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ARTICLE

Physicochemical Properties of Meat Batter Added with Edible Silkworm Pupae (*Bombyx mori*) and Transglutaminase

Yoo-Sun Park, Yun-Sang Choi¹, Ko-Eun Hwang, Tae-Kyung Kim, Cheol-Won Lee, Dong-Min Shin, and Sung Gu Han*

Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Korea

¹Food Processing Research Center, Korean Food Research Institute, Seongnam 13539, Korea

Abstract

This study was conducted to investigate the physicochemical properties of meat batters prepared with fresh pork meat, back fat, water, and salt and formulated with three different amounts (5%, 10%, and 15%) of silkworm pupae (*Bombyx mori*) powder and transglutaminase (TG). Meat batters formulated with silkworm pupae powder showed significantly higher contents of protein and ash than control batter. Addition of silkworm pupae to batter also showed significantly lower cooking loss than the control. Moreover, meat batter containing 15% silkworm pupae showed no significant difference in redness value compared to the control. In addition, pH, viscosity, hardness, gumminess, and chewiness were improved after the addition of silkworm pupae. Furthermore, meat batter formulated with TG and silkworm pupae showed improved hardness, gumminess, chewiness and viscosity compared to control batter. Addition of 1% TG with 15% silkworm pupae to meat batter resulted in significantly higher pH, textures, and viscosity. Our data suggest that both silkworm pupae and TG can be added to meat batter to improve its physicochemical properties. Therefore, combination of silkworm pupae and TG could be a new nutritional and functional source for meat products.

Keywords edible insects, silkworm pupae, Bombyx mori, transglutaminase, meat batter

Introduction

Edible insects have been consumed in more than 110 countries over the world (Rumpold and Schlüter, 2013). Mealworm (*T. molitor*), cricket (*Acheta domesticus*), and silkworm (*Lepidotera*) account for a high proportion of edible insects (Nowak *et al.*, 2016). Studies about edible insects as conventional sources of nutrition have been increased gradually (Tan *et al.*, 2016). Sah and Jung (2012) have reported that compositions of edible insects include about 45-55% protein and 40-57% fat on dry matter basis. In addition, insects have higher environmental efficiency compared to livestock. The landscape used to rear insect and greenhouse gases emission from insect rearing are less than those for rearing livestock. Therefore, it has been suggested that using insects is an effective way to fulfill future nutrient requirement (Rumpold and Schlüter, 2013). Edible insects as additives to various foods have been conducted in previous studies. For example, pasta added with mealworm powder (5-20%) has shown increased sensory preference (Kim *et*)

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*Corresponding author

Sung Gu Han Department of Food Science and Biotechnology of Animal Resources, Konkuk University, Seoul 05029, Korea Tel: +82-2-450-0526 Fax: +82-2-455-1044 E-mail: hansg@konkuk.ac.kr *al.*, 2014). In addition, pork patty added with 20% of mealworm powder has improved flavor and protein content (Kim *et al.*, 2015). Kim *et al.* (2008) have reported that mealworm powder can improve the binding properties of pork patties. Nevertheless, meat products added with silkworm pupae powder have been insufficiently examined.

Silkworm pupae is one of major edible insects consumed in Asia such as Vietnam, Indonesia, China, and Korea (Baker et al., 2016). Silkworm pupae can be obtained as a by-product from sericulture business. These by-products of sericulture business are ordinarily consumed as a street food. However, most silkworm pupae are wasted. Utilization of silkworm pupae in commercial products could result in added value. Park and Park (1986) have suggested that defatted silkworm pupa is a good protein source when it is added to emulsified food. Jeon and Park (1992) have reported that enzymatically hydrolyzed silkworm pupae can enhance the retention capacity of water and oil compared to non-treated silkworm pupae. However, consumers have raised concerns about chemically treated foods. Thus, it is necessary to use a binder for silkworm pupae to improve their functional ability without chemical treatment. To improve the binding properties of processed meat, several binders such as carrageenan, isolated soy protein, and transglutaminase have been typically used in the production of meat products (Choi et al., 2015).

