1 Polyphosphate and myofibrillar protein extract promote transglutaminase-mediated
2 enhancements of rheological and textural properties of PSE pork meat batters
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ABSTRACT

The objective of this study was to improve the rheological (storage modulus G' ; phase angle δ) and textural (hardness; breaking force) properties of nitrite-cured sausage batters prepared from pale, soft, exudative (PSE) pork with microbial transglutaminase (TG) using isolated myofibrillar protein (MP) or MP extracted by sodium tripolyphosphate (TPP) as substrates. While TG alone significantly enhanced G' , δ and hardness, its combination with TPP was more pronounced as these product quality parameters were increased to the level equal to that of the counterpart batter made from normal (red, firm, non-exudative, RFN) pork. The addition of MP had negligible such effect on TG functions. Cooking loss of TG-treated RFN and PSE meat with TPP was minimal but the batters were less bright (L^*) than other treatments. Redness (a^*) was variable between treatments. Therefore, texture-related properties of comminuted PSE meat products can be restored to the RFN level by TG cross-linking of protein that is extracted by TPP.

Keywords:

PSE pork

Sausage

Rheological property

Texture

Transglutaminase

1. Introduction

The pale, soft, exudative (PSE) characteristic that is readily noticeable is a defect phenomenon for pork, and it results directly from protein denaturation and reduced solubility (Camou & Sebranek, 1991). The occurrence of PSE meat is associated with a variety of ante- and post-mortem factors, and the adverse economic impact can be quite significant (Vermeulen, Van de Perre, Permentier, De Bieb, Verbeke, & Geers, 2015, 2016). Among the major contributors to the PSE problem are genetic, biochemical, metabolic and environmental factors (Barbut et al., 2008; Gajana, Nkukwana, Marume, & Muchenje, 2013; Scheffler & Gerrard, 2007). Recent proteomic studies have shown a strong correlation of drip loss, color, and tenderness of pork with the protein profile of the muscle exudate (Di Luca, Elia, Hamill, & Mullen, 2013; Przybylski et al., 2016). Despite an improved understanding and numerous technological attempts, however, PSE incidences remain to widely occur in practice. Therefore, processing strategies must be developed to restore the defective PSE meat to the quality level closely comparable to the level of normal meat. One possible approach is to manipulate meat processing ingredients to allow enhanced functional performance of myofibrillar protein (MP) in PSE meat.

According to Buchert et al. (2010), the poor quality of PSE meat can be improved or partially overcome by mixing of PSE meat with normal meat, using special curing formulations, and adding different ingredients, for example, cross-linking enzymes, non-meat proteins, and polysaccharides, during processing. The primary purpose of these efforts are to promote the functionality of MP because MP is largely responsible for the development of desirable texture and water binding in comminuted meat products, including sausage-type meats (Acton, Ziegler, & Burke, 1983).

The concentration of soluble protein in sausage batters strongly influences the functional properties of protein, such as gelation, binding, and emulsification capacity, and often, the efficacy of non-protein ingredients (Camou & Sebranek, 1991; Farouk, 2010). The surface protein matrix (gel) can be created by the use of food additives that promote the extraction of MP. This ingredient strategy is of particular usefulness for PSE meat that has a poor protein solubility. Hence, polyphosphate, elevation of the meat batter pH, and fine communication are commonly applied to meat processing operations.

In the present study, we aimed at building an adhesive surface protein gel matrix in sausage to allow the formation of a well-bound texture in PSE meat sausage. The study tested the hypothesis that TG, together with TPP or MP, could enhance the textural quality attributes of PSE pork sausage and possibly bring them to the level close to the normal meat.

2. Materials and methods

2.1. Materials and experimental design

The protocol of the experimental design was previously presented in the International Congress of Meat Science and Technology (ICoMST) (Lesiow & Xiong, 2009), and the layout of the study is shown in Fig. 1. A total of 15 crossbred pigs [Yorkshire × Duroc, (Yorkshire × Landrace) × Duroc] from a known halothane gene negative breed line were raised at the University of Kentucky Swine Farm on a typical commercial corn-soybean meal diet (14.0 % protein, 3.34 Mcal/kg metabolisable energy). Pigs were slaughtered at the USDA-inspected University Meat Pilot Plant located within the University of Kentucky. After fastig for for 24 h, pigs were electrically stunned, bled, scalded, then eviscerated. The weights of hot carcasses ranged from 76.6 to 89.2 kg. Loins (*Longissimus lumborum*, LL) were excised from four

