Utilization of Transglutaminase for the Development of Low-fat, Low-salt Sausages and Restructured Meat Products Manufactured with Pork Hams and Loins

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ABSTRACT : This study was performed to determine whether transglutaminase (Aciva-TG, TGase) can be used to reduce the salt level in low-fat sausages and to replace emulsified meats (10%) for the manufacture of restructured meat products (RMP). Pork hams and loins were collected from a local retail market in Gwangju, Korea and used for the manufacture of sausages and RMPs, respectively. TGase at the level of 0.1% can permit the reduction of the salt level from 1.5% to 1.0% in low-fat comminuted sausages without any quality defects, however a crumbly texture was found if the salt level was reduced below 1.0% even though it combined with certain amounts of TGase. No differences in chemical composition and physical properties were observed ($p>0.05$) among treatments. Approximately 0.3% of TGase can replace 10% emulsified meats, which are normally used for improvement of binding capacity to manufacture RMPs, without quality defects. This study suggests that TGase could be used for the manufacture of low-fat, low-salt functional meat products for the improvement of textural characteristics and binding capacity without adverse effects. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 2 : 261-265)***

Key Words : Transglutaminase, Low-fat, Low-salt Meat Products, Functional, Textural Properties

INTRODUCTION

Currently consumers need for high quality, leaner meats and meat products for their “healthy concern”. Although dietary fats play an important role in the metabolism of living organism, the excess fat and salt level in diets are highly correlated with higher chances of high blood pressure and coronary heart disease. Thus, reduced salt and fat in diets are recommended. A lot of research projects have been performed to develop a variety of ingredients, enzymes and new technologies to manufacture low-fat functional meat products for the satisfaction of consumer demand (Keeton, 1991, 1994; Chin et al., 1998a,b, 1999, 2000).

Transglutaminase (TGase: protein-glutamine ν-glutamyl transferase) catalyzes the reaction of ε-(ν-gltamyl) lysyl crosslinks among food proteins (Kumazawa et al., 1993). It improves the textural properties of certain foods, especially in meat products. Motoki and Seguro (1998) reported that the TGase could be used in several foods which require gelation for food texture. Kuraishi et al. (1997) reported that non-meat proteins functioned as substrates for TGase to have a similar binding capacity to those with salt, and sodium caseinate was the best substrate for the crosslinking to meat proteins among other non-meat proteins. In addition, TGase can be made the non-thermal gelation in processed meat products without cooking (Tony, 2000). This technology could be effective for not only the manufacture of reduced-salt and low-fat sausages, but also restructured meat products (RMPs) without addition of emulsified-meats, which are normally used for the manufacture of RMPs for the improvement of binding capacity. To have a similar binding capacity to those with emulsified-meats, TGase could bind with meat pieces or other non-meat ingredients as substrates. However, no research has been performed on this topic. Thus, this study was performed to investigate the effect of TGase on the partial substitution of salt in low-fat comminuted sausages and replacement of emulsified-meats in RMPs.

MATERIALS AND METHODS

Experiment 1: Processing and evaluation of low-fat, low-salt sausages

Pork hams were collected from a retail meat market (Hyundai Meat Retail Market, Gwangju, Korea) and trimmed of excess fat and connective tissue. They were ground through a 1.25 cm plate and then reground through a 0.32 cm plate. Then the ground meats were analyzed for proximate analysis and pH values according to AOAC (1995, Table 1) and frozen until used. Processing procedures for low-fat sausages in a model system were followed those of Chin et al. (1998a) with some modifications. Frozen pork hams, which were thawed in a refrigerator (4±1°C) for a day, were chopped for 30 s in a

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Table 1. pH, chemical composition and microbial counts of raw meats

