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Original Article

Characteristic of sausages as influenced by partial replacement of pork back-fat using pre-emulsified soybean oil stabilized by fish proteins isolate

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ABSTRACT

Substitution of animal fat with oils rich in *n*-3 is a feasible way to improve the nutritive value of comminuted meat product. The effect on the characteristics of sausages was investigated of partial replacement of porcine fat with soybean oil (SBO) using a pre-emulsification technique. Fish protein isolate (FPI) produced from yellow stripe trevally (*Selaroides leptolepis*) was used as an emulsifier to prepare pre-emulsified SBO (preSBO), and its concentration effect (1%, 2% and 3%, w/v) was observed in comparison with soy protein isolate (SPI). Substitution of porcine fat using preSBO enhanced the product stability. SPI exhibited better emulsifying ability than FPI. However, FPI was more effective at reinforcing the protein matrix of the sausages than SPI, as suggested by a lowered cooking loss and the restored textural attributes of the sausages formulated with FPI stabilized preSBO. The effective concentration of FPI to improve the product stability was 2%. This work suggested that FPI was promising in the preparation of emulsified meat products.

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Introduction

Emulsified meat products in the presence of high animal fat content contain abundant amounts of saturated fatty acids and cholesterol that may adversely affect consumer health (Jiménez-Colmenero et al., 2001). Several health benefits of *n*-3 fatty acids, for example linolenic, eicosapentaenoic and docosahexaenoic acids, are recognized such as antithrombogenic and anti-nflammatory abilities (Connor, 2000). Using oils rich in *n*-3, for example linseed, rapeseed, soybean, and fish oils, to partially replace animal fats is a feasible way to improve the nutritive profile of meat products (Josquin et al., 2012). By varying the fat sources, the characteristics of meat products could be affected (Youssef and Barbut, 2011).

Inclusion of vegetable oils through a pre-emulsification technique could enhance the stability of some meat products, such as pork frankfurter (Bloukas et al., 1997) and traditional Spanish fermented sausage (Muguerza et al., 2001). Non-meat proteins employed as emulsifier to prepare pre-emulsified oil could reinforce the protein matrix, thereby improving product stability (Youssef and Barbut, 2011). The gelation activity of non-proteins plays a crucial role in promoting the matrix strength of comminuted meat products, (Su et al., 2000). Functional properties have been reported, especially on the gelation capacity, of proteins isolated from various fishes, such as herring (Underland et al., 2002), cod (Kristinsson and Hultin, 2003) and striped bass (Tahergorabi et al., 2012). However, study on the utilization of fish proteins in emulsified meat products is still restricted. By adding protein hydrolyzate prepared from skipjack roe to fish emulsion sausages, the formation of small-sized fat globules with high uniformity was observed, which was supposedly due to the effective emulsifying activity of the protein hydrolyzate (Intarasirisawat et al., 2014). Incorporation of protein hydrolyzates prepared from Goby fish (*Zosterisessor ophiocephalus*) could delay lipid oxidation in turkey meat sausage (Nasri et al., 2013). In the present work, the emulsifying ability of fish protein isolate (FPI) employed to stabilize pre-emulsified soybean oil (preSBO) was investigated, by comparison with soy protein isolate (SPI). SPI is widely employed in manufacturing meat products (Omana et al., 2012). Nonetheless, SPI could cause a masking effect on flavor by creating a “cereal-like” off-flavor (Matulis et al., 1995). The present work aimed to investigate the effect of a partial replacement of pork back-fat using FPI-stabilized preSBO on the properties of sausages. The FPI used in the

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present work was produced from yellow stripe trevally (*S. leptolepis*), because of its abundance and low price.

Materials and methods

Materials

A yellow stripe trevally of size about 20 fish/kg, fresh pork shoulder, pork back-fat, SBO, NaCl, sugar and monosodium glutamate were purchased from a local market (Songkhla, Thailand). The SPI was provided by Abbra Co., Ltd (Bangkok, Thailand). Sodium nitrite was a product of Ajax FineChem (Auckland, New Zealand). Sodium tripolyphosphate was purchased from Aditya Birla Chemicals (Samutprakarn, Thailand). Seasoning powder was provided by Flavor Force Co., Ltd (Bangkok, Thailand).

Fish protein isolate preparation

The FPI was prepared through a pH-shift method following the procedure of Hultin and Kelleher (2000) as modified by Rawdkuen et al. (2009). Briefly, fish were taken to Prince of Songkla University within 1 h by placing in ice with a ratio of fish:ice of 1:2 (weight per weight). Upon arrival, the fish were washed and the whole meat was separated. The chemical composition of the fish flesh was determined (Association of Official Analytical Chemists, 1995). Then, the meat was minced and mixed with cold distilled water (4 °C) using a homogenizer (Ultra Turrax T25; Ika; Staufen, Germany) at 19,000 rpm for 1 min, before adjusting the pH to 11.2 using 1 M NaOH. The extraction was carried out at room temperature for 10 min. Insoluble materials were removed using a centrifuge (CR 22GIII high-speed refrigerated centrifuge; Hitachi; Tokyo, Japan) at 10,000 rpm for 20 min at 4 °C. The muscle protein was recovered by adjusting the pH of the supernatant to 5.5 and collected using a cheese-cloth. The isolated protein was neutralized, lyophilized in a freeze dryer (FTS system Flex-Dry™; SP Scientific; New York, NY, USA), and kept at 4 °C for less than 2 mth before use.