randomly-selected animals immediately after carcass splitting (about 30 min post-exanguination; loin temperature 36.3 ± 1.3 °C). The loins were separated into two equal pools (2 loins per pool) to enable two independently repeated experiments, and each loin was cut in the middle perpendicularly to the long axis. The two halves obtained from each loin were dipped in 10 mM NaN_3 (an antimicrobial) and placed in two separate plastic bags (Ziploc bags, Gal size). One bag was stored at 4 °C for 24 h to prepare RFN and the other was submerged in a 35 °C water bath for 7 h followed by chilling and storage at 4 °C for a total of 24 h postmortem to induce PSE, which was verified based on the color and pH as described previously (Lesiow & Xiong, 2013). Both RFN and PSE-like loins were subsequently vacuum packaged, stored in a -80 °C freezer, and used within 2 months.

In each of the two identical, repeated experiments (I and II), two different batches of batters were prepared (Fig. 1). Each batch began with two halves of loin from the same pork carcass to produce 7 different sausage formulations: 3 for RFN (control; TG; TPP+TG) and 4 for PSE meat (control; TG; TPP+TG; MP+TG). Sodium tripolyphosphate (TPP) was used as a treatment additive due to its strong myosin-extraction capability (Xiong, Lou, Moody, & Harmon, 2000), MP was pre-extracted from normal (RFN) pork according to Lesiow and Xiong (2013). Transglutaminase, added to cross-link the available protein, is a crude enzyme preparation (Activa-TI) with 1 % active TG and 99 % maltodextrin as carrier which was donated by Ajinomoto Food Ingredients (Chicago, IL, USA). All chemicals used for this study were reagent grade.

2.2. Preparation of meat batters

Before use, loins were thawed at 4 °C for 24 h. External fat and connective tissue were trimmed to obtain ~96% lean muscle which is then cut into small pieces and ground once through a 3-mm orifice plate. Base ingredients used in meat batter formulations included sodium chloride, sodium nitrite, and sodium erythorbate, and treatment ingredients were TPP, MP, and TG (Table 1). For all batter processing, raw meat was chopped for 1 min in a pre-chilled (4 °C) One-Touch Chopper (Model HC306, Black & Decker, Towson, Maryland, USA). After addition of cold water with dissolved ingredients (NaCl, sodium nitrite, and sodium erythorbate, with or without TPP and MP), chopping was continued for the next 1 min. The raw batter was then blended in a jar with TG for 1.5 min. Mixing time was standardized to 3.5 min and the final temperature was below 10 °C in all cases. The amount of added water and MP were calculated based on the muscle and MP concentrations so as to have a 12.5% protein concentration in all meat batters. Aliquots of 50 g of each batter were stuffed into cylindrical plastic tubes (26 mm diameter × 102 mm length). Tubes were covered with aluminum foil and kept at 4 °C for 4 h (a standardized time for TG treatment) before rheological testing or heated to form gels.

2.3. Dynamic rheological properties

The rheological properties of RFN and PSE meat batters during thermal gelation were measured with a model VOR oscillatory rheometer (Bohlin Instruments, Inc., Cransbury, NJ, USA) equipped with two parallel plates set at 1 mm apart. Samples were loaded in the space between the parallel plates and the exposed rim was covered with a thin layer of silicon oil to prevent dehydration. Meat batter gels were produced by heating from 20 to 74 °C at a heating rate of 1 °C/min, frequency of 0.1 Hz, and a maximum strain of 0.02. Changes in the storage

modulus (G' , i.e., rigidity due to elastic response of the material) and phase angle (δ , which ranges from 0 for elasticity to 90 ° for viscosity) were recorded.

2.4. Cooking loss (CL), pH, and protein content

The meat batters (50 g) stuffed into cylindrical plastic tubes (26 mm × 102 mm) and covered were isothermally heated in a 74 °C water bath for 30 min, then chilled in ice water for 20 min. A thermocouple was used to verify the sample temperature. Following overnight refrigerated storage, cooked samples were removed from the plastic tubes, blotted dry with a paper towel and weighted. CL was expressed as g of liquid expelled per 100 g of initial sample weight. Determinations were carried out in duplicate. The meat (“sausage”) samples were cut into rings of 1.5 mm height and placed into separate plastic bags for subsequent textural analysis.