Items	pH	Moisture fat protein (%)			Microorganisms (CFU/g)	
					Total bacteria	*Enterobacteriaceae*
Mean	5.92	78.0	3.99	20.7	3.9×10^4	$<10^2$
SD	0.19	1.55	0.30	0.85	-	-

commercial food processor (Crypto Peerless Ltd., EF-20, England). Salt, sodium nitrite, sodium erythorbate, and half of the ice water were blended for 2 min to extract salt soluble proteins. Flavorants, seasonings and the remaining ice water were added and mixed for 1 or 2 min at high speed until the batter reached 15°C. The ingredients used for the processing are listed in Table 2. All cooked sausages were vacuumed and stuffed into polyvinyldiene chloride (PVDC, 7325B, 40 micron gauge, 46 mm, Japan) for water cooking. The stuffed sausage batters were then transferred to a refrigerator for enzyme reaction for 2 h, and put into a water bath (75°C) and heated to an internal temperature of 71.7°C as assessed by a thermometer (Fluke 52, Everett, USA) equipped with thermocouple probe positioned in the geometric center of the sausage samples. After cooking, sausage samples were immediately chilled in an ice water and stored in the commercial refrigerator until analyzed. The total amount of sausage batter produced for each treatment was 2 kg.

Experiment 2: Processing and evaluation of restructured meat products (RMP)

The pork loins were cut into approximately 1-2 cm^3 cubes and put into the mixer with a brine solution (Table 3) for about 30 min. Then, the mixture was placed into a vacuum tumbler (Biro, Model VTS-41, Marblehead, USA) for 3-4 h (1 h intervals) until the brine solution was completely distributed into the meats. Then, they were stuffed into fibrous casings (dia, 7 cm), left to stand for 2 h for the enzyme reaction, and then cooked to an internal temperature of 71.7°C in a smoke chamber (Nu-Vu, ES-13, Food System, USA) according to the cooking procedures for typical RMPs (Table 4).

Table 2. The formulation of low-fat and low salt sausage

Items	Control[1]	Treatments		
		T1	T2	T3
Pork ham (lean)	55.0	55.0	55.0	55.0
Water	35.0	35.5	36.0	36.5
Non meat ingredients[2]	6.0	7.0	6.5	6.0
Fat replacer[3]	2.5	2.5	2.5	2.5
Salt	1.5	1.0	0.5	0.0
Activa TG-S	0	0.1	0.2	0.3

[1] Control=1.5% salt; T1=1.0% salt+0.1% TG; T2=0.5% salt+0.2% TG; T3=no salt+0.3% TG.

[2] Non-meat ingredients=sodium tripolyphosphate (STPP, 0.4%), sodium nitrite (0.02%), sodium ascorbate (0.08%), sodium caseinate (1.0%), sugar (2%), spices (1%), non-fat dry milk (1.0%), Hydrolyzed milk protein (0.5%).

[3] Fat replacer=konjac flour: carrageenan:soy protein isolate=1:1:3.

Table 3. The formation of restructured ham manufactured with either TGase or 10% emulsified meats

Items	Composition	CTL	TG treatment
Meat	Lean trimmings	1,350	1,500
	Emulsified meats	150	0
Brine soln		300	300
	Water	250	250
	Salt	39	39
	Sugar	22.5	22.5
	Sodium tripolyphosphate	6.3	6.3
	Sodium erythorbate	1.8	1.8
	Prague powder	5.7	5.7
	Activa-TG	0	4.5

Analyses performed

pH and proximate analysis : Cooked sausage and RMP samples (10 g of each) were mixed with 90 mL of double distilled (dd)-water and pH values were measured using a pH meter (Mettler Toledo, 340 Schwarzenbach, Switzerland). Moisture, fat and protein percentages (%) of the cooked sausages and RMPs were determined using dry-oven method, soxhlet fat extraction and protein determination (BUCHI, Kjeltec Auto System, B-322, Switzerland), respectively, followed by AOAC (1995).

Physico-chemical properties : Cooking losses (CL, %) for sausages and RMPs were measured by weight differences before and after cooking at 75°C for 30 min. Waterholding capacity was measured as expressible moisture (EM, %) after centrifugation at 800×g for 15 min with 1.5 g of cooked sausages or RMPs wrapped with three pieces of Whatman #3 (11 cm dia) filter paper, according to a modified method of Jauregui et al. (1981). Hunter color values were measured by a Minolta color reader (Model CR-10, Minolta Co, LTD., Japan) and expressed as hunter L (brightness), a (redness) and b (yellowness) values.

