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THE EFFECT OF TRANSGLUTAMINASE AS A BINDING AGENT IN MIXTURE OF MEAT AND NON-MEAT GELS ON WATER HOLDING PROPERTIES AT DIFFERENT COOKING CONDITIONS

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ABSTRACT

The influence of cooking methods (water bath, retort, and microwave) on the binding and water holding properties of mixed pork/casein gels with (2.5 or 5 g kg-1) or without (control) microbial transglutaminase (MTGase) was investigated using puncture test measurements. The MTGase treatment improved (P < 0.0001) water-holding capacity, reduced (P <0.0001) cooking loss (CL), purge and expressible water (EW), and increased (P <0.0001) the gel hardness and breaking strength as measured by compression and puncture tests. Microwave-cooked mixed gels had the lowest hardness but least CL and EW than retort and water bath-cooked samples. The results suggested that MTGase influenced the gel properties of pork muscle/non muscle gels, but the effectiveness of the enzyme treatment depended on the cooking methods.

***Key words*:** Meat, Casein; MTGase, Cooking Method, Water holding, Gel strength

INTRODUCTION

Non-meat ingredients derived from a variety of plant and animal sources are commonly used in comminuted meat products to modify the product functional properties, including emulsification, gelation, water- and fat-binding capacity, texture, and appearance (Whiting, 1988). The effectiveness of the different functional ingredients, however, can vary greatly with the thermal processing conditions, specially the rate of heating. For example, the breaking force and strength of meat or meat protein gels are greatly influenced by heating rate and final gelling temperatures (Foegeding et al., 1986; Xiong & Brekke, 1991). The most dramatic changes in processed meats during heating, e.g., shrinkage, tissue hardening, juice release, and discoloration, are caused by muscle protein thermal denaturation (Bowers et al., 1987). A minor shift in the heating rate could alter the thermodynamics of protein coagulation and aggregation (Xiong & Blanchard, 1994). The time-temperature relationship affects the resulting tenderness, juiciness, color and flavor of meat (Prestat et al., 2002).

A variety of thermal processing methods is available for preparing cooked meat and comminuted meat products. These include boiling and roasting (Rodriguez-Estrada et al., 1997), broiling (Cross et al., 1979), electric grill frying and microwave heating (Janicki & Appledor, 1974; Gros et al.,1986), browning (Cannell et al.,1989), charbroiling and deep fat frying (Berry & Leddy 1984), long wave irradiation and high-pressure cooking (Jimenez-Colmenero et al., 1998), high-intensity ultrasound treatment (Pohlman et al., 1997), and radio frequency cooking (Zhang et al., 2004).

Traditionally, comminuted meat products are cooked in a humidity-controlled oven, with or without smoking. However, pressure cookers are also used. For example, 'ham sausage', a popular emulsion-type meat in China, is prepared in a high-pressure retort. High-pressure conditions alter the structure and functionality of proteins, thereby affecting the properties

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(texture, structure, etc.) of meat products (Angsupanich et al., 1999; Macfarlane, 1973; Cheftel & Culioli, 1997). On the other hand, using a dielectric form of heating, polarizing electromagnetic radiation allows volumetric heating of the product such that all parts of the product in principal are heated at the same rate. The application of microwave energy is an established method of achieving rapid heating, which is widely used in the food processing industry and at homes (Giese, 1992).

It is difficult to draw a general conclusion from the previous studies since few of them have compared different cooking methods in a single study. It is doubtful that the observations obtained from model systems would reliably represent the real food processing conditions. The objective of this study was to examine the combined effect of three cooking methods, microbial transglutaminase and protein concentration on water binding and gel properties of sodium caseinate added pork batter gels. The enzyme transglutaminase was included in the meat batter formulation because of its excellent gel-strengthening and emulsion-stabilizing effects and hence, its potential utility in meat processing (Kuraishi et al., 2001; Ramirez-Suarez & Xiong, 2003).

MATERIALS AND METHODS

Materials

Pork leg meat was purchased from a local packaging plant 24-36 h postmortem. The lean muscle was ground through a 3 mm diameter orifice plate (a Model JCW 6 grinder, Shanghai Instrument Co., China). Protein content in the ground pork was determined by the AOAC (1997) procedure (928.08). Nitrogen values were converted to protein using a conversion factor of 6.25. The protein content of the pork meat was 217 g kg-1. Sodium caseinate (≥ 900 g kg-1 dry basis) was provided by Hualing Milk Products Group (Lanzhou city, Gansu, China). Microbial transglutaminase (MTGase) was purchased from Yiming Fine Chemical Plant, Gensi, Jiangsu, China. All the chemicals were of analytical grade (Sinopharm Chemical Reagent Co Ltd, Shanghai, China).