The pH of cooked batters was determined using a Model AB 15 pH meter (Accumet, Fisher Scientific, Fair Lawn, NJ, USA). An aliquot of 5 g muscle samples was mixed with 15 mL deionized water and homogenized 30 s at speed 3 (7,500 rpm) with a PT 10-35 Polytron blender (Kinematica, AG, Littau/Lucerne, Switzerland). Protein content of RFN and PSE meat and cooked meat batters was determined by AOAC (1990) procedure (method no. 981.10) using a nitrogen conversion factor of 6.25.

2.5. Color parameters

Colorimetric measurements were performed on cooked RFN and PSE sausage, 5 samples per ingredient treatment, by a Hunterlab colorimeter (LabScan XE, Hunter Associates Laboratory, Reston, VA, USA) using illuminant D₆₅ and the 10° Standard Observer. The colorimeter with a 0.64 cm port was calibrated with a black and a white tile according to the instrument manual.

Color parameters, i.e., lightness (L^*), redness (a^*), and yellowness (b^*), were measured at four different locations on the cross-section of each sliced sausage (26 mm diameter) and the average values were reported. Hue angle [$h = \tan^{-1} (b^*/a^*)$] and saturation index [$C^* = (a^{*2} + b^{*2})^{1/2}$] were also calculated.

2.6. Textural properties

Textural profile of cooked meat batters was performed using a model 4301 Instron machine (Instron Corp., Canton, MA, USA) as detailed by [Xiong, Noel, and Moody \(1999\)](#). Eight cores (diameter = 25 mm $\times$ height = 15 mm) of cooked samples from each treatment were compressed axially between two parallel plates to 20 % of their original height at a constant cross-head speed of 60 mm/min. The maximum (peak) force registered was designated as hardness. To determine breaking strength, eight samples of cooked batters (25 mm $\times$ 15 mm) were axially compressed non-stop at a rate of 60 mm/min until the sample structure was ruptured. The rupture force was defined as the sample breaking strength.

2.5. Statistical analysis

Two identical but independent experiments were conducted. In each experiment, two batches of batters, each utilizing the two halves of loin (one for RFN; one for induced PSE) from the same pork carcasses, were used as replicates. Therefore, a total of four sausage replicates were prepared and tested. Results collected from the four individual loins were analyzed using the Statistics 8.0 program (Analytical Software, Tallahassee, FL, USA). One-way analysis of variance was done to determine significant ingredient treatment effects (P at 0.05) and differences due to muscle type (RFN vs. PSE). Differences between individual means were

identified by Duncan's all-pair wise comparisons test. Correlations between different ingredient treatments on individual response variables were analyzed. Moreover, two-factor analysis of variance was performed.

3. Results and discussion

3.1. Rheological properties of meat batters

The profiles of selected rheological properties (G' as storage modulus; δ as phase angle) are displayed in Fig. 2. As a general pattern, the control and treated batters (except with TPP+TG) of both RFN and PSE were insensitive to temperature less than 40 °C. However, their G' value rose sharply after 50 °C (Fig. 2A), indicating the formation and rapid developments of an elastic protein network. Correspondingly, the δ value continuously declined after 50 °C (Fig. 2B). Table 2 summarizes the specific values of G' and δ recorded at the transition temperature (50 °C) and at the end of heating (74 °C) where the process of MP sol-to-gel transformation was complete and firm gels were formed from RFN and PSE meat batters.