Preparation and characterization of pre-emulsified soybean oil

First, the emulsifying activity of FPI was investigated by comparing with SPI. The selected protein was dissolved in 10 mM phosphate buffer pH 7 at designated concentrations, before homogenizing with SBO at 19,000 rpm for 5 min. The final emulsion contained an oil volume fraction of 0.5, and FPI (or SPI) at different concentrations (1%, 2% or 3%, weight per volume; w/v). The emulsions were kept at 4 °C overnight, before being subjected to analyses.

Emulsion size

The droplet size was measured using a laser diffraction particle size analyzer (ZetaPALS; Brookhaven Instruments; Holtsville, NY, USA), and estimated as a volume-weighted average mean diameter, $d_{43} = \sum n_i d_i^4 / n_i d_i^3$, where n_i is the number of droplets of diameter (d_i). The droplet size distribution pattern was also observed.

Thermal stability

The emulsion was heated at 70 °C for 15 min in a water bath (Memmart; Schwabach, Germany), and the thermal stability was evaluated as the percentage of oil released from the emulsified matrix (Jiménez-Colmenero et al., 1995).

Characteristics of sausages as affected by partial replacement of pork back-fat with pre-emulsified soybean oil.

Sausage preparation

All visible fat and connective tissue were removed from the porcine meat. The chemical composition of the pork meat and back-fat was investigated (Association of Official Analytical Chemists, 1995). The protein, fat, and moisture contents of porcine meat (back-fat) were 24.0 (3.1), 1.3 (83.3), and 72.9 (10.9) g per 100 g, respectively. The porcine meat and back-fat were comminuted separately to make a homogeneous mass, then packed separately in a polyethylene bag, and frozen at −18 °C for less than 2 mth. The frozen meat and fat were thawed at 4 °C overnight prior to use. The preSBO was prepared on the day before sausage preparation.

The control sausage contained 30% porcine fat and 15% protein. Partial replacement, (using SBO corresponding to a weight substitution by 25% of pork back-fat) was implemented for the studied samples. The water content of all formulations was controlled at 70%, and the water present in the preSBO was subtracted from the amount of added water. The formulations of the sausage samples are shown in Table 1.

To the prepare sausages, first the additives were mixed on the day of use by dissolving in the added water and chilled at 4 °C. The comminuted meat was chopped using a mixer grinder (MK-K77; National; Tokyo Japan) at low speed for 30 s, followed by adding one-half of the back-fat (or preSBO). One-half of the additive mixture was added before further mixing for 1 min. The rest of the additive mixture and back-fat (or preSBO) was then added and chopped for 1 min. The mixture was allowed to stand (1.5 min) for protein extraction, before further chopping for 2 min. The temperature was controlled to remain lower than 12 °C throughout the process. The batter was stuffed in a cellulose casing (15 mm in diameter), heated at 75 ± 2 °C for 30 min, and cooled in cold water (15 °C). To elucidate the effect of the oil added through the pre-emulsification technique, sausages added with SBO in pure form were also prepared for comparison. In this work, the sausages without SBO inclusion, the sausages incorporated with SBO in a pure form, FPI-stabilized preSBO, and SPI-stabilized preSBO are referred to as Control, P-SBO, FPI-preSBO and SPI-preSBO, respectively. The sausages were placed in polyethylene bags, packed without vacuum and stored at 4 °C. This method was modified from the procedure of Jiménez-Colmenero et al. (2010). Twenty sausages were prepared in each formulation for duplicate experiments and subjected to analyses.

Cooking loss

Each accurately weighed sausage was placed in a polyethylene bag and heated at 80 °C for 20 min. After cooling to room temperature, the cooking loss was calculated from the difference in weight between the unheated and heated sample and expressed as a percentage of the initial weight (Hur et al., 2008).

Water holding capacity

Each accurately weighed sausage was placed in a plastic tube and centrifuged at 1000 *g* for 10 min at 4 °C. The water holding capacity (WHC) was expressed as a percentage of the difference in weight of the sample before and after centrifugation (Hur et al., 2008).

Color

The color of a fresh-cut cross-section sample was determined using the CIE system and reported as L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) using a HunterLab colorimeter (ColorFlex; Hunter Associates Laboratory Inc.; Reston, VA, USA).

Texture profile analysis

Five cylinder-shaped samples (length 250 mm) were prepared and subjected to texture profile analysis (TPA) using a texture analyzer (TA-XT2; Stable Micro Systems; Godalming, UK) equipped

Table 1
Sausage formulas (contents per 100 g) where Control = sausages without soybean oil (SBO); P-SBO = sausages added with SBO in pure form; FPI-preSBO = sausages added with pre-emulsified SBO (preSBO) stabilized with FPI; SPI-preSBO = sausages added with preSBO stabilized with soy protein isolate (SPI).

Sample	Fat level (%)	Pork back-fat (g)	Added water (g)	Lean meat (g)	SBO (g)	preSBO (g)	Protein level (%)
Control	30	36.0	23.9	57.8	–	–	15.00
P-SBO	30	27.0	24.1	59.0	9	–	15.01
1%FPI-preSBO	30	27.0	15.1	59.0	–	18.0	15.86
2%FPI-preSBO		27.0	15.1	59.0	–	18.0	16.70
3%FPI-preSBO		27.0	15.1	59.0	–	18.0	17.55
1%SPI-preSBO	30	27.0	15.1	59.0	–	18.0	15.92
2%SPI-preSBO		27.0	15.1	59.0	–	18.0	16.84
3%SPI-preSBO		27.0	15.1	59.0	–	18.0	17.75

with a cylindrical plunger (diameter 5 mm and a depression speed of 5 mm/s).