Preparation of pork batter gels

Pork batter gels were prepared by the method of Pietrasik & Li-Chan (2002). Before processing, meat was tempered at 4 °C and the protein content adjusted to 60 and 100 g kg-1 with ice water (50:50). All formulations also contained 20 g kg-1 of sodium caseinate. The levels of sodium chloride, sodium tripolyphosphate, sodium nitrite and sodium ascorbate in all formulations were 20, 2, 0.1 and 0.5 g kg-1, respectively. Control and treated batters contained 0 and 2.5 or 5 g kg-1 MTGase preparation. Batters (300 g each) were prepared by mixing ground meat and the ingredients for 15 s on a high-speed food blender (Model DS-1, Shanghai Instrument Co., Shanghai, China).

Immediately after blending, batters were stuffed into a 25-mm diameter polyvinyl casing (Yurun Meat Co., Nanjing, China) and tied into 120-mm links. Stuffed batters were kept overnight in a 4 °C cold room and then cooked with three methods: (1) isothermally heating in a 90 °C waterbath for 15 min, (2) retorting at 121 °C for 15 min (pressure 1.05 MPa), and (3) microwaving in a microwave oven for 15 min. The microwave oven (Model Galanz WD 800T, China) was adjusted to a medium electric output power and the operating frequency was 2.45 GHz (85 °C). Cooked batters ("gels") were immediately cooled in an iced slurry until the product core temperature reached 20 °C, and were subsequently stored at 4 °C until analysis. The entire experiment was carried out in three different trials on different days, within each of which duplicate batches of batters were prepared and three measurements were taken for each specific tests (described below) to allow for statistical analysis.

Water-binding properties

Cooking loss

Cooking loss was determined 24 h after thermal processing according Visessanguan et al., (2004). Gelled batters were removed from the casing, blotted dry with a paper towel, and weighed. Percent cooking loss was expressed as:

where A was the stuffed batter weight (raw batter + casing) (g), B was cooked and blotted

gel weight (g), and C was the weight (g) of the casing.

Expressible water (EW)

Expressible moisture of gels was determined 24 h after thermal processing following the modified method of Uresti et al., (2004). For each treatment, EW was determined on 9 gel

$$\text{Cooking Loss}(\%) = \frac{(A-B)-C}{A-C} \times 100$$

cores and the mean values are reported. Samples of 5 g (± 0.1g) of cooked gels were weighed and placed on to two layers of filter paper positioned at the bottom of 50-mL centrifuge tubes. The gel samples were centrifuged at 1000 g for 5 min at room temperature. Immediately after centrifugation, the gel samples were removed and reweighed, and the amount of expressible water was calculated as:

$$EW(\%) = \frac{W_i - W_f}{W_i} \times 100$$

where W_i and W_f were, respectively, the gel weights before and after centrifugation.

Purge

Purge during storage of gels was determined by the method of Pietrasik & Li-Chan (2002). Gel samples (20 mm dia. × 15 mm thickness slices) were vacuum packaged (-800 Mbar) and placed in a single layer in a 4° C cooler. After 1-week storage, the package was removed and the gel slices were blotted and reweighed. Purge was expressed as:

$$\text{Purge}(\%) = \frac{X-Y}{X} \times 100$$

where X and Y were, respectively, the gel weights before and after storage.

Gel properties

Cooked gel samples (25 mm dia. × 30 mm height) were equilibrated to room temperature for 30 min in a plastic bag to avoid dehydration before textural analysis. The mechanical properties of the gels were determined using a TA-XT2i Stable Micro Systems Texturometer (Viena Court, England). The Ver. 1.2 analytical software supplied by the texturometer company was used to process the data.

The puncture test was performed by compressing gel samples to 75% of their initial height using a compression speed of 60 mm min^{-1} and a cylinder probe (P/20) with a 1.2 cm diameter flat-surface. The breaking force (g), deformation (cm) and gel strength (break force × deformation, g·cm) for each treatment were measured. Samples were placed on the base of the texturometer, taking care that the cylindrical probe reached the sample at the centre. Three replicates with triplicate measurements per batch were analyzed for each treatment.