Transglutaminase (TG) is an enzyme isolated from microorganism. It can conduct cross-linkage with glutamine and lysine from protein molecule. Meat products added with TG have shown improved texture and cooking yield (Choi *et al.*, 2015; Lee and Chin, 2009). Furthermore, a combination of non-meat ingredient and TG has increased the hardness and reduced the cooking loss of frankfurter (Choi *et al.*, 2016). However, the effect of TG in combination with edible insect proteins on physicochemical properties of meat products has not been reported yet. Therefore, the aim of this study was to evaluate the effect of a combination silkworm pupae and TG on physicochemical properties of meat batter to provide basic data for industrialization of silkworm pupae in meat processing. Proximate analysis was performed in the current study. In addition, pH, color, apparent viscosity, cooking loss, and texture profile analysis of meat batters were determined in this study.

Materials and Methods

Preparation of silkworm pupae powder

Silkworm pupae were purchased from a local market and then dried in vacuum dryer (DRV622DA, Toyo Seisakusho Kaisha Ltd., Japan) at 40°C. Dried silkworm pupae (L*, 49.94 \pm 0.24; a*, 7.96 \pm 0.27; b*, 33.63 \pm 0.30; pH, 6.39 \pm 0.01; Moisture, 5.50 \pm 0.17%; Protein, 54.28 \pm 1.71%; Fat, 25.69 \pm 1.99%; Ash, 4.10 \pm 0.07%) were grinded into powder, passed through a 25 mesh sieve (Chung Gye Sang Gong Sa, Korea), and stored in polyethylene bags using a vacuum packing system (FJ-500XL, Fujee Tech, Korea). Sealed powder samples were preserved at -20°C until use.

Preparation and processing of meat batter system

The method of making meat batter with TG (BISION, Korea) and silkworm pupae powder was followed the method used by Herrero *et al.* (2008). Main ingredients, pork lean meat, and back fat were purchased from a local market. All visible connective tissue and evident fat were eliminated from lean pork meat. Chilled meat was ground into 4 mm in diameter using a meat grinder (PM-70, Manica, Spain). Eight different meat batters were produced. The experimental design and formulations are shown in Table 1. The first meat batter served as control was prepared without adding silkworm pupae or TG. The follow-

Table 1. Formulations of m	eat batter samples added	with silkworm pupae and TG
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	Treatments ¹⁾								
Ingredients (units: %)	Control		r	Γ1	[Г2	Т3		
	TG non added	TG added	TG non added	TG added	TG non added	TG added	TG non added	TG added	
Pork meat	85	85	85	85	85	85	85	85	
Water	15	15	15	15	15	15	15	15	
Total	100	100	100	100	100	100	100	100	
Sodium chloride (NaCl)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	
Silkworm pupae powder	0	0	5	5	10	10	15	15	
Transglutaminase (TG)	0	1	0	1	0	1	0	1	

ing combinations of silkworm pupae were used; T1: 5% silkworm pupae; T2: 10% silkworm pupae; T3: 15% silkworm pupae. For TG added treatments, it has been reported that 1% TG addition has the most effect in improving the texture, gel strength, and cooking yield of meat products (Tseng *et al.*, 2000). Therefore, 1% TG was added to meat batter in this study. Meat batter was processed using a silent cutter (Nr-963009, Scharfen, Germany). Completed batter models were vacuum packed (FJ-500XL, Fujee Tech, Korea) and stored at 4°C 12 h before reacting with TG (Min and Green, 2008). After storage, meat batter was stuffed into collagen casing using a stuffer (Stuffer IS-8, Sirman, Italy) and heated at 75°C in a water-bath (Model 10-101, Dae Han Co., Korea) for 30 min.

Proximate analysis

Proximate composition of meat batter was analyzed using AOAC (1995) method. Moisture content was measured based on weight loss after drying in a drying oven (SW-90D, Sang Woo Scienctific Co., Korea) 24 h at 105°C in order to get rid of all moisture. Protein content of respectively cooked samples was determined using Kjeldahl method with an automatic Kjeldahl Nitrogen Analyzer (Kjeltec auto sampler system 1035 analyzer, Foss Tecator AB, Sweden). Fat content was determined using Soxhlet method with a soxhlet solvent extraction system (Büchi Extraction System B-816 ECE, Büchi AG, Switzerland). Ash content was determined following AOAC method 923.03.

pН

To determine pH values, 5 g of meat batter sample was blended with 20 mL of distilled water for 1 min using a homogenizer (Ultra-Turrax T25, Janke and Kunkel, Germany). The pH was then measured with a pH meter (Navi F-51, HORIBA, Japan). Before measuring pH, the detector was calibrated with pH 4 and pH 7 buffer. All treatments were performed in quadruples.

