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Porcine Blood Plasma Transgluataminase Combined with Thrombin and Fibrinogen as a Binder in Restructured Meat

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ABSTRACT : The purpose of this study was to use pig blood plasma transglutaminase (TGase) combined with thrombin and fibrinogen as a binder, which was applied to restructured meat, and to investigate its effect on the restructured meat quality. Pig meat was obtained 10 h post mortem from a traditional market was ground using a 10 mm aperture plate. A binder admixture was added (TGase:thrombin:fibrinogen mixed as 0.5:1:20 (v/v/v) to which was added 12% of its volume of 0.25 M calcium chloride) at 0, 5, 10, 15 and 20% of meat weight. Measurements included cooking loss, shrinkage rate, shear value, total plate count, pH value, TBA value, color difference, tension strength and sensory evaluation. The results showed that ground meat containing 20% w/w of binder admixture had higher cooking loss, shrinkage rate and shear value ($p<0.05$). Addition of different percentages of binder admixture did not affect total plate count, pH value, TBA value, and sensory evaluation of restructured meat ($p>0.05$). Tension strength was increased with increased level of binder admixture. Addition up to 15% binder admixture to restructured meat showed better scores of sensory texture, flavor and total acceptability ($p<0.05$). (**Key Words :** Porcine Plasma, Transglutaminase, Thrombin, Fibrinogen, Binder, Restructured Meat)

INTRODUCTION

The development of restructured meats, which grew out of a demand for better use of secondary carcass parts to produce middle meat steaks and chops, is progressing (Secrist, 1987; Huang and Lin, 1996). Advantages of restructured meats, in terms of controlled serving size, reduced cooking shrinkage, and better cost accounting, make them an attractive alternative to intact steaks, chops, and roasts, especially for military and institutional food services (Secrist, 1987). Consumers can buy high-value products made from restructured meats, but with a reasonable lower price (Huang and Lin, 1996; Wu, 2002). The restructuring process makes it possible to create various new products for different markets. In addition, restructured meat can be formed into dice, sticks or nuggets, of practically any shape and size (Wu, 2002). Reports have indicated that adding salt and phosphates had a marked influence on the water-holding capacity of restructured meat (Trout and Schmidt, 1987), and also on its shear force, raw and cooked texture, and juiciness (Wu, 2002).

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It is recommended that restructured meat should be kept at or near freezing point when it is being processed. Experience at the Army Natick Laboratories indicates that meat temperatures in the range of -4°C to 4°C are optimal (ABMPS, 1981).

The best-characterized part of the blood clotting process is the conversion of fibrinogen into fibrin monomer by thrombin. Fibrin monomers spontaneously assemble into an ordered array called fibrin. The clot produced by the spontaneous aggregation of fibrin monomer is stabilized by the formation of covalent cross-links, catalyzed by transglutaminase (Factor XIIIa), between the side chains of different molecules in the fibrin fiber (Stryer, 1988). By adding transglutaminase, bonding will be formed between the muscle protein side chains. This increases the gelling properties of muscle protein (Tseng et al., 1999; Wu, 2002). Through frozen reforming, the restructured meats also can be protected from discoloration and oxidation rancidity problems due to addition of nitrites, ascorbates and antioxidants (Secrist, 1987; Wu, 2002). A previous study compared binders containing porcine blood transglutaminase, thrombin and fibrinogen in different volumetric proportions and showed that the higher the amount of fibrinogen in these combinations, the stronger the gel strength (Tsai et al., 2006). The present study attempted to combine transglutaminase, thrombin and

fibrinogen as a binder to investigate the feasibility of their application in restructured meat.

MATERIAL AND METHODS

Restructured meat treatment

The pork meat was obtained at 10 h post-mortem from a carcass purchased from a local market, ground using a 10 mm plate, and mixed with binder solution of TGase:thrombin: fibrinogen (TTF 0.5:1:20 v/v/v) at 0, 5, 10, 15 and 20% of meat weight. To every TTF preparation was added 12% v/v of 0.25 M calcium chloride and it was then added into the minced meat samples. Each binder solution had 5 replicates. After thoroughly blending, meat mixture was stuffed into a fibrous casing, chilled at 4°C for 24 h, and then frozen in a -40°C freezer to reach an internal temperature of approximately -2°C to -5°C. The restructured meat samples were sliced to a 10 mm thickness and then they were roasted at 190°C by electrical oven until the internal temperature reached 71°C. The roasted samples were analyzed for cooking loss, shrinkage rate, shear value, total plate count, pH value, 2-thiobarbituric acid value, color, tension strength and sensory evaluation.