Statistical analysis

The experimental design was a complete randomized 2×3×3 factorial with two muscle protein levels (60 and 100 g kg^{-1} treated with 20 g kg^{-1} sodium caseinate), three MTGase levels (MTGase free as control, 2.5 and 5.0g kg^{-1}) and three cooking methods (Water bath, Retort and Microwave). Data were analyzed by analysis of variance (ANOVA) using the generalized linear model procedure of Version 8.1 SAS system for windows. When ANOVA showed significant treatment effects ($P<0.05$), mean separation was done by using the Duncan test.

RESULTS AND DISCUSSION

Water binding properties

Table 1 shows the effects of MTGase, protein level and cooking method on batter stability and water-binding properties of sodium caseinate added pork batter gels. Statistical analysis revealed that 2.5 g kg^{-1} addition of the MTGase had a significant ($P<0.0001$) effect on all parameters characterizing water-binding properties of the gels. The water-holding capacity is directly associated with

the percent water expressed by centrifugation (the lowest % of water extracted means the highest water binding capacity) (Ramírez *et al*., 2002). The addition of MTGase to the sodium caseinate added pork homogenates significantly (P<0.0001) decreased expressible water from the resulting gels. In all treatments more moisture was retained in gels containing 2.5 g kg^{-1} MTGase as compared with those without MTGase addition. The percent water extracted varied in the range of 3.87 to 22.98%. The lowest percentage of water extracted corresponded to 100 g kg^{-1} muscle protein gels with 20 g kg^{-1} sodium caseinate and cooked in retort using 5 g kg^{-1} MTGase and the highest value of water extracted was obtained by the control with 60 g kg^{-1} muscle protein gel cooked in same cooking method. Trout and Schmidt (1986) reported that aggregation of meat protein with increasing temperature caused the meat protein matrix to shrink. This limited the amount of water the matrix could bind and reduced the strength of the forces immobilized in the water. The addition of muscle protein and MTGase significantly (P<0.0001) improved the expressible water of meat gels. Although both water bath and retort cooking has no significant (P<0.05) effect on expressible water while microwave cooking has significant (P<0.05) decrease in extracted water.

Cooking loss results are the most important test for the meat industry to predict the behaviour of the product during cooking due to non-meat ingredients or other factors (Pietrasik et al., 2002). In present study, the addition of muscle protein and MTGase significantly (P<0.0001) affected cooking losses and purge losses. Cooking losses originate from a lateral and/or longitudinal shrinkage of the myofibrils. The denaturation of myosin increased myofibrillar shrinkage. Offer & Knight (1988) cited that shrinkage/swelling of a protein network structure within the muscle is brought about by the formation/breakage of short-range bonds (electrostatic or hydrogen bonds) between groups in the same or neighboring polypeptide chains. They mentioned that in this way the space between the polypeptide chains are diminished/enlarged and therefore less or more water is held by the network.

The greatest cooking loss was observed in MTGase free 60 g kg^{-1} muscle protein gel cooked in retort method while lowest cooking loss was observed in gel sample cooked in water bath containing 2.5 g kg^{-1} MTGase and 100 g kg^{-1} muscle protein. However, addition of 5 g kg^{-1} MTGase was not able to decrease cooking loss and storage losses of gels containing sodium caseinate with compared to the 2.5 g kg^{-1} MTGase added sample. This behaviour suggests that an excess of MTGase increased protein-protein interactions with a decrease of protein-water interactions, which induces an increase in cooking loss. An inverse relationship between transglutaminase addition and cooking loss was reported by Tseng, Liu & Chen (2000) in reduced salt (1%) chicken meatballs produced with 0-1% pig plasma transglutaminase. The significant (P<0.0001) effect on cooking losses was obtained among the three cooking methods. The lowest cooking loss (3.38%) recorded in water bath cooking while highest cooking loss (15.3%) reported in retort treatment. The treatment formulated with 2.5 g kg^{-1} MTGase showed significantly (P<0.0001) lower purge losses after 1-week storage in comparison with the samples manufactured without MTGase. However, addition of 5 g kg^{-1} MTGase does not show significant (P<0.0001) improvement of purge losses in batter samples. After the enzyme concentration reaches a certain point in myofibrillar proteins, it becomes inversely correlated with the WHC, because the higher the enzyme concentration, the greater is the number of inter and intrachain peptide cross-links and the lower the protein–water interaction (Gaspar & Góes-Favoni, 2015). The highest purge losses were observed in samples cooked in microwave while lowest purge losses was noted in retort cooking. Upon alteration of muscle cell structure entrapped or immobilized water can eventually escape as purge (Offer & Knight, 1988). Further, limited degradation of cytoskeletal proteins may result in increased shrinking of the overall muscle cell, which is ultimately translated in to drip loss (Huff-Lonergan & Lonergan, 2005). In addition, statistical analysis shows significant interactive effects between muscle protein, MTGase