Color measurement

Batter sample was placed in a petri dish to record color values using a calibrated colorimeter (Minolta Chroma meter CR-210, Minolta Co., Japan; illuminate C, calibrated with a white plate, CIE L*=+97.83, CIE a*=-0.43, CIE b* =+1.98). Cross section samples of cooked meat batters were measured. The following criteria were used: CIE L* (+ lightness, - darkness), CIE a* (+ redness, - greenness), and CIE b* (+ yellowness, - blueness).

Apparent viscosity

Meat batter sample was filled into a 25 mL metal cup and fixed under standard cylinder sensor (SV-2). Viscosity was measured using a rotational viscometer (HAKKE Viscotester® 500, Thermo Electron Corporation, Germany) under constant shear at s⁻¹ for 30 s before each reading was taken.

Cooking loss

Stuffed meat batters were weighed before heated at 75°C in a water-bath (Model 10-101, Dae Han Co., Korea) for 30 min. Before measuring cook loss, cooked samples were cooled at room temperature for 30 min. Cooking loss was estimated using the following formulation:

Cooking loss (%) = [(the weight of raw sample (g) - the weight of cooked sample (g) / weight of raw sample (g)] \times 100

Texture profile analysis (TPA)

Cooked batter was cut into samples with height of 2.5 cm. TPA of unified sample was then measured with a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Surrey, England). The following conditions were used for texture analysis: pre-test speed, 2.0 mm/s; post-test speed, 5.0 mm/s; head speed, 2.0 mm/s; and distance, 4.0 mm. The analysis was performed at room temperature and repeated 6 times for each sample. The average and standard deviation were then calculated from the six measurements.

Statistical analysis

For all data analyses, SPSS 20 (SPSS Inc., USA) was used. Analysis of variance (ANOVA) was used to determine the significance of main effect. Duncan's multiple range test was used to determine differences between means. Student's *t*-test was used to determine significant difference between TG non-added and TG added treatments. Statistical significance was considered at p<0.05.

Results and Discussion

Proximate composition

Results of proximate compositions of meat batters added with different levels of silkworm pupae powder in the presence or absence of TG are shown in Table 2. Moisture contents of meat batters were significantly decreased with increasing levels of silkworm pupae powder, in agreement with results of Hwang and Choi (2015) showing that moi-

¹⁾ Treatments	Moistu	ıre (%)	Prote	in (%)	Fat	(%)	Ash (%)	
	TG non	TG added	TG non	TG added	TG non	TC addad	TG non	TG added
	added	10 auteu	added	added		10 audeu	added	10 auteu
Control	72.96 ± 0.08^{a}	$72.40{\pm}0.07^{a}$	18.25 ± 1.69^{b}	19.93±0.58°	5.61 ± 0.35^{b}	5.22±0.27	2.44±0.05°	$2.52{\pm}0.03^{b}$
T1	69.38 ± 0.24^{b}	67.97 ± 0.17^{b}	19.34 ± 0.20^{bB}	22.43 ± 0.22^{bcA}	$6.20{\pm}0.38^{ab}$	6.51±1.05	2.49 ± 0.01^{bc}	$2.53{\pm}0.03^{b}$
T2	66.19±0.33°	$64.41 \pm 0.01^{\circ}$	19.91 ± 1.08^{bB}	$23.74{\pm}1.70^{bA}$	$7.07{\pm}0.57^{a}$	7.43 ± 1.07	$2.56{\pm}0.04^{ab}$	$2.68{\pm}0.05^{b}$
Т3	63.11 ± 0.35^{d}	62.10 ± 0.10^{d}	$26.58{\pm}1.53^{aB}$	29.89 ± 2.10^{aA}	$7.09{\pm}0.53^{a}$	7.73±1.57	$2.63{\pm}0.04^{a}$	$3.08{\pm}0.22^{a}$

Table 2. Approximate analysis of meat batters formulated with silkworm pupae at various levels with or without TG

All values are mean±SD of three replicates.