Despite the initial surge, the G' of the PSE control batter began to level off and showed a trend of declining after reaching 64 °C, indicating the disruption of the gel network formed at lower temperatures. In comparison, the G' of the RFN control batter ascended continuously, reaching an ultimate level of 21600 Pa at 74 °C which is 30% higher than the PSE control. In PSE meat, MP is partially denatured and the solubility is low (Kazemi, Ngadi, & Gariepy, 2011; Lesiow & Xiong, 2013), which explains the poor gelling properties of PSE batters than the RFN counterpart. Treatment with TG alone significantly improved the gelling ability of both control batters and reduced the G' difference at 74 °C between RFN and PSE. However, the addition of TPP, which improves MP extraction and solubility by as much as 3-fold (Xiong et al., 2000)

through the dissociation of bound myosin and actin (Robe & Xiong, 1993), markedly enhanced the efficacy of TG as a protein cross-linking catalyst. This is evident from the earlier onset of gelation (at 40 °C instead of 49 °C) and the diminishment of the difference of G' between RFN and PSE batters (TPP+TG) at all temperatures when compared with other treatments. Although TPP can extract actomyosin, it has a direct negative effect on MP gelation due to its electrostatic repulsion (Robe & Xiong, 1993; Torley & Young, 1995). Therefore, the enhanced gelling ability of TPP-treated batters by TG was attributed to the indirect effect of TPP, i.e., increasing the available substrate for the cross-linking enzyme. On the other hand, the partial replacement of PSE meat by MP extracted from RFN meat plus TG (PSE+MP+TG) produced no obvious benefit when compared with the PSE+TG treatment without MP. This was unexpected but can be explained by the protein concentration effect, namely, the amount of substitutional “good” protein (0.98 g/100 g batter) incorporated into the PSE batter (final protein concentration 12.5 g/100 g batter) was insufficient to promote TG, therefore, incapable of eliciting a measurable impact. Perhaps pre-blending (solubilization) of MP may help with the effectiveness of TG and should be explored in future studies.

The microbial TG used in the study has a broad temperature range for activity and remains effective even at refrigerator temperatures. Not only does it cross-link Gln-Lys via acyl transfer, it promotes both intra- and intermolecular disulfide bond formation thereby polymerizing myosin and other MP at cold temperatures (Kuraishi et al., 1997). The pre-generated MP aggregates appeared to play an essential role in the subsequent formation and development of a three-dimensional elastic gel network induced upon heating. Transglutaminase functions optimally at 50 °C and completely loses activity at above 60 °C (Sakamoto, Kumazawa, & Motoki, 1994). The fact that the G' values of control and TG-treated RFN or PSE batters exhibited no

appreciable difference at 50 °C but yet, differed substantially ($P < 0.05$) at increasing temperatures, confirmed that protein aggregates generated at lower temperatures were a prerequisite for subsequent formation of an elastic gel. The accentuation of such protein-protein interactions induced by TG at mild temperatures that only appeared at high temperatures when a continuous protein gel network was formed was particularly prominent when TPP was used to extract MP (TPP+TG). Through the promotion of novel covalent bonds between myosin molecules, TG seems to counteract and greatly overcome the negative impact caused by myosin denaturation in PSE muscle. It is noteworthy that despite its ability to dissociate the actomyosin complex, hence, extract myosin, TPP actually is antagonistic for MP gelation due to its strong charge interaction with protein (Robe & Xiong, 1993). However, this gel-weakening effect was largely off-set by the increased protein substrate concentration to allow a more efficient TG performance.

As another demonstration of the rheological response of pork batters to different ingredient treatments, changes in the phase angle of the batters during cooking are plotted (Fig. 2B) and some key values are shown in Table 2. The most striking observation was that with TPP+TG, batters prepared from RFN and, to a lesser extent, from PSE, showed a rather high δ value (20-30 °), an indication of high viscosity of the original batter due to of presence of extracted protein by TPP. When heated to about 30 °C, the δ began to decrease and plunged from 35 to 45 °C, evidence of the transformation from a viscous protein sol to an elastic semi-solid (Davis, Ding, Forster, Pinkas, & Barron, 2010). Although not conspicuous, the δ value of PSE+TG meat batter at 50 °C was significantly lower and at 74 °C higher than that of RFN+TG meat batter ($P < 0.05$). The δ values of PSE meat batter with TPP+TG at both temperatures were not significantly different from those of RFN meat batter (Table 2). The partial replacement of PSE meat by

extracted protein (MP+TG) had no additional influence on δ at 50 °C, and the value was not significantly different when compared with the corresponding δ for PSE with TG only. As previously discussed, the low protein concentration was likely the reason for the unanticipated result. At 74 °C, the PSE+MP+TG batter exhibited a slight, yet, significant increase of elasticity (lower δ) than the PSE+TG sample.

