of TGase, thrombin and fibrinogen stored at 4°C for 5 weeks

The activity of TGase and thrombin stored at 4°C for 5 weeks (Figures 5 and 6), decreased as the storage time increased ($p<0.05$). The TGase activity descended, which maybe due to its sulfur hydroxy group (-SH) in active site being subjected to oxidizing. At sixth week, thrombin activity descended sharply to 25 units, its activity was already too low, so there was no actual exploitation value, therefore this study was a 5 week's trial.

The change of pH value of TGase, thrombin and fibrinogen stored at 4°C for 5 weeks (Figure 7), showed pH

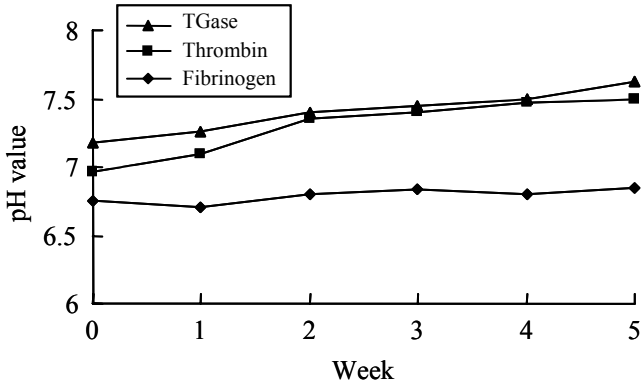

Figure 7. Changes in pH value of transglutaminase, thrombin and fibrinogen during storage at 4°C for 5 weeks

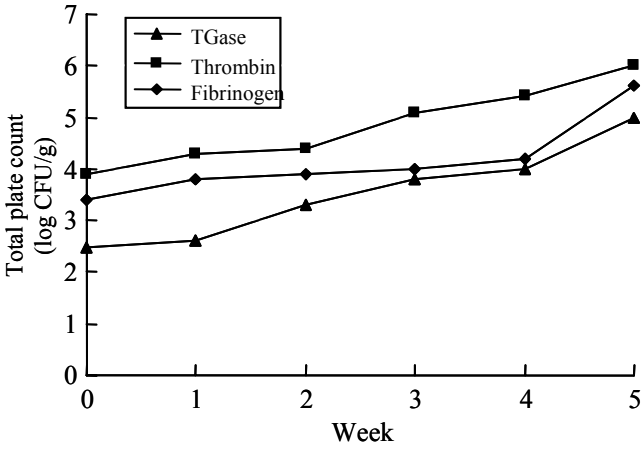

Figure 8. Changes in total plate count of transglutaminase, thrombin and fibrinogen during storage at 4°C for 5 weeks.

value of TGase and thrombin slightly increased with storage time increased, the pH of the fibrinogen vaired not greatly.

The total plate count of TGase, thrombin and fibrinogen stored at 4°C for 5 weeks (Figure 8) had a trend to increse with storage time increased. May be the TGase and thrombin were a aquar state and while at refrigerator condition, so bacteria speeded up easily, made total plate count increase.

TGase, thrombin and fibrinogen stored at 4°C for 5 weeks, with their different volume ratios admixed to prepare a kind of binder, determined their changes of pH value, clotting time and gel strength

Figure 9 showed the gelling status of TGase, thrombin and fibrinogen admixed with different volume ratios. All of nine combination ratios of those three elements might form a gel. But the larger gel strength exited in the more fibrinogen ratio. Their pH value slightly changed, while stored at 4°C for 5 weeks, were shown in Figure 10. They had the same trend of a little rising of pH along with storage time increased. However, the pH difference between individual TGase, thrombin and fibrinogen (Figure 7) and

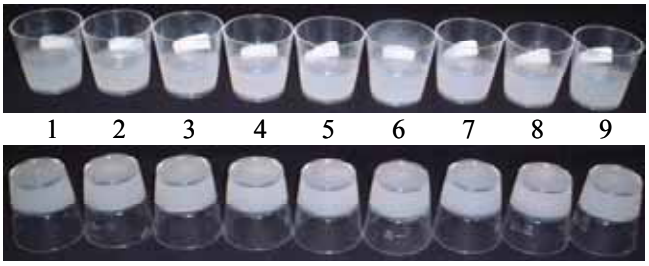

Figure 9. Gel status of a binder prepared by transglutaminase, thrombin and fibrinogen with various ratio during storage at 4°C for 5 weeks. TGase:thrombin:fibrinogen (V/V/V). (1) 0.5:1:15, (2) 0.5:1:20, (3) 0.5:1:25, (4) 1:1:15, (5) 1:1:20, (6) 1:1:25, (7) 1.5:1:15, (8) 1.5:1:20 and (9) 1.5:1:25.

