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Effect of Thai hoary basil (*Ocimumcanum* Sims.) seed mucilage on fat reduction and quality characteristics of chicken salt soluble protein gel and low-fat meat products

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Abstract

Mucilage from Thai hoary basil seed (HBM) from solvent extraction was investigated (ratio of seed to water, soaking temperature, soaking time) to determine the optimal conditions for using as fat replacer in Chinese sausage and frankfurter sausage. The optimal extraction conditions were a ratio of seed to water of 1:30 and soaking at 40°C for 30 min. A gel model of HBM, pork back fat (BF) and chicken salt soluble protein gel (SSPC) was developed at 2.0% and 2.5% NaCl, which improved the water holding capacity (WHC), textural parameters and gel strength of the SSPC. The gel model with 40% fat reduction containing 8.11% HBM, 12.16% BF, 79.73% SSPC and 2.5% NaCl had the highest storage modulus under frequency period (0.1–100 rad/s), indicating the strongest gel structure, and had the highest WHC (71.85%) and similar textural properties to the model with BF alone. This model produced Chinese sausage with increased hardness, gumminess and chewiness; however, the color, water activity, microbial count and sensory scores were not significantly different from the control. On the other hand, this gel model significantly reduced the hardness, gumminess, chewiness and WHC of frankfurter, which resulted in increased cooking loss. The color, microbial count and sensory scores were not significantly different from the control. The final products were acceptable with liking scores of 6.0–6.6. The quality changes were not detected by consumers due to the synergistic texture contribution of HBM and BF being comparable to some tested commercial products (20% fat).

Introduction

Mucilage is one of the important hydrocolloids in food formulation usually used in gelling, thickening and stabilizing agents in many food products (Hosseini-Parvar et al., 2010; Karazhiyan et al., 2011). Thai hoary basil is a common name for the culinary herb *Ocimum canum* Sims. of the Family Lamiaceae. The seed of this plant is small with a black husk containing mucilage. This mucilage is easily extracted using water because it is able to swell 45 times in water and has been reported as a novel hydrocolloid of interest for use in food products (Khazaei et al., 2014). The mucilage from Thai hoary basil seed (HBM) is mainly composed of two major fractions of glucomannan (43%), with the ratio of glucose to mannose being 10:2, and (1→4)-linked xylan (24.29%) and a minor fraction of glucan (2.31%). HBM is

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a good swelling hydrocolloid and is a high water binding agent suitable for use as a thickening and stabilizing ingredient in food systems (Rafe et al., 2012) such as processed cheese (Hosseini-Parvar et al., 2015), edible film (Khazaei et al., 2014) and low fat ice cream (Javidiet al., 2016). However, there has been limited information published on the application of HBM in meat products.

Pork back fat (BF) is normally used in meat products to provide flavor and texture to the product; however, it contains a high saturated fatty acid content (Vural and Javidipour, 2002). The frequent consumption of saturated fat has been reported as a major cause of some non-communicable chronic diseases such as cardiovascular disease and coronary heart diseases (Vural and Javidipour, 2002; Özvural and Vural, 2008). Several studies have made great efforts to develop meat products with reduced fat contents. The replacement of BF with the other ingredients directly affects the overall qualities of meat products; hence the amount of fat replacer can be limited. The results of Abiola and Adegbaju (2001) indicated that up to 66% BF can be replaced with rind in pork sausage without any adverse effect on the processing yield. Furthermore, the replacement of oatmeal and tofu in low fat sausage has also been studied where BF was replaced with 25% hydrated oatmeal or 25% tofu without a significant change in acceptability (Yang et al., 2007). Moreover, Mora-Gallego et al. (2014) reported that 3% sunflower oil was suitable as a substitute for BF in fermented sausages, conferring desirable sensory properties similar to sausages with 100% BF. The applications of gum as fat replacer in meat products has also been reported with xanthan gum and locust bean gum used as fat replacer in ostrich meat emulsion (Chattong et al., 2015). Rather et al, (2016) reported the successful utilization of 0.5-1% guar gum as fat replacer in traditional Indian meat product. The application of tragacanth gum from *Astragalus gossypinus* and *Astragalus compactus* in emulsion-type sausage were also reported and the suitable concentration was 0.5% (Abbasi et al., 2019). The review by Naji-Tabasi and Razavi (2017) showed the successful application of basil seed gum as fat replacer in pistachio butter, yogurt, mayonnaise sauce and ice cream. These previous citations in total show that the reduction of BF in meat product can be done successfully with the addition of fat replacer. The current research investigated the proper extraction conditions of HBM using water as the solvent and determined the effect of HBM as fat replacer in a model of chicken salt, soluble protein gel (SSPC) and in a real system of meat products—Chinese sausage (dried sausage) and frankfurter sausage (cooked sausage). Three main extraction factors were studied (ratio of seed to water, water temperature and soaking time) and HBM at various replacement ratios was added to the gel model and the sausages.