3.2. Protein content, cooking loss, pH, and textural properties

The protein content of raw RFN and PSE meat was found to be 20.22 ± 0.13 g/100 g and 19.99 ± 0.15 g/100 g, respectively.

Generally, RFN meat batters exhibited a greater cooking yield (lower cooking loss) than the respective PSE meat batters (Table 3), indicating stronger water-holding capacity. Interestingly, although TG improved the gelling ability (Fig. 2), it did not reduce the cooking loss for RFN and actually caused more cooking loss for PSE batter ($P < 0.05$). Moreover, the addition of extracted MP with TG did not improve the water retention in cooked PSE batter. There is no congruency in the literature with regard to the influence of TG on the cooking yield in meat gels and meat products. Pietrasik, Jarmoluk, and Shand (2007) and Shang and Xiong (2010) reported that TG had no significant effect on cooking loss of pork muscle or protein gels. However, some researchers found that TG enhanced water-holding capacity of pork batters (Pietrasik & Li-Chan, 2002), while some other studies showed increased moisture loss in comminuted pork products treated with TG (Carballo, Ayo, & Jiménez-Colmenero, 2006; Katayama, Chin, Yoshihara, & Muguruma, 2006). These discrepancies in reported TG effects on water-binding properties can be partly explained by different levels of TG used (Pietrasik & Jarmoluk, 2003) and the conditions under which TG is applied (reaction temperature and time, meat particle size and

disruption methods, presence of other ingredients, meat source, etc.). The negative impact of TG on the meat system can be due to reduced water-binding capacity of salt-solubilized meat proteins as a result of cross-linking, which could inhibit a uniform development of protein networks and hence ineffective entrapment of water (Carballo et al., 2006).

The addition of TPP with TG promoted water-binding in cooked RFN and PSE batters showing a substantial reduction of cooking loss to 3.14 and 13.33 g/100g, respectively ($P < 0.05$). Our results were in agreement with that reported by Torley, D'Arcy, and Trout (2000) on the effect of polyphosphate. The less recovery of cooking loss by TPP for PSE meat when compared with RFN samples probably resulted from reduced cleavage of actomyosin by TPP in PSE muscle, therefore, limited myosin extraction (Izumi, Ito, & Fukazawa, 1977). Because of the increased moisture retention, the protein content in cooked batters with TPP+TG was lower than other ingredient treatments for both RFN and PSE, a “dilution” effect. The slightly higher pH in these products also contributed to the increased cooking loss (Table 3).

Hardness and breaking force of cooked RFN and PSE meat batters are presented in Table 3. While hardness of control PSE and RFN sausages was no different ($P > 0.05$), those treated with TG with or without TPP were harder for PSE than for corresponding RFN ($P < 0.05$). Moreover, the presence of TG with or without extracted MP increased hardness of the PSE control batter ($P < 0.05$). There was no direct relationship between cooking loss and hardness values of sausage. In fact, the TPP+TG treatment, which allowed the greatest moisture retention in cooked batters, produced the largest hardness value of all other treatments. The results were consistent for PSE meat but surprisingly not for RFN meat with the findings of Pietrasik et al. (2007) who reported hardness improvement for normal pork gels by TG. Perhaps the leanness (fatter

semimembranosus vs. leaner *longissimus*) of muscles and the level of TG addition (0.6 vs. 0.5%) are contributing factors the disparity.

Differing from hardness, the breaking force of RFN control and TPP+TG sausages was significantly higher than the respective PSE samples ($P < 0.05$). The most striking observation was that within the same muscle (RFN or PSE), TPP+TG remained to produce the strongest gel network that withstood approximately 13 times the compressive force than control batters before structural failure (Table 3). Sausages treated with TG+TPP were also much more resistant to breakage than without TPP. These results are in a similar trend to those of the rheological measurement (Fig. 2), suggesting the importance of adequate soluble MP for building a TG-induced gel matrix. However, the dynamic rheological testing result that G' values of the PSE batter formulated with TPP+TG at temperature 74 °C were not significantly different from that of the RFN batter (Table 2) is not reflected in the breaking force obtained from the Instron compressive testing (Table 3). The disagreement may be explained because the latter test was done with set gels (cooled to room temperature after heating to 74 °C) and registers mainly the compressive force, while the former test was performed on the gelling paste during the gel network formation where only shear force was recorded. Hence, these two tests do not always correlate with each other (Ferry, 1980).