Table 1: Water binding properties of pork batter gels as influenced by muscle protein (MP) level, MTGase treatment, and cooking method*

Sample	*Cooking loss (%)*		*Expressible water (%)*		*Purge (%)*	
	6% MP	*10% MP*	*6% MP*	*10% MP*	*6% MP*	*10% MP*
Waterbath						
Control	9.50a	3.62e	18.30a	9.96c	22.98af	15.00g
2.5 g kg^{-1} MTGase	4.52bi	3.38e	15.35b	7.12f	17.91b	13.98hj
5.0 g kg^{-1} MTGase	4.91b	3.88efi	10.22c	7.03f	17.41b	13.43h
Retort						
Control	15.30c	12.11g	22.98d	6.69f	21.91c	12.07i
2.5 g kg^{-1} MTGase	10.40d	6.02h	12.59e	6.37fh	17.96b	12.05i
5.0 g kg^{-1} MTGase	8.80a	6.29h	9.03ci	3.87g	16.54d	11.98i
Microwave						
Control	5.10b	4.47bf	16.47b	7.66fi	23.35a	14.43gj
2.5 g kg^{-1} MTGase	4.85b	3.51c	13.80e	4.42g	22.32cf	13.66h
5.0 g kg^{-1} MTGase	4.86b	4.75b	9.58c	5.00gh	26.34e	17.52b
A: Effect of muscle protein level**						
6% MP	8.47a		14.98a		20.75a	
10% MP	5.34b		6.46b		14.00b	
P-value	$P < 0.0001$		$P < 0.0001$		$P < 0.0001$	
B: Effect of MTGase treatment**						
Control	9.78a		14.70a		18.29a	
2.5 g kg^{-1} MTGase	5.37b		9.53b		16.32b	
5.0 g kg^{-1} MTGase	5.54b		7.93c		17.51c	
P-value	$P<0.0001$		$P < 0.0001$		$P < 0.0001$	
C: Effect of cooking method**						
Waterbath	4.97a		11.33a		16.79a	
Retort	11.41b		11.34a		15.74b	
Microwave	4.32a		9.49b		19.60c	
P-value	$P < 0.0001$		$P < 0.05$		$P < 0.0001$	
Interaction *P*-value						
A×B	$P < 0.0001$		$P < 0.0001$		$P < 0.0001$	
A×C	$P < 0.0001$		$P < 0.01$		$P < 0.0001$	
B×C	$P < 0.0001$		$P < 0.01$		$P < 0.0001$	
A×B×C	$P < 0.005$		$P < 0.0001$		$P < 0.001$	

***Different letters in the same column, within each main effect, indicate significant difference ($P < 0.05$).**

****Each number within the same main effect represents the mean value of all the samples.**

Table 2: Puncuture test parameters of pork batter gels as influenced by muscle protein (MP) level, MTGase treatment, and cooking method*