¹⁾Treatments: Control, meat batter formulated without silkworm pupae powder and TG; T1, meat batter formulated with 5% silkworm pupae powder; T2, meat batter formulated with 10% silkworm pupae powder; T3, meat batter formulated with 15% silkworm pupae powder. ^{a-d}Means with different superscripts within a same column differ significantly (p<0.05).

 A,B Indicate significant differences in average scores among groups compared (p<0.05).

sture content was decreased in food treated with mealworm powder. However, addition of TG had no significant effect on moisture content of meat batter. Many researchers have reported that TG has no effect on moisture content of meat products (Lee and Chin, 2009; Pietrasik et al., 2007). Protein content of meat batter was increased with increasing levels of silkworm pupae. Protein content of silkworm pupae powder was 54.28%. This might have resulted in the increase of protein content in meat batters added with silkworm pupae. Treatments with TG added resulted in significant increase in protein contents than treatments without any addition of TG. However, control samples without any addition of silkworm pupae showed no significant difference in protein content between TG added and non-added treatments. It has been reported that proteins cross-linked by TG have higher molecular weight than those not treated by TG. Such cross-linking reactions might have resulted in more compact protein molecules (Gujeral and Rosell, 2004). Therefore, the increase in protein content after treatment with TG might be due to higher amount of linkage between TG and free amino groups of silkworm pupae. Fat contents of meat batters added with silkworm pupae powder (6.20% to 7.09%) were higher than those (5.61%) of the control. However, fat contents were not significantly different among TG treatments. Choi et al. (2016) have reported that the incorporation of sea mustard and TG does not change fat contents of frankfurters. Ash content in silkworm pupae was 4.10%. Ash content in meat batter was gradually and significantly increased with increasing level of silkworm pupae addition. These results were in agreement with results of Min et al. (2016) showing that protein, fat, and ash contents in foods added with mealworm powder were higher than those in control foods. Silkworm pupae contain minerals and amino acids, including essential amino acids and sulphur-containing amino acids (Zhou and Han, 2006) that could increase ash content of products.

pH and color evaluation

Results of pH, L* values (lightness), a* values (redness), and b* values (yellowness) of meat batters are summarized in Table 3. The pH value of meat batter containing silkworm pupae was higher than that of the control. This might be to the fact that the pH of silkworm pupae was 6.39. It has been reported that silkworm pupae and mealworm can increase the pH of meat batter, suggesting that addition of insect can directly influence the pH of meat emulsion (Kim *et al.*, 2016). The pH of meat batter treated with TG was higher than that of meat batter without TG treatment. This might be due to the fact that TG has a wide range of pH (from 6.5 to 7.0) (Meiying *et al.*, 2002).

Regarding color evaluation of meat batters, L* value was decreased whereas b* value was increased with the addition of silkworm pupae powder. Similar trends in L* value and b* value have been reported in a study of Hwang and Choi (2015) showing that addition of different levels of mealworm into foods has reduced L* value but increased b* value. Min et al. (2016) have also reported that the addition of mealworm powder to foods can reduce the L* value. It has been shown that increased concentration of termite can darken the color of food due to maillard reaction occurring among amino acids, peptides, and proteins which causes browning (Kinyuru et al., 2009). The a* value of meat batter and cooked meat is important. It represents the appearance of meat product which determines consumer preference (Steenkamp and van Trijp, 1996). Control and 15% silkworm pupae added samples (T3) after cooking had higher a* values than other treatments without any addition of TG. Silkworm is one of those insects that produce melanin in nature (Sug-