3.3. Cooked color parameters

The colorimetric parameters L^* , a^* , and b^* of cooked RFN and PSE meat batters of control and TG-treated were similar and showed no difference between the two muscle sources (Table 4). On the other hand, RFN sausages with TPP+TG exhibited significantly lower L^* , a^* and b^* values when compared with respective PSE samples. The PSE sausage with added MP+TG was

less red (lower a^* value) than PSE control or PSE with only TG. Furthermore, L^* , a^* , and b^* of cooked RFN batter with TPP+TG and L^* and b^* of cooked PSE batter with TPP+TG were significantly lower than the respective meat batters of control and with TG. The reduced colorimetric values can be explained by the “dilution” effect, since the presence of TPP substantially increased sausage cooking yield (Table 3). TPP has also been found to promote myoglobin denaturation, which would contribute to color loss (Trout, 1989).

3.4. Correlations and two-factor analysis

Correlation analysis was done on major sausage quality parameters and measurements of RFN and PSE meat and the results are presented in Table 5. The data applied for the analysis were pooled results from all the four batches (Fig. 1). There were significant correlations ($P < 0.05$) between formulation treatments on pH for all RFN and PSE meat batters, implying that the pH of both types of meat responded similarly to the protein-extraction and cross-linking ingredients which was consistent from batch to batch. The values for cooking loss between individual PSE products were also significantly correlated, but correlations for RFN sausage were observed only between the control and TG-treated samples (Table 5). This suggests a greater impact of ingredient treatments on PSE meat. The negative correlation between TG and TPP+TG treatment PSE sausages is attributed to the strong water-binding effect of the polyphosphate itself and the gels formed by TPP-extracted protein (Torley et al., 2000; Trout, 1989), which prevented the moisture loss caused by TG alone. Similarly, the positive effect of the processing ingredients on sausage hardness was largely limited to PSE meat. The results demonstrate a more consistent, positive batch-batch response of PSE meat, when compared with RFN meat, for the quality parameters of pork sausage products.

Two-factor analysis (meat type; formulation treatments) was also performed and the results are summarized in Table 6. The type of meat (RFN and PSE) had no influence on batter rheological values (G' at both 50 and 74 °C; δ at 74 °C), pH, and colorimetric parameters. It substantiates the obtained results for that batter quality indicators presented in Tables 2–4 with one exception that some color parameters for RFN meat batters were significantly lower than the corresponding ones for PSE meat batters. On the other hand, treatments (control, TG, or TPP+TG) had significant influence for all batter quality indicators. Moreover, there were significant interactions between control, TG, and TPP+TG for all batter indicators.

4. Conclusions

The rheological properties of PSE pork batter weak gels were markedly improved by the combination of TPP and TG to the level comparable to RFN batter gels that were similarly treated, which was attributed to the enzyme cross-linking of increased concentrations of extracted protein. The addition of isolated MP plus TG did not show such significant improvement of PSE batter gelation. However, hardness of cooked PSE sausage with TPP+TG as well as its water-holding potential remained significantly lower than RFN sausage. More research is required to reduce the cooking loss of PSE sausage, for example, applying an efficient water-binding agent which would work cooperatively with TG and TPP to produce texture- and juiciness-improved comminuted meat products from low-quality PSE pork.

Acknowledgments

This is publication No. 16-07-092 of the Kentucky Agricultural Experiment Station, and the study is supported by the USDA National Institute of Food and Agriculture (Hatch project

1005724). The sabbatical opportunity at University of Kentucky for the primary author (TL) is acknowledged.

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Table 1

Formulation (g/100g batter) of samples from RFN (red, firm, non-exudative) and PSE (pale, soft, exudative) pork meat

Treatment ¹	Meat	Water	NaCl	N/E ²	TPP	TG	MP	Total
RFN control	62.5	35.9	1.5	0.1	-	0.0	-	100
RFN+TG	62.5	35.4	1.5	0.1	-	0.5	-	100
RFN+TPP+TG	62.5	34.9	1.5	0.1	0.5	0.5	-	100
PSE control	62.5	35.9	1.5	0.1	-	0.0	-	100
PSE+TG	62.5	35.4	1.5	0.1	-	0.5	-	100
PSE+TPP +TG	62.5	34.9	1.5	0.1	0.5	0.5	-	100
PSE+MP+TG	54.2	24.1	1.5	0.1	-	0.5	19.6	100

¹ TG: transglutaminase; TPP: sodium tripolyphosphate; MP: myofibrillar protein isolate (adjusted to an 8.5% protein concentration).