Microstructure

The morphology of the sausages was investigated using a scanning electron microscope (Quanta 400; FEI Europe BV; Eindhoven, the Netherlands), following the method of Jiang and Xiong (2013) with a slight modification. A cubic sample ($2.5 \times 2.5 \times 2.5$ mm) was fixed using glutaraldehyde (2%) for 24 h at 4 °C. Then, dehydration was conducted by a series of ethanol solutions with concentrations of 50%, 70%, 80% and 90% once each for 10 min, and twice in absolute ethanol for 10 min. The samples were then subjected to critical point drying (model CPD 030; BAL-TEC; Balzers, Liechtenstein) using CO_2 as the transition fluid. The dried samples were sputter-coated with gold and observed at an accelerating voltage of 15 kV.

Statistical analysis

All experiments were run in triplicate and mean values with standard deviations were reported. A completely randomized design was used for the experiments. Statistical analysis was performed using analysis of variance and mean comparisons were tested using Duncan's multiple range test (SPSS for Windows; SPSS Inc.; Chicago, IL, USA) at a confidence level of 95%.

Results and discussion

Emulsifying activity of fish protein isolate

The fish meat consisted of protein (21.3%), fat (1.6%), moisture (75.2%) and ash (1.2%). The protein recovery efficiency was 63.7 ± 1.5%. The alkaline condition of the pH shift protein extraction method could maintain sarcoplasmic proteins, resulting in effective protein isolation (Rawdkuen et al., 2009). Extensive washing as used in the conventional method enhanced sarcoplasmic protein solubility, thereby lowering the protein recovery yield (Rawdkuen et al., 2009). From the gel electrophoresis analysis, the predominant protein bands of FPI had molecular weights (MWs) of about 35–50 kDa (data not shown), and was assumed to be actin. The lowest solubility of FPI was found at pH 5.5 (about 20 mg/g), as this was expected to be the isoelectric point of FPI (Kim et al., 2003). Greater solubility of FPI was observed under alkaline conditions compared to acidic conditions, and the highest solubility was found at pH 12 (about 410 mg/g). This behavior was in accordance with the solubility of the proteins isolated from other fish species such as Pacific whiting (*Merluccius productus*; Kim et al., 2003) and Tilapia (*Oreochromis niloticus*; Kristinsson and Ingadottir, 2006).

The droplet size and thermal stability of the emulsions stabilized by FPI and SPI at different concentrations (1%, 2% or 3%) are shown in Table 2. Larger-sized oil droplets were found for the emulsions stabilized with FPI than for SPI ($p \leq 0.05$). Increasing the SPI concentration to 2% could decrease the droplet size ($p \leq 0.05$). Nonetheless, the FPI concentration had no effect on the droplet size of the emulsions ($p > 0.05$). Broader droplet size distribution patterns, suggesting greater polydispersity of the emulsion, were observed with increasing protein concentrations (Fig. 1). By increasing the concentration, the proteins might not have been entirely adsorbed to the oil-water interfaces, and the presence of unadsorbed protein residues localized between oil drops and in the aqueous phase could be expected (Euston et al., 2000). These unadsorbed protein residues might have enhanced the aggregation of the dispersed oil droplets (Euston et al., 2000). A large size and higher polydispersity of oil droplets was observed for the FPI-stabilized emulsions compared to the SPI-stabilized emulsions might suggest a lower adsorbed amount with FPI than for SPI.

Better thermal stability was found when the emulsions were stabilized by FPI compared to SPI, as indicated by the clearly lowered released oil content of the former emulsions at all protein concentration levels ($p \leq 0.05$). As a result of the heating process, the proteins underwent partial denaturation, thereby increasing the surface hydrophobicity of the molecules. Then, association between neighboring protein residues could be enhanced via hydrophobic interactions (Kulmyrzaev et al., 2000). This phenomenon strengthened the interfacial protein film and resulted in improved thermal stability of the emulsion (Feng and Xiong, 2002). It has been suggested that the protein molecules of yellow stripe trevally underwent unfolding at 40 °C (Arfat and Benjakul, 2012). A lower thermal stability of the SPI stabilized emulsions might be postulated based on the insufficient unfolding of SPI at the observed temperature (70 °C), so that restricted interaction between the protein residues could be expected. The denature temperatures of the major components of SPI were reported as 75 °C and 91 °C for the 7S and 11S soy proteins, respectively (Scilingo and Anon, 1996). For the FPI-based emulsions, the increased protein concentration tended to provide the emulsions with better thermal stability. This effect is postulated to have been due to the presence of unadsorbed proteins in a continuous phase of the emulsion with increased protein concentration. In the heat-treated emulsion, the protein aggregation was more extensive and proceeded more rapidly with the presence of unadsorbed proteins attributed to greater interactions between the protein residues (Euston et al., 2000). With the greater degree of protein interaction, the interfacial films covering the oil droplets might be strengthened, thereby enhancing the thermal stability of the emulsions. Nonetheless, any differences in the thermal stability of the emulsions stabilized by SPI at different concentrations were negligible ($p > 0.05$). Better thermal stability of FPI-based emulsions than SPI-based emulsions was coincident with a large size and higher polydispersity of the oil drops of the former emulsions, presumably since there was a greater amount of unadsorbed FPI compared to SPI present in the emulsion system.