¹⁾ Treatments		pH		L	*	8	l*	b*		
		TG non	TG added	TG non	TG added	TG non	TC addad	TG non	TG added	
		added	10 audeu	added	10 added	added	10 audeu	added	10 added	
	Control	5.69 ± 0.01^{dB}	5.80±0.01 ^{dA}	61.55±1.40 ^{aA}	58.84±0.73 ^{bB}	9.37 ± 0.44^{aB}	13.31±0.87 ^{aA}	11.18 ± 0.92^{d}	12.38 ± 0.34^{d}	
Uncooked	T1	$5.82{\pm}0.01^{cB}$	$5.91 {\pm} 0.02^{cA}$	54.77±0.67 ^{cB}	$62.31{\pm}0.71^{aA}$	7.36±0.33°B	$8.35 {\pm} 0.24^{bA}$	18.52±0.42°	18.68±0.34°	
	T2	$5.94{\pm}0.01^{bB}$	$5.97 {\pm} 0.01^{bA}$	57.08 ± 1.50^{b}	55.80±0.78°	$8.43{\pm}0.52^{bA}$	7.12 ± 0.30^{cB}	$20.72{\pm}0.83^{\text{b}}$	$20.95{\pm}0.62^{\text{b}}$	
	T3	$6.01{\pm}0.03^{aB}$	$6.08{\pm}0.01^{aA}$	$55.67{\pm}0.38^{\circ}$	55.57±0.74°	$7.48{\pm}0.34^{\text{cA}}$	6.69 ± 0.20^{cB}	$22.28{\pm}0.79^{a}$	$22.45{\pm}0.24^{a}$	
	Control	5.89 ± 0.07^{dB}	6.04 ± 0.02^{dA}	72.12±0.63 ^{aA}	69.92±0.44 ^{cB}	5.12±0.41 ^a	5.33±0.26 ^a	10.87 ± 0.45^{d}	10.67 ± 0.22^{d}	
Cooked	T1	6.05 ± 0.01^{cB}	6.13±0.01 ^{cA}	62.98±1.39 ^{bB}	$66.28{\pm}0.46^{\text{dA}}$	4.53±0.21 ^b	4.52 ± 0.12^{b}	15.75±0.70°	16.24±0.42°	
	T2	6.13 ± 0.01^{bB}	6.17 ± 0.01^{bA}	63.69 ± 0.52^{bA}	$62.43{\pm}0.98^{bB}$	$4.46{\pm}0.08^{b}$	4.56 ± 0.14^{b}	$18.78 {\pm} 0.36^{b}$	$18.82{\pm}0.38^{b}$	
	T3	$6.18{\pm}0.01^{a}$	$6.20{\pm}0.03^{a}$	59.40±0.30 ^{cA}	$58.39{\pm}0.61^{aB}$	$4.97{\pm}0.37^{a}$	5.31±0.24ª	$21.07{\pm}0.46^{a}$	$21.21{\pm}0.69^{a}$	

Table 3. Results of pH and color measurement of meat batters formulated with various levels of silkworm pupae with or without TG

All values are mean±SD of three replicates.

¹⁾Treatments: Control, meat batter formulated without silkworm pupae powder and TG; T1, meat batter formulated with 5% silkworm pupae powder; T2, meat batter formulated with 10% silkworm pupae powder; T3, meat batter formulated with 15% silkworm pupae powder.

^{a-d}Means with different superscripts within a same column differ significantly (p<0.05). ^{A,B}Indicate significant differences in average scores among groups compared (p<0.05).

umaran, 2002). Its yellow to reddish brown pheomelanin might have increased a* and b* values of treatments. Results of color evaluation for meat batters with or without TG treatment showed no significant difference. However, meat batter added with TG showed reduced L* value except T1. Similar results have been reported by Lee and Chin (2009) showing that pork sausage added with TG has decreased L* value due to formulation of additives and TG.

Apparent viscosity

Results of apparent viscosities of meat batters added with various concentrations of silkworm pupae with or without TG are shown in Fig. 1. Viscosity was increased by the addition of silkworm pupae powder and TG. T3 treatment showed the highest viscosity among different meat batters without TG. Various edible insects have functional properties such as water and oil absorption capacity and emulsifying and foaming ability that could improve viscosity (Assielou *et al.*, 2015; Park and Park, 1986). Higher pH than isoelectric point could improve binding characteristics of meat emulsions because myosin and actomyosin gel strengths are increased with higher pH (optimum 5.5-6.0) (Zorba *et al.*, 2005). The pH value of meat batter added with silkworm pupae was increased from



Fig. 1. Changes in apparent viscosity of meat batters formulated with various levels of silkworm pupae with or without TG. ¹⁾Treatments: Control(\bigcirc): meat batter without silkworm pupae powder without TG, Control-1(\bigcirc): meat batter without silkworm pupae powder without TG, T1-1(\blacksquare): meat batter with 5% silkworm pupae powder without TG, T1-1(\blacksquare): meat batter with 5% silkworm pupae powder without TG, T2-1(\diamondsuit): meat batter with 10% silkworm pupae powder without TG, T2-1(\diamondsuit): meat batter with 10% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powder without TG, T3-1(\bigstar): meat batter with 15% silkworm pupae powde