² N/E: sodium nitrite 0.0156g/100g; sodium erythorbate 0.055g/100g.

Table 2

Shear storage modulus (G') and phase angle (δ) of RFN (red, firm, non-exudative) and PSE (pale, soft, exudative) pork meat batters

Treatment ¹	G' (Pa) (50 °C)	G' (Pa) (74 °C)	δ (deg) (50 °C)	δ (deg) (74 °C)
RFN control	3370 ^c ±295	21100 ^{bc} ±803	11.9 ^a ±0.1	5.6 ^b ±0.1
RFN+TG	4288 ^{bc} ±177	25500 ^{ab} ±926	10.7 ^b ±0.2	5.0 ^c ±0.1
RFN+TPP+TG	6655 ^a ±173	30550 ^a ±823	8.7 ^d ±0.3	3.8 ^d ±0.2
PSE control	3493 ^c ±444	16365 ^c ±3517	11.3 ^{ab} ±0.2	6.2 ^a ±0.1
PSE+TG	4830 ^b ±380	21550 ^{bc} ±2775	9.9 ^c ±0.1	5.7 ^{ab} ±0.1
PSE+TPP+TG	6633 ^a ±714	29550 ^a ±3581	8.7 ^d ±0.4	3.8 ^d ±0.3
PSE+MP+TG	4273 ^{bc} ±370	21950 ^{bc} ±2192	10.0 ^c ±0.2	5.1 ^c ±0.3

¹ TG: transglutaminase; TPP: sodium tripolyphosphate; MP: myofibrillar protein isolate (adjusted to an 8.5% protein concentration).

^{a-d} Means ($\pm$ standard errors) within the same column without a common letter differ significantly ($P < 0.05$).

Table 3

Cooking loss, pH, protein content, hardness, and breaking force (BF) of RFN (red, firm, non-exudative) and PSE (pale, soft, exudative) cooked pork batters

Treatment ¹	Cooking loss (g/100g)	pH	Protein (g/100g)	Hardness (N)	Breaking force (N)
RFN control	22.9 ^c ±0.7	5.8 ^b ±0.1	19.7 ^c ±0.3	11.2 ^{ef} ±0.3	24.3 ^c ±0.6
RFN+TG	24.5 ^c ±1.0	5.8 ^b ±0.1	19.9 ^c ±0.2	11.7 ^{de} ±1.2	26.5 ^c ±0.7
RFN+TPP+TG	3.1 ^e ±0.2	6.2 ^a ±0.0	16.7 ^c ±0.2	12.8 ^{bc} ±0.3	339.4 ^a ±4.9
PSE control	28.3 ^b ±0.9	5.8 ^b ±0.0	21.6 ^b ±0.3	10.8 ^f ±0.3	14.4 ^d ±0.3
PSE+TG	31.1 ^a ±0.8	5.8 ^b ±0.0	22.6 ^a ±0.4	13.4 ^b ±0.3	18.7 ^{cd} ±0.3
PSE+TPP+TG	13.3 ^d ±0.3	6.1 ^a ±0.0	18.3 ^d ±0.4	16.3 ^a ±0.5	189.1 ^b ±5.5
PSE+MP+TG	30.3 ^{ab} ±0.7	5.9 ^b ±0.0	21.5 ^b ±0.2	12.3 ^{cd} ±0.3	22.2 ^{cd} ±0.4

¹ TG: transglutaminase; TPP: sodium tripolyphosphate; MP: myofibrillar protein isolate (adjusted to an 8.5% protein concentration).

^{a-f} Means (± standard errors) within the same column without a common letter differ significantly ($P < 0.05$).