Materials and Methods

Materials

HBM seed from Raitip® brand (Thai Cereal World Co. Ltd., Bangkok, Thailand) was used in this study. Chicken breast meat (a certified commercial brand) was purchased from a supermarket in Bangkok, Thailand. The visible fat and connective tissue were removed and the remaining meat was cut into small pieces, packed in a plastic bag and stored at -18°C.

Production and characteristics of Thai hoary basil seed

Dust and chaff were removed from the different Thai hoary basil seed samples. The cleaned seeds were kept in separate plastic bags and vacuum sealed to prevent any quality changes. The experiment was carried out using 3×3 factorial design in a completely randomized design. The three main factors were: ratio of seed to water (1:10 weight per volume; w/v, 1:20 w/v, 1:30 w/v), soaking temperature (40°C, 50°C, 60°C) and soaking time (30 min, 60 min, 90 min). HBM was produced by soaking the seeds in distilled water at the different soaking ratios and soaking temperatures and for the different soaking times. Then, the soaked seeds were drained and blended using a Waring® blender at 11,000 rpm for 20 s. The seed mucilage was separated from the husk by squeezing through a double-layered muslin cloth and then was stored at 4°C for use within 48 hr.

The extracted HBM after separation was weighed and then the yield was determined using Equation 1:

$$\text{Yield (\%)} = \frac{\text{Weight of HBM}}{\text{Weight of soaked seed}} \times 100 \tag{1}$$

Subsequently, the absorption capacities of the HBM samples were analyzed using the method of Phimolsiripol et al. (2011) with some modifications. A sample (10 g) was weighed and put into a centrifuge tube with the addition of 20 mL of water or soybean oil or butter. The mixture was stirred and then allowed to stand for 30 min before centrifugation at 4,000 rpm (20 min). Water or oil capacities were expressed as grams of water or oil bound per gram of sample on a wet basis and calculated using Equation 2:

$$\text{Absorption capacity (\%)} = \left(\frac{W_a - W_b}{W_b}\right) \times 100 \tag{2}$$

where W_b is the weight of the centrifuge tube and sample (measured in grams) before adding solution and W_a is the weight of the centrifuge tube and sample (measured in grams) after decanting the supernatant.

Gel model of Thai hoary basil seed extract mucilage, pork back fat, chicken salt soluble protein gel and NaCl

Preparation of chicken salt soluble protein gel

SSPC was extracted from chicken breast meat according to Kachanechai et al. (2008)with minor modifications. All preparation steps were carried out by controlling the system at 4°C. The salt content was determined using Mohr's method (Skoog et al., 1996). The protein concentration was determined using Biuret's method (Copeland, 1994). The SSPC sample was kept at 4°C for further use.

Preparation of gel model of Thai hoary basil seed extract mucilage, pork back fat, chicken salt soluble protein gel at 2.0% and 2.5% NaCl

The homogeneous gel mixtures of HBM, BF, SSPC and NaCl were prepared by fixing the concentration of SSPC at 79.73% and varying the concentration of HBM and BF from 0 to 20.27% and using salt concentrations of either 2.0% or 2.5%. The gel model was weighed precisely, mixed homogeneously and allowed to stand at 4°C for 10 min to ensure homogeneity. Complete gel setting was performed by leaving the gel model in the refrigerator at 4°C for 12 hr prior to analysis.