Sample	*Breaking force (g)*		*Deformation (cm)*		*Gel strength (g·cm)*	
	6% MP	*10% MP*	*6% MP*	*10% MP*	*6% MP*	*10% MP*
Waterbath						
Control	323.95a	1101.05cf16	0.74a	1.13c	241.20a	1245.85cg
2.5 g kg^{-1} MTGase	857.52bi	97.69g	1.01be	1.34f	870.91bh	2268.28e
5.0 g kg^{-1} MTGase	1056.24c	1992.82h	1.16c	1.30fg	1224.84cg	2602.68f
Retort						
Control	729.98d	903.44i	1.05b	1.16c	763.76b	1051.28gh
2.5 g kg^{-1} MTGase	797.77bdi	1356.09j	1.03be	1.43h	823.85b	1934.79i
5.0 g kg^{-1} MTGase	1193.17ef	1664.85g	1.18c	1.45h	1409.39cd	2405.19ef
Microwave						
Control	298.93a	1231.40e	0.71a	1.28g	211.23a	1572.74d
2.5 g kg^{-1} MTGase	726.34d	2119.29k	0.94d	1.47h	681.87b	3128.99j
5.0 g kg^{-1} MTGase	762.02bd	2461.09l	0.98ed	1.44h	743.77b	3572.08k
A: Effect of muscle protein level**						
6% MP	749.55a		0.98a		774.53a	
10% MP	1614.19b		1.33b		2197.99b	
P-value	$P < 0.0001$		$P < 0.0001$		$P < 0.0001$	
B: Effect of MTGase treatment**						
Control	764.79a		1.01a		847.67a	
2.5 g kg^{-1} MTGase	1259.12b		1.21b		1618.12b	
5.0 g kg^{-1} MTGase	1521.70c		1.25b		1992.99c	
P-value	$P<0.0001$		$P < 0.0001$		$P < 0.0001$	
C: Effect of cooking method**						
Waterbath	1171.55a		1.11a		1408.96a	
Retort	1107.55b		1.22b		1398.04a	
Microwave	1266.51a		1.14a		1651.78b	
P-value	$P < 0.05$		$P < 0.05$		$P < 0.05$	
Interaction *P*-value						
A×B	$P < 0.01$		$P < 0.05$		$P < 0.001$	
A×C	$P < 0.0001$		$P < 0.0001$		$P < 0.0001$	
B×C	Ns		Ns		Ns	
A×B×C	Ns		$P < 0.01$		Ns	

***Different letters in the same column, within each main effect, indicate significant difference ($P < 0.05$). Ns = Not significant**

****Each number within the same main effect represents the mean value of all the samples.**

and cooking method on all water binding parameters. Once the muscle is harvested the amount of water and the location of that water in meat can change depending on numerous factors related to the tissue itself and how the product is handled (Honikel, 2004; Honikel & Kim, 1986). When meat is cooked, the thin filaments start to disintegrate and the thick filaments coagulate at about 60° C, and at 80°C filaments can no longer be recognized (Schmidt & Parrish, 1971).

Gel properties

Mechanical properties of heat induced gels comprised with sodium caseinate and 60 and 100 g kg^{-1} muscle proteins are shown in Table 2, as a function of MTGase and cooking method. There were, as expected, significant differences (P<0.0001) between muscle protein level and MTGase treatment on puncture test parameters of batter gels. Under the same thermal treatment conditions additions of MTGase exhibited higher breaking force and gel strength. Analysis of variance demonstrated that protein; MTGase and cooking method had significant (P<0.0001, & P<0.05) effects on all puncture test characteristics. Breaking force and gel strength were significantly increased (P<0.0001) in both types of protein formulation by addition of 2.5 & 5 g kg^{-1} MTGase (Fig. 2). There were no interactions between MTGase×cooking methods on all puncture test parameters. However, significant interactions occurred for protein×MTGase (P<0.01, P<0.001 & P<0.05, respectively) and protein × cooking method (P<0.0001) on all mechanical properties. The lowest breaking force (903.4 g) and gel strength (1051.3 g×cm) were recorded in 100 g kg^{-1} muscle protein batter cooked in retort. Pressurization may cause denaturation and/or aggregation (Cheah & Ledward, 1996), which could limit heat-gelation in meat batters (Carballo *et al*., 1996). The disruption of myofibrillar proteins is mainly accountable for the toughening effect (Obuz *et al*., 2003) and responsible for the heat-induced aggregation involved in gelling and binding mechanism (Bouton *et al*., 1977). These heat-induced changes are time and temperature dependent, and the net effect of this toughening or tenderization depends on cooking conditions.

CONCLUSIONS

Concluded that non-muscle/muscle protein level and MTGase level is critical in ensuring water binding and gel characteristics of processed batter gels. The different cooking method also had detrimental effect on water binding and gel properties. Non-muscle/ muscle protein batter gel displayed a wide range of water binding and gel characteristics due to differential temperature and heating rate encounter in different cooking method. Therefore use of non-muscle/muscle protein, MTGase level and cooking method proved to enhance the water binding and gel properties.

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