Textural properties : Textural properties were evaluated using a Texture meter (TA-XT2, Stable Micro System, Hasemere, England) for sausages and RMP samples as

Table 4. Smoking and cooking conditions for restructured meat products

Steps	Time (min)	Temperature (°C)	Relative humidity (%)	Smoking
Reddning	30	50	100	off
Drying	30	50	0	off
Smoking	30	55	0	on
Cooking 1	30	68	40	off
Cooking 2	30	77	60	off
Cooking 3*	60	82	100	off
Cooling with cold shower				

* The final cooking procedure continues until products reach an internal temperature of 71.7°C

described in Bourne (1978). The samples were prepared to give cores with 13 mm height and 10 mm diameter, and compressed to 75% of their original height in order to measure hardness, springiness, cohesiveness, chewiness and gumminess.

Microbiological analysis : For sausage and RMP samples determinations were made of the total plate counts (TPC) and of coliform bacteria using plate count agar (Difco, USA) and violet red bile agar (VRB, Difco, USA), respectively. A 10 g of sample was blended with 90 mL of sterilized dd-water and diluted several times and incubated at 37°C for 48 h. Results were expressed with $\log_{10}$ No of colony forming units (CFU) per gram of cooked sausage and RMP.

Statistical analysis

The two experiments were conducted in triplicates and mean values of triplicate determination of each analytical measurement were compared among treatments in sausage products or between a control and a TG treatment in RMPs. Data were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedures of SAS (1989). Three TGase levels (0.1-0.3) with reduced salt level from 1.5 to 0.5% in low-fat sausages were the main effects that were compared with the control (without a TGase). In the 2nd experiment, RMPs with 0.3% TG were compared with samples with the 10% emulsified-meats as a control. If the main effects were significant ($p<0.05$), data were analyzed using Student-Newman-Kuels (SNK) procedure for multiple comparisons.

RESULTS AND DISCUSSION

Low-fat sausage characteristics

The pH, proximate analysis and water holding capacity (WHC) values of low-fat sausages are listed in Table 5. As we expected, increased level of TGase with reduced salt content didn't affect ($p>0.05$) pH values, chemical composition and water holding capacity values. pH values of all sausages groups ranged from 6.14 to 6.26 and they had 77-79% moisture, <3% fat and 13-14% protein without changes of WHC. Sakamoto et al. (1994) reported that the optimum temperature and pH for the reaction of TGase as substrates for the soy protein and sodium caseinate was 50°C and 9.0, respectively. Soy protein isolates as well as meat proteins might be substrates for TGase, however, the optimum pH range was not suitable for meat products due to the lower pH range of 6.0-6.5 in most meat products. The chemical composition of low-fat sausage in this study was similar to that in a previous study (Chin and Choi, 2001). However, these results differ from other reports such as, Moon et al. (1996) and Bloukas et al. (1997), who reported that the fat contents of their products were higher than ours. The moisture: total protein (M:P) ratio in this study was approximately 5.5-5.6 and these M:P values have been reported the better formulation to have a similar textural characteristics to those of regular-fat counterparts (Chin et al., 1998b).

No differences in hunter color values were observed ($p>0.05$) as varied levels of TGase (data not shown). Although no differences were observed in hardness and cohesiveness (Table 6), low-fat sausages containing less than 1% salt had lower springiness ($p<0.05$) than those with higher than 1% salt, resulting in a crumbly texture (Table 6). This result indicated that the low-fat sausages having lower than 1% salt may have problems related to the textural characteristics, even though certain amount of TGase was added to the formulation. Thus, the TGase could not be substituted with salt completely, but salt can be reduced by 0.5% if it is replaced with 0.1% TGase. All treatments had microbial counts lower than 10^2 cfu/g due to the heat treatment (71.7°C).

Characteristics of restructured meat products (RMP)

The pH, chemical composition, physico-chemical and textural characteristics of low-fat restructured meat products (RMP) manufactured with TGase were measured and compared to the control (Table 7). Generally, emulsified meats (10%) are added to the formulation of RMPs to improve the binding capacity, but this requires extra inputs of time and labor. Thus, this study was performed to determine if TGase could replace the emulsified meats.

pH and chemical composition didn't differ between the treatment and a control (Table 7). As compared to the low-fat sausage (Table 5), RMPs had more protein and less moisture content (%). When the RMPs were cooked in a smoking chamber, the cooking loss (CL, %) was more than 13-15% and EM values was approximately 25-27%

Table 5. pH, chemical composition and waterholding capacity values of low-fat comminuted sausage