Table 2
Mean diameter of oil droplets (d_{43}) and percentage of oil released in the emulsions stabilized by fish protein isolate (FPI) and soy protein isolate (SPI) at different protein concentrations.

Protein concentration (%, weight per volume)	d_{43} (nm)		Oil released (%)	
	FPI	SPI	FPI	SPI
1	$5271.4 \pm 267.2^{aA*}$	4241.9 ± 260.9^{aB}	1.01 ± 0.27^{aAB}	1.72 ± 0.01^{aA}
2	4993.8 ± 399.4^{aA}	2758.8 ± 396.9^{bB}	0.79 ± 0.01^{abB}	2.11 ± 0.15^{aA}
3	4633.0 ± 348.8^{aA}	2775.4 ± 338.4^{bB}	0.48 ± 0.01^{bB}	2.11 ± 0.42^{aA}

Means ± SD are shown ($n = 3$).
* In each tested parameter, different uppercase, superscript letters indicate significant differences between means in the same row ($p \leq 0.05$), and different lowercase, superscript letters indicate significant differences between means in the same column ($p \leq 0.05$).

Characteristics of sausages affected by partial replacement of porcine fat with pre-emulsified soybean oil

Fig. 2 shows the cooking loss and WHC of the sausages as affected by the substitution of porcine fat using SBO in alternative forms, involving P-SBO, FPI-preSBO and SPI-preSBO. P-SBO had a lower cooking loss compared to the Control ($p \leq 0.05$), suggesting improved stability in the sausages from the SBO inclusion. Partial replacement of beef fat using canola oil could enhance the stability of the comminuted meat products as indicated by a lowered fluid loss and the restored texture parameter, which was explained by a lower melting point of canola oil compared to beef fat (Youssef and Barbut, 2011). Paneras et al. (1998) also reported a firmer texture of low-fat frankfurters incorporated with vegetable oils compared to those made with animal fat. Compared to the Control, a lowered cooking loss was observed for the FPI-preSBO, whereas SPI-preSBO had a higher cooking loss ($p \leq 0.05$). Oil introduced via the pre-

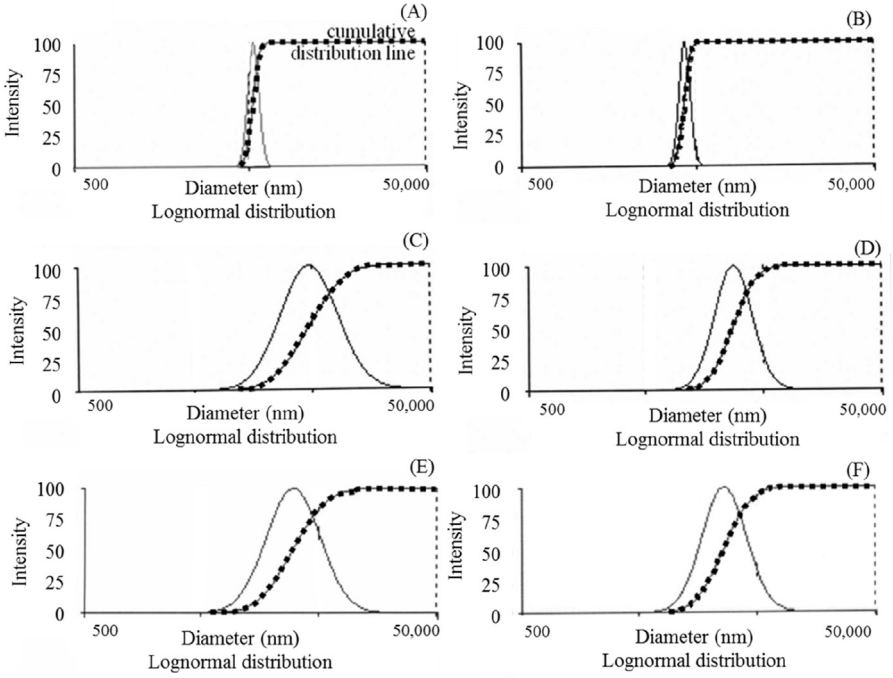


Fig. 1. Droplet size distribution patterns of the emulsions stabilized using fish protein isolate at different concentrations (weight per volume): (A) 1%; (C) 2%; (E) 3% and using soy protein isolate at different concentrations (weight per volume): (B) 1%; (D) 2%; (F) 3% (dashed curve shows the cumulative distribution.).