Texture profile analysis and water holding capacity of gel model

TPA was performed using a texture analyzer (Stable Micro System, TA. XT Plus, UK) equipped with a 2 kg load cell. Prior to testing, the gel samples were heated to 90°C for 20 min and cut into cubes with each side 2.0 cm. The gels were subjected to compression testing using a cylindrical probe (36 mm in diameter). The probe compressed the sample to 50% of its original height twice at a speed of 2.0 mm/s. From the resulting force/deformation curves, the textural parameters were calculated for hardness, cohesiveness, springiness, gumminess and chewiness (Chen et al., 2007). The determination was performed in 10 replicates.

For WHC analysis, a centrifugation method (10,000 rpm for 15 min at 4°C) was used (Pramualkijja et al., 2016). The determination was performed using three replicates. The WHC was expressed as a percentage of the initial water content of the gel and calculated using the Equation 3:

$$\text{WHC (\%)} = \left(\frac{W_s - W_w}{W_s}\right) \times 100 \qquad (3)$$

where W_s is weight of the initial sample (measured in grams) and W_w is the weight of water loss (measured in grams).

Dynamic rheological testing of gel model

Dynamic rheological experiments were performed using a rotational rheometer (Gemini 200 HR Nano; Malvern-Bohlin Instruments; UK) equipped with parallel plate geometry (diameter 25 mm) and the gap between the plate and sample was 1.0 mm. Prior to a dynamic experiment, the detection of storage modulus (G′) and loss modulus (G″) were performed through an amplitude sweep test at strain levels from 0.01% to 10% over a frequency of 10 rad/s measurements. A strain sweep was tested. The low strain, (not destroying the gel structure) was selected in the linear range of the rheogram. All oscillatory tests were determined at a strain of 0.1% strain (within the linear viscoelastic region) over the frequency range 0.01–100 rad/s, at 4°C. Temperature sweeps were studied in the range 4–90°C at a heating rate of 5°C/min. The determination was performed in triplicate.

The gel model with the best elastic characteristic was selected as the model for use in the real system with the meat products of Chinese chicken sausage and frankfurter sausage.

Application of gel model in Chinese chicken sausage and frankfurter sausage

Production of meat products

The formulation of Chinese chicken sausage is shown in Table 1. The product was produced using the method of Tan et al. (2007) with slight modifications. After mixing, the batter was stuffed, linked and dried in a tray dryer (XMT-152A, Mechanical and Electronic Industry Research Institute of Ningbo; China) at 50°C for 15 h. After cooling down to room temperature, the sausage was weighed to calculate the yield and then vacuum packaged and stored at 4°C. Each sample was selected randomly for analysis of its composition, color, TPA, water activity (a_w), microbial count and sensory evaluation.

For the frankfurter sausage, the analysis was performed using a similar procedure to that used for the Chinese sausage except for the drying process. The sausage was cooked in hot water until the internal temperature reached 75°C. Sausages were cooled down in an ice bath, vacuum packed and stored at 4°C for further analysis. Each sample was randomly selected to analyze the same properties using the same methods applied to the Chinese sausage samples. Furthermore, the samples were also analyzed for WHC and cooking loss.

Proximate composition and color measurement

The moisture, protein, fat and ash contents were determined using the methods of Association of Official Analytical Chemists (2000). Color was objectively measured at the center of freshly sampled slices (2.0 cm thick) using a spectrophotometer (D_{65} light source, 10° standard observer; Model CM-3500d; Minolta Camera Company; Osaka; Japan) and expressed in terms of L*, a*, and b* scores. Color evaluation was conducted in duplicate.

Texture profile analysis and water activity

The texture of sausage samples was determined using a method similar to gel model measurement. The water activity of sausage samples was determined at room temperature using a water activity analyzer (Model CX3TE; Aqua Lab; USA). The measurement was carried out in triplicate.