5.82 to 6.01. Observed results with control batters having the lowest viscosity whereas treatments samples added with silkworm pupae and TG having higher viscosity values might be due to cross-link between TG and silkworm pupae protein. The viscosity of the meat batter added with TG in T3 was higher than that of any other treatments with TG. Hong et al. (2012) have observed that viscosity of pork myofibrillar protein is increased by the addition of TG, consistent with our results. Ionescu et al. (2008) have shown that the increase in viscosity of myofibrillar protein from beef with TG treatment is due to catalyzed TG with protein chains. The cross-linkage between TG and protein can affect the viscosity of food, making it stiffer and more rigid than the control, thus improving the viscosity (Gujral and Rosell, 2004). This finding signifies that silkworm pupae can improve the viscosity of meat batter while the addition of TG also enhances the rheological property of meat batter.

Cooking loss

Results of cooking loss of meat batters containing different amounts of silkworm pupae with TG treatment are shown in Fig. 2. Cooking loss of meat batter was the highest in the control. It was decreased after silkworm pupae powder was added. Kim *et al.* (2016) have suggested that silkworm pupae and mealworm can reduce the cooking loss of emulsion sausage due to reduced moisture content. Similar result of reduced cooking loss with increasing edible insect level has been reported by Kim *et al.* (2008) for pork patties added with silkworm powder. The significant decrease of cooking loss for meat batter added with silkworm powder might be due to pH and viscosity. High pH can affect the degree of net electrostatic repulsion between myofilaments that regulate water to locate in meat matrix, thus resulting in reduced cooking loss (Kim et al., 2010). In general, cooking loss is associated with viscosity, an indicator of binding ability of meat batter. Choe et al. (2013) have reported that viscosity of meat batter is increased when cooking loss is decreased. However, TG addition had no significant effect on cooking loss of meat batter, similar to results of Stevenson et al. (2013) on chicken protein gels and those of Dimitrakopoulou et al. (2005) on pork. These results are also in agreement with those of Chin et al. (2009) showing that there is no significant difference in cooking loss of protein gel after it is treated with a combination of TG and konjac powder. This result was probably due to increased salt content by adding silkworm pupae (salinity, 1.8%). High salt concentration can affect water-entrapment in protein matrix structure, thus decreasing cooking loss (Chin et al., 2009).

Texture profile analysis (TPA)

Results of texture profile analysis for meat batters formulated with silkworm pupae powder and TG are summarized in Table 4. Generally, texture in food makes a significant contribution to its overall quality (Bourne, 2002). Hardness, gumminess, and chewiness of meat batters were increased when silkworm pupae level was increased. Gumminess and chewiness showed the same trend as hardness. In fact, there are hardness-related test based on the definition of gumminess and chewiness (Lin *et al.*, 2000). Similar results have been obtained by Min *et al.* (2016) showing that increasing mealworm level has significantly increased the hardness of foods. It has been reported that



Fig. 2. Cooking loss of meat batters formulated with various levels of silkworm pupae with or without TG. ¹⁾Treatments: Control, meat batter formulated without silkworm pupae powder and TG; T1, meat batter formulated with 5% silkworm pupae powder; T2, meat batter formulated with 10% silkworm pupae powder; T3, meat batter formulated with 15% silkworm pupae powder. The vertical bars show the standard deviation. ^{a-c} Means in treatments with different letters are significantly different (p<0.05).