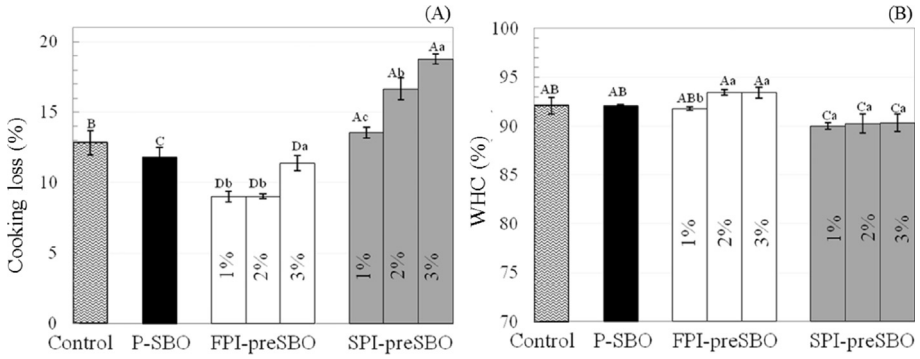


Fig. 2. (A) Cooking loss; (B) water holding capacity (WHC) of different sausages. (percentages indicate concentration as weight per volume of fish protein isolate (FPI) and soy protein isolate (SPI); Control = sausages without soybean oil (SBO); P-SBO = sausages added with SBO in pure form; FPI-preSBO = sausages added with pre-emulsified SBO (preSBO) stabilized with FPI; SPI-preSBO = sausages added with preSBO stabilized with SPI; error bars show ± SD; in each subfigure, different letters indicate significant differences ($p < 0.05$) between means, with uppercase letters showing effect of SBO inclusion and lowercase showing effect of FPI and SPI concentrations).

emulsification technique could enhance the protein matrix strength by the participation of non-meat proteins in the gel network (Wu et al., 2009; Youssef and Barbut, 2011). The more compact, finer and homogeneous microstructure of the heat-induced meat gel was observed by adding pea protein isolates, presumably due to the cross linking between the pea proteins and the muscle proteins via hydrophobic interactions induced by the heating process (Sun et al., 2012). Nonetheless, different non-meat proteins used as emulsifier greatly affected the properties of the meat products. Na-caseinate had effective emulsifying capacity, but there was no improvement in stability observed in the sausage added with Na-caseinate-stabilized, pre-emulsified canola oil (Youssef and Barbut, 2011). This was explained by the inferior gelation activity of Na-caseinate under the elevated temperature conditions in sausage preparation (Youssef and Barbut, 2011). In the present work, the SPI-preSBO had more than about 1.5 times higher cooking loss compared to the FPI-preSBO. The higher cooking loss for the SPI-preSBO than for the FPI-preSBO was coincident with the lowered thermal stability of the emulsions stabilized by SPI than for those stabilized by FPI (see Table 2). A higher purge loss of low-fat