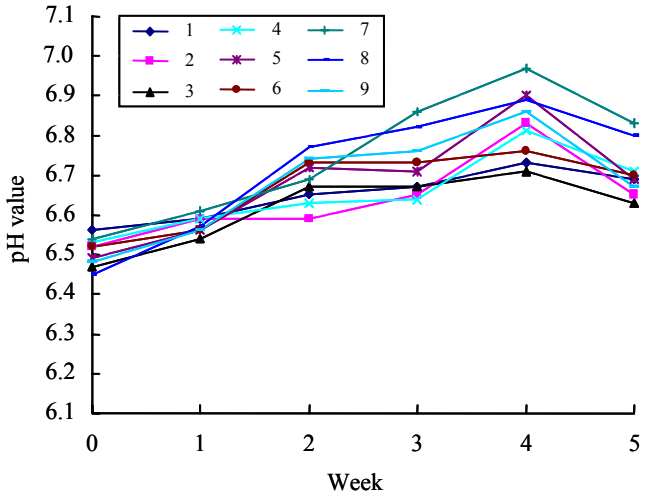

Figure 10. Changes in pH value of a binder prepared by transglutaminase, thrombin and fibrinogen with various ratio during storage at 4°C for 5 weeks. TGase:thrombin:fibrinogen (V/V/V). (1) 0.5:1:15, (2) 0.5:1:20, (3) 0.5:1:25, (4) 1:1:15, (5) 1:1:20, (6) 1:1:25, (7) 1.5:1:15, (8) 1.5:1:20 and (9) 1.5:1:25.

above three combined for preparing a binder (Figure 7) were owing to different concentration of TGase, thrombin and fibrinogen, therefore, its pH value were alsso not the same after admixture.

The change of clotting time of admixture of TGase, thrombin and fibrinogen with differenct volume ratios, which were individually stored at 4°C, combined at every week till fivth week, is shown in Figure 11. As a result, each treatment had a same tendency that the clotting time increased as storage time increased ($p<0.05$). It might be due to the enzyme activity of TGase and thrombin were decreased with storage time increased ($p<0.05$) (Figures 5 and 6) The gel strength of different combination of TGase, thromb in and fibrinogen stored at 4°C for 5 weeks (Figure 12), as a result showed that treatment 1, 4 and 7, treatment 2, 5 and 8, treatment 3, 6 and 9 were similar tendency. The higher addtion of fibrinogen, the stronger gel strength ($p<0.05$). Kuraishi et al. (1997) pointed out that gel strength

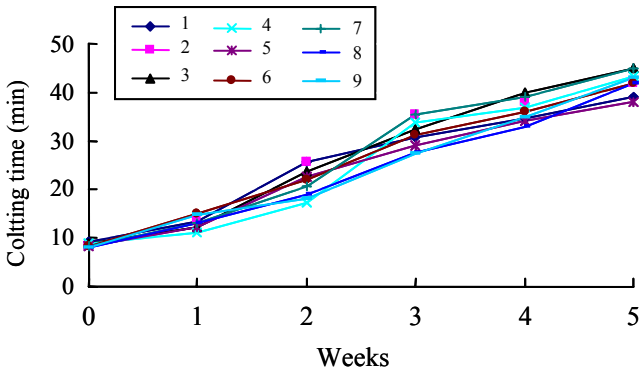

Figure 11. Changes in clotting time of a binder prepared by transglutaminase, thrombin and fibrinogen with various ratio during storage at 4°C for 5 weeks. TGase:thrombin:fibrinogen (V/V/V). (1) 0.5:1:15, (2) 0.5:1:20, (3) 0.5:1:25, (4) 1:1:15, (5) 1:1:20, (6) 1:1:25, (7) 1.5:1:15, (8) 1.5:1:20 and (9) 1.5:1:25.