Table 1 Formulation of Chinese sausage and frankfurter sausage

Ingredient	Chinese sausage (%)		Frankfurter sausage (%)	
	Control	Developed	Control	Developed
Breast meat	64.33	64.33	64.33	64.33
Pork back fat (BF)	16.65	9.99	16.65	9.99
Fat to mucilage	-	6.66	-	6.66
Salt	1.63	1.63	1.63	1.63
Sugar	15.55	15.55	1.36	1.36
Soy sauce	1.52	1.52	-	-
Coriander root	0.16	0.16	-	-
Pae-lo powder	0.16	0.16	-	-
Ice	-	-	15.00	15.00
Pepper	-	-	0.49	0.49
Garlic	-	-	0.29	0.29
Cinnamon	-	-	0.12	0.12
Coriander seed	-	-	0.12	0.12
Total	100.00	100.00	100.00	100.00

Water holding capacity and cooking loss

The WHC was determined using the centrifugal method as described in the previous section (gel model). Cooking loss was determined for five individual samples and calculated based on the weight differences before and after cooking (Choi et al., 2014) using Equation 4:

$$\text{Cooking loss (\%)} = \left(\frac{W_r - W_c}{W_r}\right) \times 100 \quad (4)$$

where W_r is theweight of uncooked meat batter (measured in grams) and W_c is the weight of cooked meat batter (measured in grams).

Microbiological analysis

A sample of 10 g was randomly picked, homogenized and diluted for microbial analysis (total plate count and yeast mold) using the pour plate technique (Kok and Park, 2007). The results were expressed as log numbers of colony forming units per gram of sample (log cfu/g). The samples were analyzed at day 1 after production. The analysis was carried out in duplicate.

Sensory evaluation

Fresh Chinese sausage was sliced into 2.0 cm widths, deep fried in palm oil at 75°C for 30 s and then cooled to 25°C. The frankfurter sausages were reheated until the core temperature reached 75°C in a steam pot, cooled to 25°C, cut into small pieces (2.0 cm in length) and were then served to the panelists randomly. Each sample was coded with a randomly selected 3-digit number. The 30 untrained panelists were recruited for evaluating four samples of two Chinese sausage samples (the control and the developed product) in terms of color, aroma, oily mouth feel, texture and overall liking, and two frankfurter sausage samples (the control and the developed product) in terms of appearance, color, aroma, texture and overall liking, using a 9-point hedonic scaling test. The criteria for panelist recruitment were age (18-60 years old), with a reasonable frequency of meat product consumption, no allergy symptoms and good experience with meat product. Overall consumer acceptance was also graded.

Statistical analysis

All data were analyzed using analysis of variance. A confidence level of 5% was used to compare means ($p < 0.05$) between treatments. The mean values were compared using Duncan's new multiple range test. Statistical analysis of results was performed using the SPSS package (SPSS 12.0 for Windows, SPSS Inc.; Thailand).

Results and Discussion

Effect of ratio of seed to water, soaking temperature and soaking time on characteristics of Thai hoary basil seed extract

The soaking ratio, soaking temperature and soaking time significantly affected the extraction yield (Fig. 1) and absorption capacities (Fig. 2). These results were in agreement with other researchers (Amid and Mirhosseini, 2012; Muñoz et al., 2012; Campos et al., 2016) who reported that the extraction conditions were the major factors affecting all properties of the extracts.

Extraction yield

At 40°C, the increase in the soaking ratio elevated the water absorption of the seeds and resulted in an increased extraction yield. The highest yield was in seed soaked at a ratio of 1:30 (Fig. 1). The presence of excessive water for extraction led to a greater binding of water soluble components present in the seed endosperm, thereby increasing the extraction yield (Sepúlveda et al., 2007; Amid and Mirhosseini, 2012). Furthermore, Koocheki et al. (2009) explained that the addition of solvent increased the driving force of leaching mucilage out of the seed and made it easy to extract. The high extraction yield was an advantage for industry after transferring the mucilage to commercialized products.

The extraction yield increased with increasing soaking temperature from 40–60°C ($p < 0.05$), except for the sample with a soaking ratio of 1:30 at 60°C (Fig. 1). At high temperature, a reduction in the mucilage viscosity occurred, resulting in easier mass transfer of water-soluble polysaccharide from the cell wall into the extract (Wu et al., 2007;