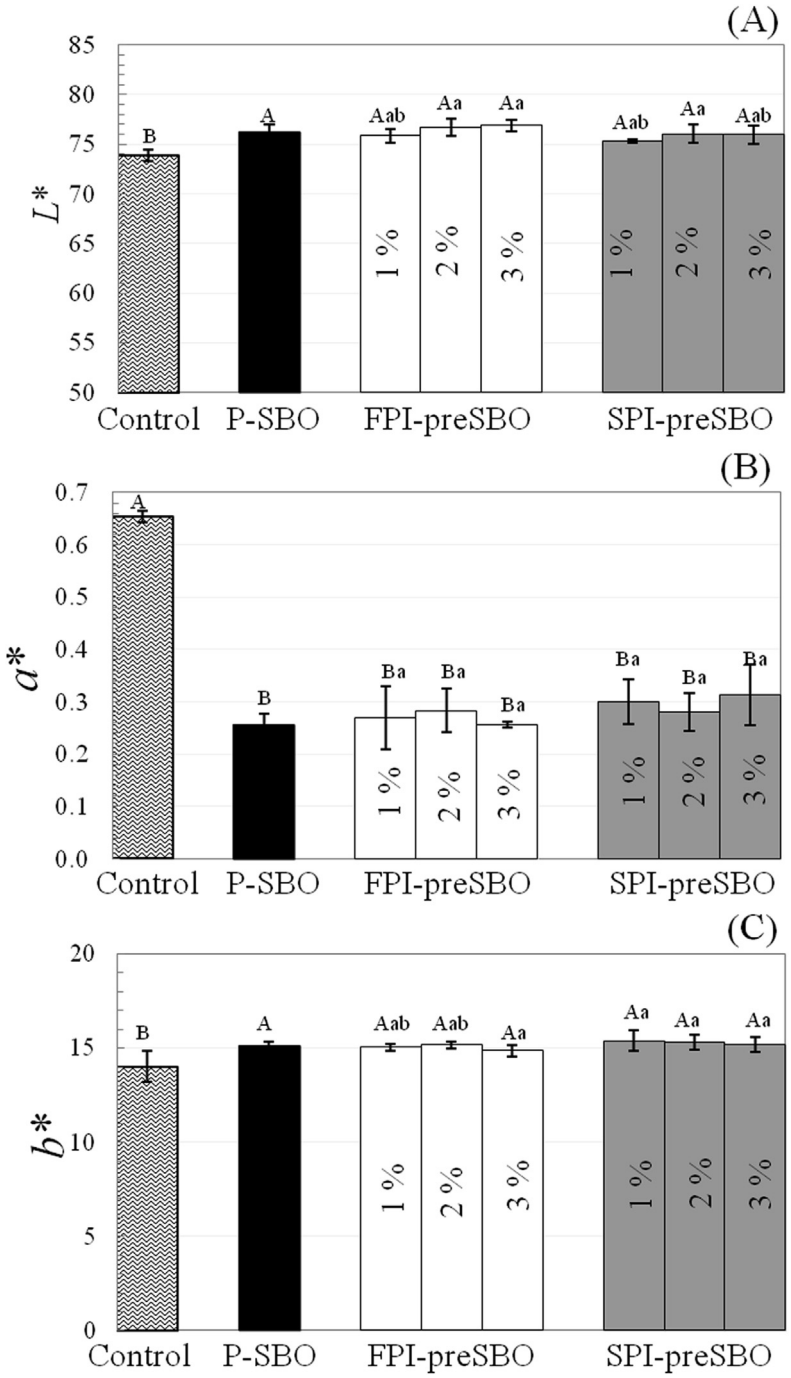


Fig. 3. (A) Lightness (L^*); (B) redness (a^*); (C) yellowness (b^*) of sausages. (percentages indicate concentration as weight per volume of fish protein isolate (FPI) and soy protein isolate (SPI); Control = sausages without soybean oil (SBO); P-SBO = sausages added with SBO in pure form; FPI-preSBO = sausages added with pre-emulsified SBO (preSBO) stabilized with fish protein isolate (FPI); SPI-preSBO = sausages added with preSBO stabilized with soy protein isolate (SPI); error bars show ± SD; in each subfigure, different letters indicate significant differences ($p < 0.05$) between means, with uppercase letters showing effect of SBO inclusion and lowercase showing effect of FPI and SPI concentrations).

frankfurter was observed when SPI was incorporated in the products than for those added with muscle protein isolate (Yang et al., 2001). In the present work, SPI showed better emulsifying ability than FPI as implied by the smaller d_{43} of the SPI-stabilized emulsions (Table 2), but the better ability of FPI to strengthen the sausage matrix was evident. By introducing oils through the pre-emulsification technique, the gelation and water retention capacities of non-meat proteins played crucial roles rather than the emulsifying ability in determining meat product stability (Su et al., 2000). The lowest WHC was also observed for the SPI-preSBO ($p \leq 0.05$), whereas there was no difference in the WHC between the Control with P-SBO and FPI-preSBO ($p > 0.05$).

Increasing the FPI concentration to 2% led to a higher WHC ($p \leq 0.05$), whereas the SPI concentration had no effect on the WHC of the sausages ($p > 0.05$). Reinforcement of the heat-set emulsified meat matrix by increasing the protein concentration has been previously reported (Feng and Xiong, 2002). For the FPI-preSBO, using FPI at 3% led to a higher cooking loss than at 1% and 2% ($p \leq 0.05$). However, there was a considerable cooking loss with an increased SPI concentration for the SPI-preSBO ($p \leq 0.05$). A reduction in the muscle protein gel hardness was observed with increased non-meat protein content: By increasing the pea protein isolate (PPI) to 3.5% (4%), the strength of chicken breast (chicken thigh) protein gel was diminished (Sun et al., 2012). This behavior was expected since self association occurred between the muscle proteins and PPI at an increased PPI concentration, thereby reducing the stabilizing effect of PPI (Sun et al., 2012).

Partial replacement of porcine fat with SBO noticeably increased the lightness and yellowness as well as reducing the redness of the sausages ($p \leq 0.05$; Fig. 3). This tendency was in accordance with the study of Bloukas et al. (1997), who reported increased lightness and yellowness of sausages when animal fat was partially substituted with olive oil. Pre-emulsified vegetable oil droplets tended to have a smaller size than animal fat globules (Youssef and Barbut, 2011). With the larger surface area of the smaller-sized oil drops, preSBO might reflect light more effectively than the porcine fat globules, thereby increasing the color lightness of the samples (Youssef and Barbut, 2011). Markedly diminished redness of bologna was observed when porcine fat was substituted with pre-emulsified corn oil (Bishop et al., 1993). Increased yellowness of the sausages with SBO inclusion might have been due to the yellow color of the vegetable oil. There was no noticeable difference in the color of the FPI-preSBO and SPI-preSBO samples, irrespective of the protein concentration ($p > 0.05$).

Fig. 4 shows the texture parameters of the sausages as affected by SBO inclusion. The P-SBO and FPI-preSBO had the highest hardness ($p \leq 0.05$). Replacement of beef fat using canola oil increased the hardness of the comminuted meat matrix (Youssef and Barbut, 2011). This behavior was explained as due to the more pronounced interaction between protein molecules occurring in the presence of smaller-sized canola oil drops with larger surface areas compared to the beef fat globules, thereby reinforcing the protein matrix (Youssef and Barbut, 2010). Compared to the Control, the sausages formulated with SBO had higher gumminess and chewiness ($p \leq 0.05$), whereas there were no noticeable differences in springiness and cohesiveness ($p > 0.05$).

Generally, the substitution of porcine fat with SBO restored the texture attributes as indicated by the higher hardness, gumminess