Table 4
Color parameters of cooked pork meat batters

Treatment ¹	<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>C</i> *	<i>h</i>
RFN control	76.3 ^a ±0.4	5.0 ^{ab} ±0.2	10.4 ^{ab} ±0.1	11.6 ^a ±0.2	64.3 ^b ±0.8
RFN+TG	76.0 ^a ±0.5	5.1 ^a ±0.2	10.4 ^{ab} ±0.1	11.6 ^a ±0.2	63.8 ^{bc} ±0.8
RFN+TPP+TG	72.8 ^c ±0.4	4.0 ^c ±0.2	7.4 ^d ±0.2	8.5 ^c ±0.3	61.9 ^{cd} ±0.9
PSE control	76.2 ^a ±0.3	5.6 ^a ±0.1	10.1 ^b ±0.1	11.6 ^a ±0.1	61.2 ^d ±0.5
PSE+TG	75.8 ^a ±0.3	5.5 ^a ±0.1	10.3 ^{ab} ±0.1	11.7 ^a ±0.1	61.8 ^{cd} ±0.3
PSE+TPP+TG	74.5 ^b ±0.3	5.1 ^{ab} ±0.2	8.7 ^c ±0.1	10.1 ^b ±0.2	60.0 ^d ±0.6
PSE+MP+TG	76.6 ^a ±0.3	4.5 ^{bc} ±0.2	10.6 ^a ±0.1	11.6 ^a ±0.1	67.0 ^a ±0.8

¹ RFN: red, firm, non-exudative; PSE: pale, soft, exudative; TG: transglutaminase; TPP: sodium tripolyphosphate; MP: myofibrillar protein isolate (adjusted to an 8.5% protein concentration).

^{a-d} Means (± standard errors) within the same column without a common letter differ significantly ($P < 0.05$).

Table 5

Correlation coefficients (r) between selective individual formulation treatments on some quality parameters and measurements of RFN (red, firm, non-exudative) and PSE (pale, soft, exudative) pork meat batters

Treatment correlation ¹	Cooking loss	pH	<i>L</i> *	Hardness	Breaking force	G' (50 °C)	G' (74 °C)
RFN control/ RFN+TG	0.897*	0.983*	0.877*	0.270	0.817*	0.525	-0.234
RFN+TG/ RFN+TPP+TG	0.345	0.886*	0.883*	0.117	0.050	0.925*	0.631
PSE control / PSE+TG	0.986*	0.938*	0.769*	0.766*	0.263	0.835	0.829
PSE+TG/ PSE+TPP+TG	-0.736*	0.938*	0.836*	0.179	0.063	0.303	0.551
PSE+TG/ PSE+MP+TG	0.890*	0.958*	0.750*	0.582*	0.354*	0.986*	0.903*

¹ TG: transglutaminase; TPP: sodium tripolyphosphate; MP: myofibrillar protein isolate (adjusted to an 8.5% protein concentration).

* **Bold-faced values indicate significant correlations ($P < 0.05$).**

Table 6

Two-factor analysis of variance for experimental variables, considering RFN (red, firm, non-exudative) and PSE (pale, soft, exudative) pork meat batters as one factor and treatments C (control), TG (transglutaminase), TG+TPP (sodium tripolyphosphate) as the second factor.

Parameter	Meat type RFN/PSE	Control batters	TG batters	TPP+TG batters
G' (50 °C)	-	**	** ***	** ***
G' (74 °C)	-	**	***	** ***
δ (50 °C)	*	**	** ***	** ***
δ (74 °C)	-	**	** ***	** ***
Cooking loss	*	**	** ***	** ***
pH	-	**	***	** ***
protein	*	**	***	** ***
Hardness	*	**	** ***	** ***
Breaking force	*	**	***	** ***
L^*	-	**	***	** ***
a^*, b^*, C^*, h	*	**	***	** ***

* Significant difference ($P < 0.05$) for meat type.

** Significant difference ($P < 0.05$) for control vs. TG or control vs. TPP+TG.

*** Significant difference ($P < 0.05$) for TG vs. TPP+TG.

Fig. 1. Schematic representation of the experimental design. A total of 4 batches of sausage were prepared. RFN (red, firm, non-exudative); PSE (pale, soft, exudative).

Fig. 2. Heating-induced changes in storage modulus G' (A) and phase angle δ (B) of RFN-normal (red, firm and non-exudative) and PSE meat batters treated with or without transglutaminase (TG) in the presence or absence of sodium tripolyphosphate (TPP) and myofibrillar protein (MP) extract.