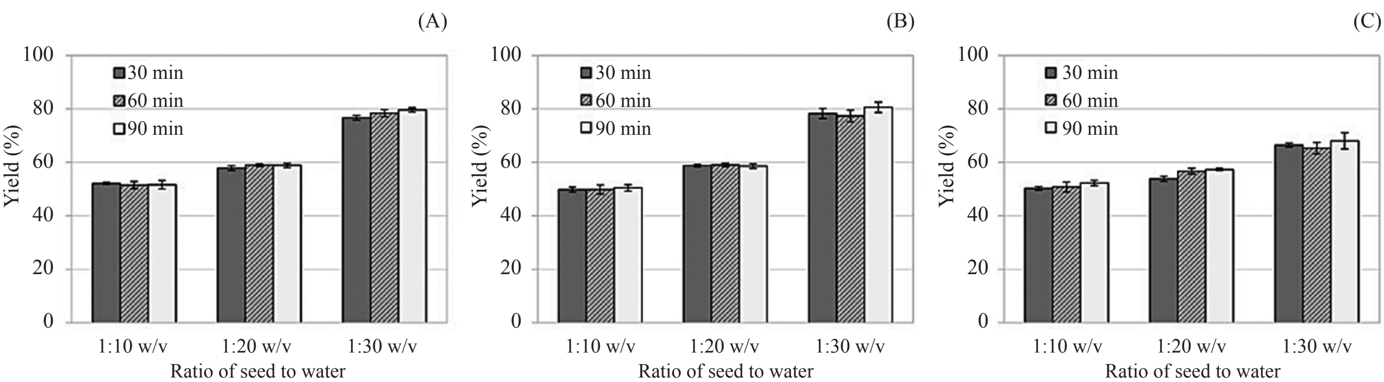

Fig. 1 Extraction yield of Thai hoary basil seed mucilage (HBM) under different extraction conditions: (A) = Yield 40°C; (B) = Yield 50°C; (C) = Yield 60°C; error bars indicate SD.

Behbahani et al., 2017). However, excessive heat occurred when soaking at high temperature which resulted in the degradation of glucomannan and a decreased extraction yield. On the other hand, the increase in the soaking time did not significantly affect the extraction yield. Soaking seeds for 30 min at 40°C was appropriate for producing HBM with a high extraction yield.

Water absorption capacity

Water absorption of HBM was significantly affected by the seed:water ratio and soaking temperature, whereas the soaking time did not affect this property (Fig. 2A–C). HBM had good water absorption ability. During extraction, distilled water was added for soaking the seeds at the amount varied by the seed:water ratio. The HBM obtained from the lowest ratio (1:10 w/v) had the highest water absorption ability because the water used for extraction was not sufficient to fully swell the HBM, which still had intact mucilage that was able to swell and absorb more water during the analysis. The HBM samples obtained from the other two ratios (1:20 w/v and 1:30 w/v) were able to absorb small amounts of water because they had already absorbed sufficient water during extraction. These samples had lower water binding ability, resulting in their low water absorption capacity compared with the ratio of 1:10. This might have occurred due to the extraction process of HBM. If the soaking water used in the extraction process was not sufficient, the seeds would be able to absorb more water and this would result in their high water absorption ability. In contrast, soaking the seeds with excess water resulted in fully absorbed seeds and fully swollen HBM and hence, less water binding ability would be observed (Montero et al., 2000). Moreover, water absorption of the mucilage was dependent on the soaking temperature (Behbahani et al., 2017). Fig. 2A–C show the thermal oxidation of mucilage at the elevated soaking temperature and the reduction in water absorption by the sample (Massiot and Renard, 1997). In this study, the mucilage extraction using a high soaking ratio at a low temperature resulted in a high water absorption capacity, similar to that reported by Amid and Mirhosseini (2012). The extraction conditions affected the extraction yield due to the different amounts of polar hydroxyl groups and the extent of the hydrodynamic interactions of samples (Koocheki et al., 2012).