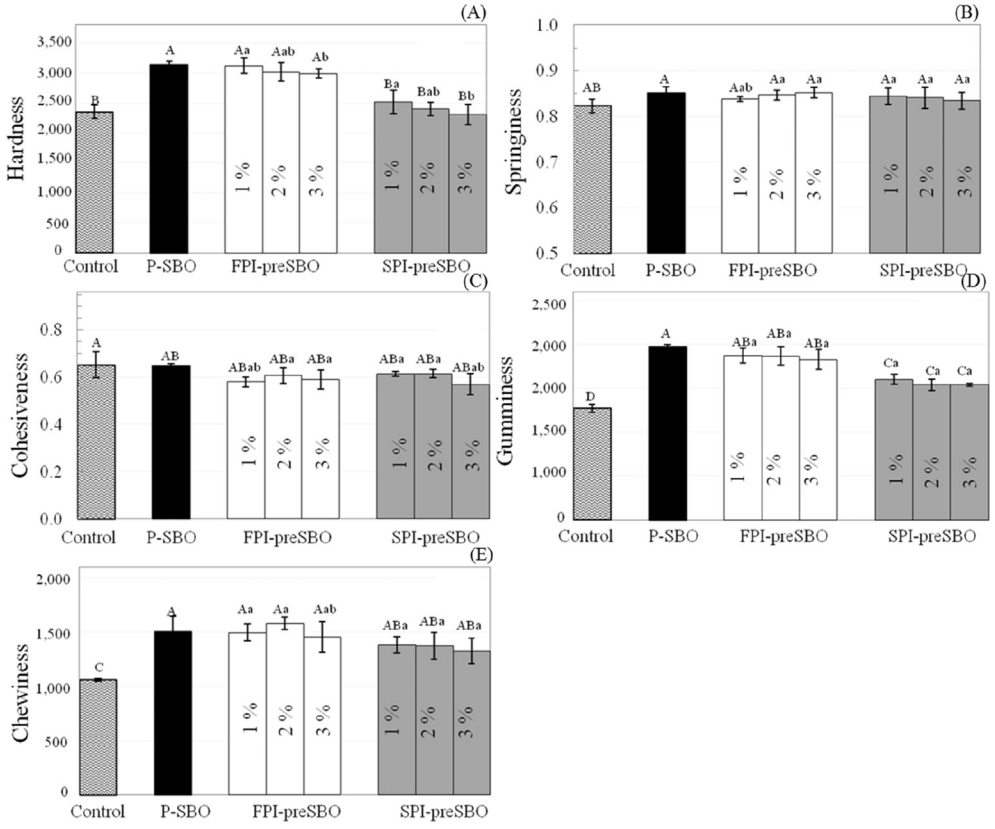


Fig. 4. Texture attributes of the sausages. (percentages indicate concentration as weight per volume of fish protein isolate (FPI) and soy protein isolate (SPI); Control = sausages without soybean oil (SBO); P-SBO = sausages added with SBO in pure form; FPI-preSBO = sausages added with pre-emulsified SBO (preSBO) stabilized with fish protein isolate (FPI); SPI-preSBO = sausages added with preSBO stabilized with soy protein isolate (SPI); error bars show ± SD; in each subfigure, different letters indicate significant differences ($p < 0.05$) between means, with uppercase letters showing effect of SBO inclusion and lowercase showing effect of FPI and SPI concentrations).