Analytical methods

The cooking loss and shrinkage rate was determined by the method of Huang and Wang (1997) :

$$\text{Cooking loss (\%)} = \frac{\text{Before cooking weight - After cooking weight}}{\text{Before cooking weight}} \times 100$$

$$\text{Shrinkage rate (\%)} = \frac{\text{Area before cooking - Area after cooking}}{\text{Area before cooking}} \times 100$$

Total plate count : According to Huang and Wang (1997), a 10 g ground sample was put into a sterilized bottle, sterilized-distilled water (90 ml) added, shaken well (Funnel Shaker, Shin Kuang, Taipei, Taiwan) at 150 rpm for 2 min, and then the remaining procedures such as diluting, plating, incubation, counting colonies and recording were followed according to Gilliland et al. (1976). The plate count agar (Difco™, Becton, Dickinson and Company, Sparks, MD 21152, USA) was chosen, its colony-forming unit (log CFU/g) was recorded and the mean of 5 replicates was calculated.

pH value : To 10 g of meat sample was added 100 ml distilled water, the mixture homogenized (Homogenizer PH91, SMT, Japan) at 10,000 rpm for 2 minutes, and pH meter electrodes (pH meter SP-701, Suntex, Taiwan) inserted to measure the pH value (Ockerman, 1972) and the mean calculated for 5 replicates.

TBARS (Thiobarbituric acid reactive substances value) : The method of Faustman et al. (1992) was followed to assay the oxidation of fatty acids. A spectrophotometer (Metertek SP-830, Metertek Inc., Taiwan) was used to measure the absorbance at wavelength 532 nm, and the mean of 5 replicates was calculated.

Hunter L, a, b-value : Color difference measurement of restructured meat samples was done by a color difference apparatus (Handy Colorimeter NR-3000, Nippon Denshoku, Tokyo, Japan), calibrated with the standard whiteboard (D65/10, X = 81.67, Y = 86.32, Z = 89.12), to measure its L. a. b-value, and the mean of 5 replicates was calculated.

Shear value : Following the Huang and Wang (1997) method, the restructured meat samples were cooled for 15 minutes after baking, cut into 1 cm^3 meat cubes and mounted on a load table of a rheometer (Fudoh Rheometer DRT-2002, Rheotech, Japan) to determine the shear value. Measuring conditions were as follows : Power 2 kg, adapter No. 31, carrying speed 30 cm/min, and one-way return number. The power that was needed to break down the meat cube, was indicated as kg/cm^2, and the mean of 5 replicates was calculated.

Tensile strength : Restructured meat samples were prepared as above and tension strength measured with a rheometer (Fudoh Rheometer DRT-2002, Rheotech, Japan). Measured condition as follows : The distance between top and bottom clip 10 mm, Power (force) 2 kg, adapter No. 17, carrying speed 30 cm/min, and one way return number. The power that pulls the meat cube to break down, was indicated as tensile strength g/cm^2, and then a mean was calculated for 5 replicates.

Sensory evaluation : Following Carmack et al. (1995), the intact restructured meat samples were roasted, cut into $2\times2\times1$ cm^3 cubes and then served to 10 panelists for the sensory evaluation of color, appearance, texture, taste, juiciness and overall acceptance. The 7 Helladic points were used.

Statistical analysis

Statistical analysis was performed with SAS (Statistical Analysis System, 1996), using the general linear model procedure and Duncan's multiple range test to compare the significance of differences between treatments.

RESULTS AND DISCUSSION

The various amounts of binder had effects on cooking loss, shrinkage rate, shear value, pH value, TBA value, color difference, total plate count and tensile strength of restructured meat

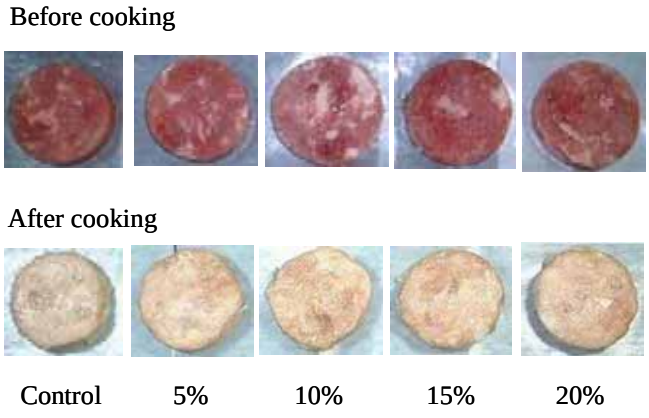

Figure 1. Changes in appearance of restructured pork prepared with addition of various amount of the binder before and after cooking.