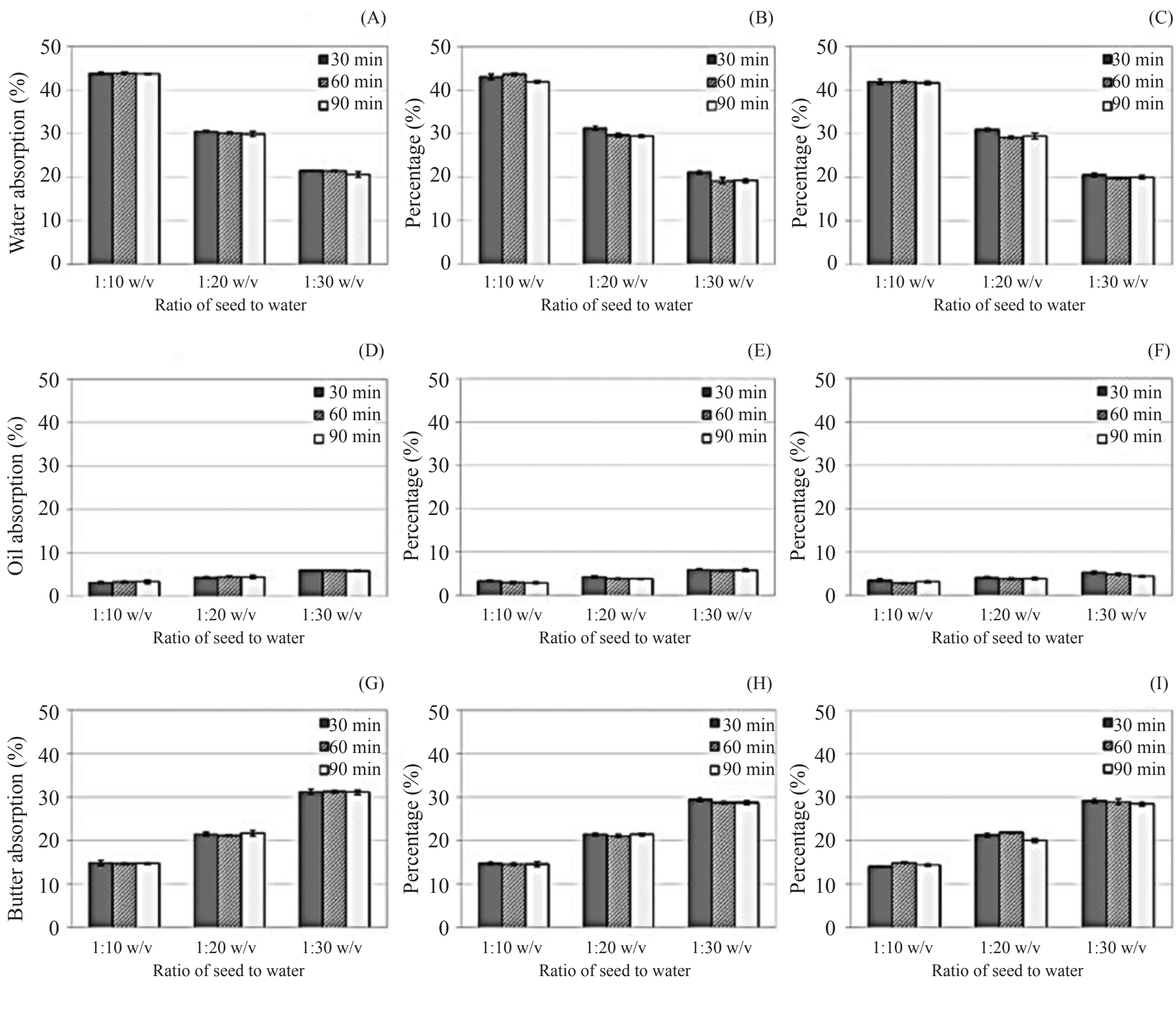

Fig. 2 Absorption capacity of Thai hoary basil seed mucilage under different extraction conditions: (A–C) = water absorption capacity at 40°C, 50°C and 60°C, respectively; (D–F) = oil absorption capacity at 40°C, 50°C and 60°C, respectively; (G–I) = butter absorption capacity at 40°C, 50°C and 60°C, respectively, and error bars indicate SD.

Oil absorption capacity

The oil absorption ability of HBM depended on the extraction conditions, especially the soaking ratio (Fig. 2D–F). HBM obtained from the 1:30 w/v had better oil absorption than from the other soaking ratios. The highest oil absorption capacity was a result of the high content of nonpolar molecules that can trap many oil particles (Sathe and Salunkhe, 1981). HBM extracted at high temperature had lower oil absorption ability ($p < 0.05$) compared to that of the HBM extracted at low temperature. This result agreed with Amid and Mirhosseini (2012). The increase in the soaking time from 30 min to 90 min resulted in decreasing oil absorption capacity because of the degradation of the HBM monomer (mainly glucomannan) during extraction at high temperature. With the structure surrounded with hydroxyl (-OH) groups, the major functional group (glucomannan) had good water binding. It was not clear that HBM was able to bind with water readily and so it exhibited low oil absorption ability. Conditions with the highest soaking ratio at the lowest temperature were the best for extraction suited to produce HBM, which is a good fat replacer in meat product as it can help to reduce fat along with minimizing the loss of flavor and oil during cooking (Thebaudin et al., 1997).