	Hardness (kg)		Cohesiveness		Springiness		Gumminess		Chewiness	
¹⁾ Treatments	TG non	TG added	TG non	TG added	TG non	TG added	TG non	TG added	TG non	TG added
	added	10 audeu	added	10 audeu	added	10 audeu	added		added	10 audeu
Control	0.25±0.03 ^{dB}	0.48±0.03 ^{cA}	0.47 ± 0.02^{B}	0.50±0.03 ^A	$0.81{\pm}0.06$	0.81 ± 0.04^{b}	0.12 ± 0.01^{dB}	0.24±0.02 ^{cA}	0.09±0.01 ^{dB}	0.20±0.02 ^{cA}
T1	0.45 ± 0.02^{cB}	$0.73{\pm}0.04^{bA}$	$0.47{\pm}0.02^{\rm B}$	$0.52{\pm}0.02^{A}$	0.83 ± 0.04	$0.82{\pm}0.05^{b}$	0.21±0.01 ^{cB}	$0.38{\pm}0.02^{bA}$	0.18 ± 0.02^{cB}	$0.31{\pm}0.03^{bA}$
T2	$0.70{\pm}0.02^{bB}$	$0.85{\pm}0.03^{aA}$	0.48 ± 0.02	0.50 ± 0.01	0.83 ± 0.05	$0.84{\pm}0.03^{ab}$	$0.34{\pm}0.02^{bB}$	$0.42{\pm}0.02^{aA}$	0.28 ± 0.03^{bB}	$0.35{\pm}0.02^{aA}$
Т3	$0.77{\pm}0.03^{aB}$	$0.86{\pm}0.04^{aA}$	0.48 ± 0.01	0.51 ± 0.03	0.86 ± 0.06	$0.88{\pm}0.03^{a}$	$0.37{\pm}0.01^{aB}$	$0.44{\pm}0.04^{aA}$	$0.32{\pm}0.02^{aB}$	$0.38{\pm}0.05^{aA}$

Table 4. Texture profile analysis (TPA) of meat batters formulated with various levels of silkworm pupae with or without TG

All values are mean±SD of three replicates.

¹⁾Treatments: Control, meat batter formulated without silkworm pupae powder and TG; T1, meat batter formulated with 5% silkworm pupae powder; T2, meat batter formulated with 10% silkworm pupae powder; T3, meat batter formulated with 15% silkworm pupae powder. ^{a-d}Means with different superscripts within a same column differ significantly (p<0.05).

 A,B Indicate significant differences in average scores among groups compared (p<0.05).

patties added with 10% mealworm powder have higher hardness, chewiness, and gumminess values than control patties (Kim et al., 2015). These values are increased as mealworm addition level is increased (Kim et al., 2015). Kim et al. (2016) have reported that emulsion sausages added with silkworm pupae and mealworm have higher hardness values than control sausages due to reduced moisture contents after the addition of edible insects. Meat batter added with 15% silkworm pupae (T3) had higher texture properties than control batter due to its lower moisture content. This result is in agreement with that of Lin et al. (2000) showing that the low moisture content of soy protein meat has resulted in hard texture. Hard texture of meat batter could be due to reduced cooking loss. Many researchers have shown that meat products with decreased cooking loss have high values of hardness (Choi et al., 2012; Pietrasik and Duda, 2000). Incorporation of TG into meat batter significantly improved its texture properties except springiness. These results were in agreement with those of Lee and Chin (2009) reporting that the hardness, gumminess, and chewiness values of pork sausages added with TG were higher than those of control sausages. Crosslink with TG and proteins have also improved and maintained texture properties of foods (Motoki and Seguro, 1998). Choi et al. (2016) have shown that hardness of sausage is increased by the addition of TG. Atilgan and Kilic (2017) have shown that a combination of TG and additives can improve textural properties of ground meat. Non-meat proteins modified by TG can improve the breaking stress of sausage due to cross-linkage between TG and non-meat proteins (Muguruma et al., 2003). Rosell et al. (2003) have reported that 1% TG with lysine in protein can increase the extensibility of foods. Lysine content in silkworm pupae is 47.3-50.0 mg/g. It might be able to interact with TG and improve the bind ability of meat products (Rumpold and Schlüter, 2013). Intensive bonds between TG and silkworm pupae might have resulted in high TPA values in this study.

Conclusions

In the present study, silkworm pupae showed potential as a valuable additive for meat products. The addition of silkworm pupae increased nutrition values such as protein, fat, and ash contents but reduced the cooking loss of meat batter. Insufficient binding ability of silkworm pupae containing meat batter was improved by the addition of TG. Meat batter containing silkworm pupae and TG showed increased pH, viscosity, and texture properties compared to the control. Further studies are needed to determine the effect of silkworm pupae as a meat replacement in meat products.

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