The appearance of fresh and cooked restructured meat, made with different percentages of binder addition to meat by weight, is shown in Figure 1. The color and shape of restructured meat slices were acceptable, when they were prepared (before cooking). But after cooking, they turned into a gray color and had shrunk in shape. The cooking loss, which was affected by the level of addition of binding solution, is shown in Table 1. The results showed that the highest cooking loss, shrinkage rate and shear value existed in those meats containing 20% addition of binder; this might be caused by higher moisture content resulting in more drip loss during the cooking period, and so more weight loss of these restructured meats. As shown in Table 1, no differences in pH and TBARS were observed between treatments.

From the color aspect, shown in Table 2, L-values were increased as the percentage of meat binder solution increased. In contrast, a- values were decreased with the additional percentage. This might be due to the higher moisture content of meat with higher percentage of binder solution resulting in higher L value and lesser a value.

Table 2. Effect of various amount of binder on the color of restructured pork*

Color difference	Treatments					SEM**
	0%	5%	10%	15%	20%	
L	24.28^{e}	25.32^{d}	25.53^{c}	26.12^{b}	27.52^{a}	0.03
a	8.36^{a}	8.12^{b}	7.69^{c}	7.23^{d}	7.13^{e}	0.01
b	6.29^{e}	6.34^{d}	6.43^{c}	6.56^{b}	6.75^{a}	0.01

* Means within the same row without the same superscript are significantly different ($p<0.05$).
** SEM: standard error of means.

The effect of adding various percentages of binder on the total plate count and tensile strength of restructured meat is shown in Table 3. Different binder percentage did not influence the total plate count. However, the tensile strength for all restructured meat groups containing 5-20% binder solution were significantly higher than the control ($p<0.05$), suggesting binder solution with thrombin, fibrinogen and TGase gave better tensile strength to restructured meat. The porcine plasma TGase, used in this study, was a calcium-depending enzyme and should be accompanied by adding calcium ion in the meat processing procedure to initiate this enzyme. Nielsen et al. (1995) reported that phosphate together with calcium formed insoluble phosphate when used in a meat piece model; this will make calcium unavailable for F XIIIa and hence decrease the activity of the enzyme. Ockerman and Hansen (1988) also pointed out that adding calcium chloride to meat would reduce myoglobin.

Liu (1999) pointed out that porcine plasma TGase crude extract powder added to reformed pork chops could improve their hardness, coagulation, elastic and stretching strength. Nielsen et al. (1995) pointed that adding 0.4% F XIIIa, 0.2% phosphate and 1% salts to reformed meat and storage at 37°C for 90 mins could increase its tensile

Table 1. Effect of various amount of binder on the cooking loss, shrinkage rate, shears value, pH value and TBA value of restructured pork*

Item	Treatments					SEM**
	0%	5%	10%	15%	20%	
Cooking loss (%)	21.9^{c}	27.8^{b}	27^{b}	26.9^{b}	29.5^{a}	0.174
Shrinkage rate (%)	19.26^{e}	21.06^{d}	22.8^{c}	28.06^{b}	29.82^{a}	0.065
Shear value (kg/cm^2)	1.34^{b}	1.42^{b}	1.45^{b}	1.49^{b}	1.72^{a}	0.012
pH value	5.83	5.74	5.85	5.86	5.83	0.008
TBA value (mg/kg)	0.31	0.34	0.33	0.31	0.27	0.006

* Means within the same row without the same superscript are significantly different ($p<0.05$).
** SEM: standard error of means.

Table 3. Effect of various amount of binder on the total plate count and tension strength of restructured meat*

Item	Treatments					SEM**
	0%	5%	10%	15%	20%	
Total plate count (log CFU/g)	4.08	4.17	4.22	4.04	4.15	0.06
Tensile strength (g/cm^2)	60^{e}	100^{d}	130^{c}	230^{b}	300^{a}	6

* Means within the same row without the same superscript are significantly different ($p<0.05$).
** SEM: Standard error of means.