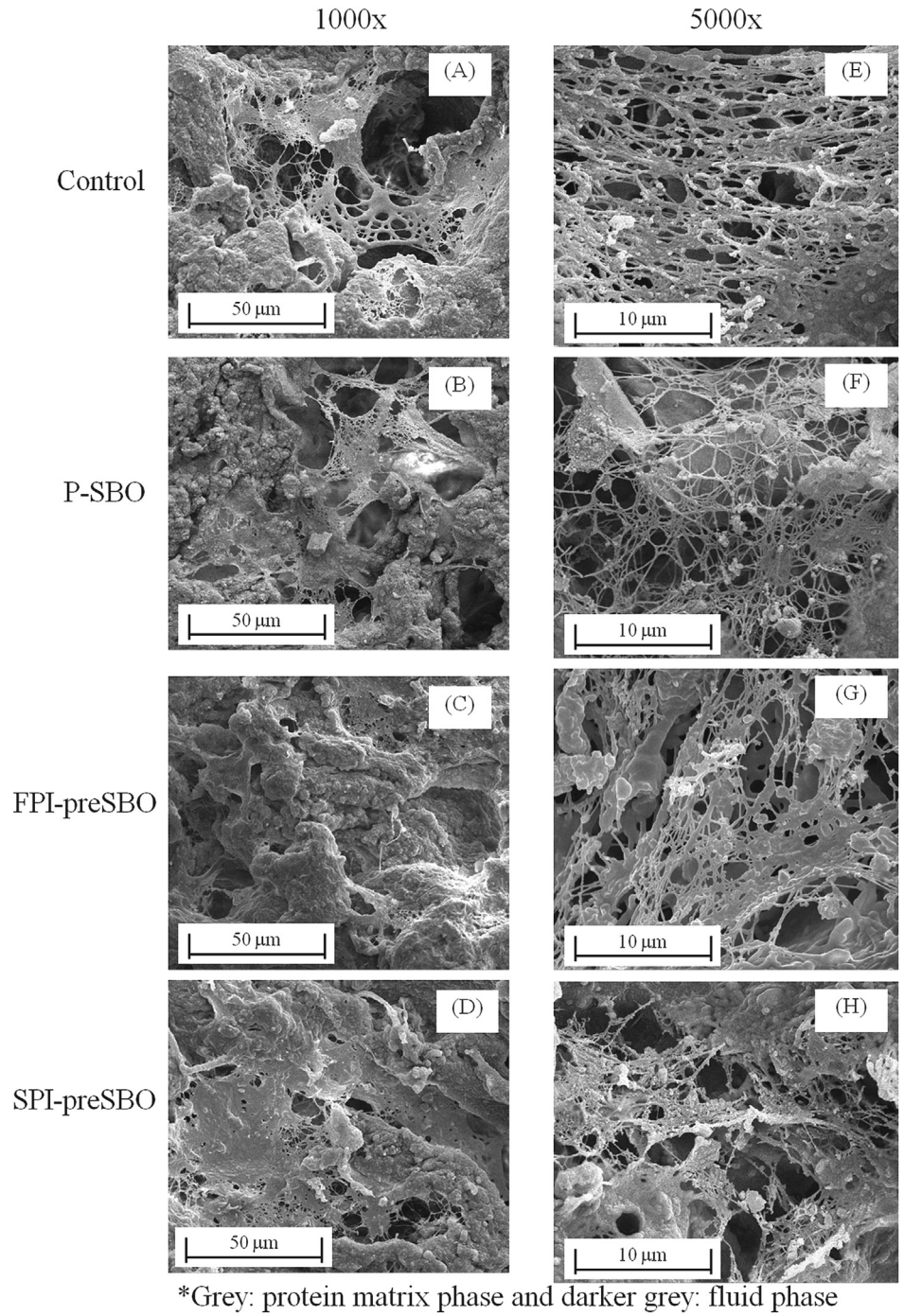


Fig. 5. Microstructure of (A, E) Control = sausages without soybean oil (SBO); (B, F) P-SBO = sausages added with SBO in pure form; (C, G) FPI-preSBO = sausages added with pre-emulsified SBO (preSBO) stabilized with fish protein isolate (FPI); (D, H) SPI-preSBO = sausages added with preSBO stabilized with soy protein isolate (SPI).

and chewiness, especially when SBO was incorporated in pure form and as FPI-stabilized preSBO. This tendency was in accordance with previous work studying frankfurters (Paneras et al., 1998) and fermented sausages (Josquin et al., 2012). Significantly lower hardness was found for the SPI-preSBO compared to the P-SBO and FPI-preSBO samples, which was coincident with the higher cooking loss of the former sample (Fig. 2). The temperature of 65–75 °C that is generally used in the manufacturing of meat products might not be sufficient to denature the main protein components of SPI (Feng and Xiong, 2002), especially in the presence of salt (Feng and Xiong, 2002) and sugar (Kulmyrzaev et al., 2000). Restricted partial denaturation of the SPI might hinder its interactions with myofibrillar proteins (Feng and Xiong, 2002), thereby limiting its gelling property in the emulsified meat matrix (McCord et al., 1998). Considering the effects of the non-meat protein concentration, increasing the FPI and SPI contents up to 3% significantly reduced the hardness of the samples. This might be expected due to the diminished stabilizing effect of the non-meat proteins caused by their self association with muscle proteins at the excess concentration (Sun et al., 2012). The reduction in the hardness of the FPI-preSBO and SPI-preSBO samples at the 3% protein level was in agreement with their higher cooking losses (Fig. 2). Nonetheless,

the effect of the emulsifier concentration on the other texture parameters was not noticeable ($p > 0.05$).

Using FPI (SPI) at the level of 2% (1%) could produce sausages with good stability, as suggested by the lower cooking loss and higher WHC (Fig. 2). To better elucidate the characteristic of the sausages, the microstructures of the Control, P-SBO, FPI-preSBO (at an FPI concentration of 2%), and SPI-preSBO (at an SPI concentration of 1%) were explored as shown in Fig. 5. A protein network with higher structural density and small cavities was observed for the FPI-preSBO, which was concurrent with its better stability as indicated by the lowered cooking loss and restored textural attributes. The fish sausage with a more intense protein network formation also possessed higher textural parameters (hardness, chewiness and resilience) than in the lowered ones (Intarasirisawat et al., 2014). Upon isolation via the pH-shift method, FPI was exposed to an alkaline environment that accelerated the partial denaturation of the protein (Rawdkuen et al., 2009; Yongsawatdigul and Park, 2004). Unfolding of FPI might facilitate its interaction with muscle proteins, thereby enhancing the protein matrix strength of the sausage (Jiang and Xiong, 2013; Knudsen et al., 2008). The rigidity of the protein gel was determined by the strength of the interactions between the particles in the gel network (Narine and Marangoni, 1999). Alkaline conditions induced partial unfolding of SPI that could also have enhanced its emulsifying capacity and led to reinforcing the pork myofibrillar protein gel strength (Jiang and Xiong, 2013).

For the SPI-preSBO, a less dense protein network with the presence of large cavities was observed, which might have been related to the higher cooking loss and less retained texture parameters. Moreover, with its large cavities, the SPI-preSBO could be expected to have a lowered WHC. It has been suggested that the emulsified meat matrix with more uniform cavity formation could restrict some water in the network more effectively, thereby increasing the WHC of products (Zhao et al., 2014).

Substitution of porcine fat with SBO in pure form and FPI-stabilized preSBO could improve the stability of the sausages as indicated by the lower cooking loss and the restoration of all texture attributes. In the partial substitution of porcine fat with SBO via the pre-emulsification technique, non-meat protein employed as emulsifier had an important role in determining the stability of the sausages. Although SPI exhibited better emulsifying ability than FPI, a stronger protein network was observed for the sausages added with FPI-stabilized preSBO. Using FPI at the concentration of 2% could effectively maintain the stability of the sausage. The present work suggested that the FPI prepared from yellow stripe trevally could be promisingly employed as an emulsifier for meat product preparation.

Conflicts of interest

The authors declare there is no conflict of interest.

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