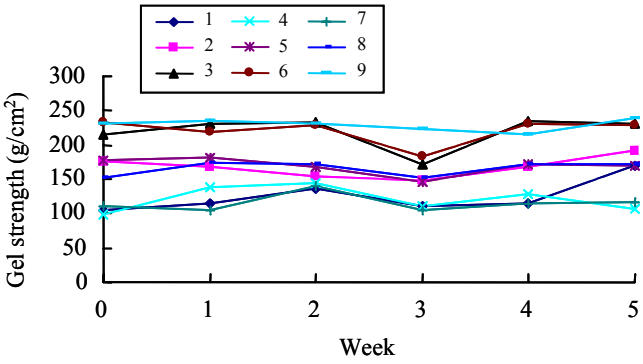

Figure 12. Changes in gel strength of a binder prepared by transglutaminase, thrombin and fibrinogen with various ratio during storage at 4°C for 5 weeks. TGase: thrombin:fibrinogen (V/V/V). (1) 0.5:1:15, (2) 0.5:1:20, (3) 0.5:1:25, (4) 1:1:15, (5) 1:1:20, (6) 1:1:25, (7) 1.5:1:15, (8) 1.5:1:20 and (9) 1.5:1:25.

96 g/cm^2 could aid to enhance the binding of restructured meats. Based our study, the nine combination of different volume ratios of TGase, thrombin and fibrinogen solutions could be useful in the improvement the binding of restructured meat.

CONCLUSION

The total plate count of thrombin had significantly higher ($p<0.05$), the higher pH value was then with TGase ($p<0.05$). In SDS-PAGE aspect, the extracted TGase, thrombin and fibrinogen of this study, had all same protein bands with its commercial product (Sigma), and nine combinations of blending binder, those protein components havd no obvious difference. The enzyme activity of TGase, thrombin decreased as storage time increased. The total plate count and clotting time of TGase, thrombin and fibrinogen, all increased along with storage time increased. On gel strength aspect, treatment 1, 4 and 7

(TGase:thrombin:fibrinogen = 0.5, 1 and 1.5:1:15), treatment 2, 5 and 8 (TGase:thrombin: fibrinogen = 0.5, 1 and 1.5:1:20), and treatment 3, 6 and 9 (TGase:thrombin: fibrinogen = 0.5, 1 and 1.5:1:25) all present a same trend, such as the higher addition of fibrinogen, the gel strength was also stronger.

REFERENCE

Anbe, H. 1961. Japanese Handbook of hematology. (6-II) pp. 788-791. Japan.

Ando, H., M. Ahachi, K. Umeda, A. Matsuura, M. Nonaka, R. Uchio, H. Tanaka and M. Motoki. 1989. Purification and characteristics of a novel transglutaminase derived from microorganisms. Agric. Biol. Chem. 53:2613-2617.

Binnie, C. G. and S. T. Lord. 1993. The fibrinogen sequences that interact with thrombin. Blood 81:3186-3192.

Chen, J. S. K. and K. Mehta. 1999. Tissue transglutaminase: an enzyme with a split personality. Int. J. Biochem. Cell Biol. 31:817-836.

Chung, S. I., M. S. Lewis and J. E. Folk. 1974. Relationships of the catalytic properties of human plasma and platelet transglutaminase (activates blood coagulation factor XIII) to their subunit structures. J. Biol. Chem. 249:940-950.

Divakavan, S. 1982. Animal blood processing and utilization. Central Leather Research Institute Madras India.

Fargemand, M., J. Otte and K. B. Qvist. 1998. Emulsifying properties of milk prtoeins cross-linked with microbial transglutaminase. Int. Dairy J. 8:715-723.

Folk, J. E. 1970. Transglutaminase. In "Method in Enzymology", (Ed. H. Tabor and C. W. Tabor), 17:889-894. Acadenic Press, New York.

Folk, J. E. 1980. Transglutaminase. Ann. Rev. Biochem. 49:517-531.

Gornall, A. G., C. J. Bardwill and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177:751-766.

Greenberg, C. S. and M. A. Shuman. 1982. The zymogen forms of blood coagulation factor XIII bind specifically to fibrinogen. J. Biol. Chem. 257:6069-6101.