Table 4. Effects of various amount of binder on the sensory evaluation of restructured meat*

Item	Treatments					SEM**
	0%	5%	10%	15%	20%	
Appearance	4.67	5.00	5.67	5.33	5.33	0.23
Color	4.33	4.67	4.00	4.33	4.33	0.23
Texture	4.00^{c}	4.67^{bc}	5.33^{ab}	6.33^{a}	6.33^{a}	0.31
Flavor	4.00^{c}	5.00^{bc}	5.33^{b}	6.33^{a}	6.33^{a}	0.28
Juiciness	4.33	4.33	4.33	5.00	5.33	0.42
Total acceptability	4.20^{c}	4.67^{bc}	5.00^{b}	6.00^{a}	6.00^{a}	0.23

Seven point scale with 7 = like extremely and 1 = dislike extremely.

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

** SEM: Standard error of means.

strength, however, color deterioration of the product was observed when adding F XIIIa.

The addition of commercial F XIIIa could effectively reduce the dosage of salt and phosphate, and increase the tensile strength of restructured meat. Kuraishi et al. (1997) reported that adding 0.05-0.1% microbial TGase and 0.5-1.0% sodium caseinate to restructured meat for reacting at 5°C for 2-5 h, made an excellent restructured meat with adequate binding without salt and heating.

Tseng et al. (1999) pointed out that low-salt restructured meat with TGase addition had greater binding strength than the control ($p<0.05$), and showed regularity of the microstructure gel matrix with scanning electron microscopy (SEM). The gel strength of low salt chicken meat-ball increased with incremental added TGase. Its microstructure had a firmer and more regular gel structure than the control (Tseng et al., 2000).

Liu (1999) pointed out that crude enzyme liquid of transglutaminase added to a low-salt pork ball could increase its flexibility. Addition of 1% sodium caseinate and crude enzyme powder, to a cold plate type of gelling meat product increased flexibility and anti-breaking strength, and could decrease the use of animal derived gelatin and reduce its poor flavor.

Kilic (2003) pointed out that SDS-PAGE showed that addition of MTGase with or without sodium caseinate, created cross-linking between meat proteins. Texture measurements indicated that the effect of the enzyme on binding properties of chicken meat is more effective if it is used with sodium caseinate.

Seguro et al. (1995) pointed out that adding 0.03% MTGase to kamaboko gels from Alaska Pollock surimi could increase gel properties and breaking strength. Tsukamasa et al. (1993) reported that the ε-(γ-glutamyl) lysine content would be raised with the MTGase concentration, and the gel breaking strength would increase with ε-(γ-glutamyl) lysine content, therefore the product quality was improved.

Liu (1999) pointed out that the crude enzyme powder of pig blood plasma TGase added to a simulated fish kamaboko product could increase the breaking strength, hardness, coagulation and flexibility of the product. Yeh (2000) pointed out that *Streptoverticillium kentuckense* CCRC 12429 TGase affected the rheological change of chicken breast meat colloid acting under 37°C for 60 mins, while 0.5 and 1% crude TGase extract powder or 0.05, 0.1 and 0.3% of further purified TGase powder could all effectively improve gel strength, breaking strength and hardness of chicken breast meat colloid.

Effects of various amounts of binder on sensory evaluation

The result of panel tests of restructured meats is shown in Table 4. There were no significant difference in the appearance, color and juiciness of each treatment. However, the panel scores for texture, flavor, juiciness and total acceptability for those treatments admixed with 15% and 20% binder mixture were significantly higher ($p<0.05$).

CONCLUSION

Addition of different percentages of binder admixture did not affect total plate count, pH value, TBA value, and sensory evaluation of restructured meat significantly ($p>0.05$). Tension strength increased as the percentage of binder admixture increased. The sensory evaluation of texture, flavor and total acceptability showed that 15 and 20% binder admixture treatments were better ($p<0.05$). But when 20% binder admixture in restructured meat showed higher cooking loss, shrinkage rate and shear value. In summary, porcine blood plasma transglutaminase combined with thrombin and fibrinogen as a binder could be used in restructured meat, their admixture ratio (T:T:F as 0.5:1:20 v/v/v) and addition up to 15% of raw meat by weight could provide a better result in binding strength of restructured meat.

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