Treatments[1]	pH[2]	Moisture	Fat	Protein	WHC
		(%)			
CTL	6.14±0.11	77.1±0.50	2.40±0.95	14.1±0.32	44.9±2.05
T1	6.19±0.09	77.5±0.75	2.71±0.60	13.8±0.47	44.1±2.99
T2	6.26±0.11	78.0±0.59	2.98±0.54	13.9±0.40	44.6±2.62
T3	6.23±0.14	78.4±0.35	2.99±0.45	14.1±0.87	45.4±2.46

[1] Treatments : Control= 1.5% salt; T1=1.0% salt + 0.1% TG; T2=0.5% salt + 0.2% TG; T3=no salt+0.3% TG.

[2] Mean±SD for triplicates.

Table 6. Textural properties of low-fat, low-salt comminuted sausage using TGase

Treatments[1]	Texture parameters				
	Hardness[2]	Gumminess	Chewiness	Springiness	Cohesivness
CTL	4,471±314	898±98	598±23	0.67±0.07[a]	0.23±0.03
T1	4,369±328	856±62	558±110	0.66±0.09[a]	0.23±0.03
T2	4,513±579	759±127	530±48	0.46±0.04[b]	0.24±0.01
T3	4,433±703	766±124	550±39	0.46±0.09[b]	0.29±0.04

[a,b] Means in a same column with different superscripts are different ($p<0.05$).

[1]Treatments; Control=1.5% salt; T1=1.0% salt+0.1% TG; T2=0.5% salt+0.2% TG; T3=no salt+0.3% TG.

[2] Mean±SD for triplicates.

Table 7. pH, proximate analysis, physico-chemical and textural properties of restructured meat products using TGase

Parameters	With TG	Without TG
pH	5.97±0.07	6.00±0.14
Moisture (%)	70.0±0.21	71.5±0.21
Fat (%)	3.12±0.37	2.61±0.64
Protein (%)	25.4±1.63	25.6±1.13
Expressible moisture (%)	25.9±0.14	26.5±0.35
Cooking loss (%)	14.4±0.28	13.6±0.71
Hunter L	64.7±3.46	67.9±3.32
Hunter a	12.4±1.84	11.9±0.21
Hunter b	3.47±0.80	4.95±1.02
Hardness	12,879±569	12,439±682
Springiness	0.57±0.03	0.60±0.03
Cohesiveness	0.30±0.01	0.27±0.03
Gumminess	3,349±88	2,827±415
Chewiness	1,931±168	1,663±287

(Table 7). Since the CL values are higher than the typical CL (<10%) due to higher cooking temperatures, a reduced internal temperature of 68-70°C might increase the CL values, however such a change would favors to microbial growth including pathogens. In this study microorganisms were not found after cooking (<10^2 cfu/g). More moisture loss during cooking procedure probably leads to less loss of moisture content during storage. No differences ($p>0.05$) in hunter color values and textural properties were determined between a control and a TGase treatment. This result indicated that the low-fat RMPs could be successfully manufactured without addition of emulsified-meats as a binding agent without textural defects and it may be significant for the reduction of labor and normal tumbling time to be required. In previous TGase application, Kumazawa et al. (1993) developed dried fish ("Himono") manufactured with myosin heavy chain and TGase by crosslinking reaction. Kuraishi et al. (1997) reported that restructured meat was manufactured without salt and cooking, however the addition of salt improved binding capacity. They also recommended that sodium caseinate in combined with TGase showed better binding capacity and was superior substrate for the crosslinking to meat proteins than others. In future studies, TGase will be applied to the manufacture a RMPs with low-quality pork meat, such as, pale, soft, exudative (PSE) to determine whether the binding capacity can be improved.

CONCLUSION

The addition of 0.1% TGase with reduced salt content from 1.5 to 1.0% did not affect the chemical composition, physico-chemical properties and textural characterstics of low-fat sausages, as compared to a control (1.5% salt). However, the salt level should be at least 1% in low-fat sausages to obtain similar textural characteristics to those with the control. Emulsified-meats are normally used for the improvement of binding for the manufacture of restructured meat products (RMF). This study also indicated that 0.3% TGase successfully replaced 10% emulsified-meats in the manufacture of RMPs and might result in reduced labor needs and shorter tumbling times.

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