Harding, H. W. J. and G. E. Rogers. 1972. Formation of the ε- (γ-glutamyl) lysine cross- link in hair proteins. Investigation of transamidases in hair follicles. Biochemistry 11:2858-2862.

Herrick, S., O. Blanc-Brude, A. Gray and G. Laurent. 1999. Fibrinogen. Int. J. Biochem. Cell Biol. 31:741-746.

Huang, C. C. and T. Y. Wang. 1997. Effect of fresh and frozen chicken meat on the quality of restructured chicken steaks. J. Chin. Soc. Anim. Sci. 26:347-358.

Huang, H. J. 1987. Studies on the tested preparation of hemostat from porcine blood and its hemostating effect. Master Thesis. National Chung-Hsing University, Taichung, Taiwan.

Huang, H. J., D. C. Liu, H. L. Guo and M. T. Chen. 1992. Preparation of hemostat from porcine blood-study on properties of fibrinogen and thrombin. J. Chin. Soc. Anim. Sci. 21(2):203-211.

Imm, T. Y., P. Lian and C. M. Lee. 2000. Gelation and water binding properties of transglutaminase-treated skim milk powder. J. Food Sci. 65:200-205.

Kang, H. and Y. D. Cho. 1996. Purification and properties of transglutaminase from soybean (Glycine max) leaves. Biochem. Biophys. Res. Commun. 223:288-292.

Kuraishi, C., J. Sakamoto, K. Yamazaki, Y. Susa, C. Kuhara and T. Soeda. 1997. Production of restructured meat using microbial transglutaminase without salt or cooking. J. Food Sci. 62:488-490,515.

Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature. 227:681.

Lee, C. C. 1991. Enzymatic modification on fish proteins to improve the gelation by transglutaminase. Master Thesis. National Ocean University, Keelung, Taiwan.

Lin, S. M. 1995. Isolation, purification and characterization of microbial transglutaminase and its application in surimi products. Master Thesis. National Ocean University. Keelung, Taiwan.

Liu, H. H. 1999. Study on separation of transglutaminase from blood and its application in preparation of meat products. Master Thesis. Tunghai University, Taichung, Taiwan.

Machovich, R. 1986. Choices among the possible reaction routes catalyzed by thrombin. Ann. N. Y. Acda. Sci. 485:170-183.

Motoki, M. and K. Seguro. 1994. Trends in Japanese soy protein research. Inform. 5:308-313.

Ninomi, R., S. Saido and U. Inada. 1984. Basal study on effective utilization of animal blood. In: Result report of supporting study on meat. pp. 296-301. Ido memorial financial group. Japan.

Nonaka, M., S. Toiguchi, H. Sakamoto, H. Kawajiri, T. Soeda and M. Motoki. 1994. Changes caused by microbial transglutaminase on physical properties of thermally induced soy protein gels. Food Hydrocolloids 8:1-8.

SAS. 1996. SAS User's Guide: Statistics. Cary, NC. SAS Institute.

Schwartz, M. L., S. V. Pizzo, R. L. Hill and P. A. Mckee. 1973. Human factor XIII from plasma and platelets. J. Biol. Chem. 248:1395-1407.

Tsai, M. H. 1987. Blood transfusion scopy. In: Clinical hematology. pp. 111-177. Yi Hsien Publishing Co.

Tseng, T. F. 1999. Purification and characteristics of transglutaminase and its application. Dissertation. National Chung-Hsing University. Taichung. Taiwan.

Tseng, T. F., D. C. Liu and M. T. Chen. 1999. Characteristics of transglutaminase derived from pig plasma. J. Agric. Assoc. China. 1(4):452-458.

Wijngaards, G. and E. J. C. Paardekooper. 1987. Preparation of a composite meat product by means of an enzymatically formed protein gel. In (Ed. P. S. Van Roon and J. H. Houben) Trends in Modern Meat Technology 2, Proceeding of the International Symposium, Den Dolder, Netherlands, 23-25 November, pp. 125-129, Wageningen: Pudoc.

Yeh, Y. W. 2000. Purification, biochemical character and application of transglutaminase from *Streptoverticllium kentuckense* CCRC 12429. Master Thesis. National Chung-Hsing University. Taichung. Taiwan.