Butter absorption capacity

The butter absorption capacity is presented in Fig. 2G–I which show that the seed:water ratio and soaking temperature significantly affected this property. For a soaking ratio of 1:30, the increase in soaking temperature from 40°C to 60°C reduced the butter absorption capacity of HBM (Fig. 2G–I). This sample showed the ability to bind more water which is one of the necessary properties of meat products. Surprisingly, the highest oil and butter absorption capacity was in the treatment with a soaking ratio at 1:30 w/v. This treatment had the lowest water absorption capacity but the highest oil and butter binding properties as reaction with either oil or butter is possible by using their hydrophobic groups in their side chains. It has been suggested that the emulsifying, stabilizing and absorption properties are not ascribed to the surface active protein moiety but could also be attributed to the hydrophobic character of the polysaccharide itself (Galla and Dubasi, 2010). The protein remaining in the HBM, which was crude mucilage, is also responsible for this property (Phimolsiripol et al., 2011; Rafe et al., 2012; Khazaei et al., 2014). The purification of the mucilage reduced its adsorption activity, indicating the role of protein in the mucilage.

Effect of pork back fat to Thai hoary basil seed mucilage ratio and concentration of NaCl on properties of chicken salt soluble protein gel

SSPC was extracted with appropriate conditions as described in Kachanechai et al. (2008). The SSPC had a protein content of 7.54 mg bovine serum albumin (BSA)/g, a salt content of 1.74% and pH at 6.0. The protein content was controlled at 7.50 mg BSA/g prior to mixing with BF or HBM or both.

Texture profile analysis and water holding capacity

The analysis of cooked gel texture showed that the concentration of NaCl and the substitution ratio of BF:HBM significantly affected the gel texture (Table 2). Cooked gels without HBM had a gel-like property, while the addition of HBM reduced the hardness of the SSPC gel which was exhibited as a high-viscous solution. The softest gel resulted when the HBM was increased to 100%. Some studies have shown that BF had a function in the reinforcement of meat product texture and the addition of some hydrocolloids resulted in a product with softer texture (Mendoza et al., 2001; Totosaus and Pérez-Chabela, 2009). BF is the prominent ingredient in an emulsified meat network (Felisberto et al., 2015). On the other hand, an increased NaCl concentration improved the gel hardness. This result could be explained by the properties of NaCl which enhanced the protein solubility and induced structural changes through electrostatic interactions between muscle protein, sodium and chloride ions (Totosaus and Pérez-Chabela, 2009), resulting in the swelling of myofibrils, depolymerization of myofilaments and dissociation of the actomyosin complex.

A similar result also occurred with the WHC of the gels. The texture and WHC of the cooked gel model were improved when the salt concentration was increased to 2.5%. Moreover, Mora-Gallego et al. (2016) suggested that the effect of BF reduction had a greater

Table 2 Texture parameters of chicken salt soluble protein gel gels with various ratios of pork back fat (BF) to Thai hoary basil seed mucilage (HBM) and NaCl concentrations

NaCl (%)	BF (%)	HBM (%)	Texture profile analysis					WHC (%)
			Hardness (N)	Springiness (mm)	Cohesiveness	Gumminess	Chewiness (N.mm)	
2.0	20.27	0	13.32±1.97^{ab}	0.86±0.08^{a}	0.67±0.05^{a}	8.87±1.45^{abc}	7.60±1.49^{ab}	71.60±0.99^{b}
	16.22	4.05	11.34±2.80^{bc}	0.83±0.05^{a}	0.67±0.04^{a}	7.62±2.12^{cd}	6.40±2.18^{bcd}	70.93±0.64^{b}
	12.16	8.11	13.30±1.62^{ab}	0.88±0.05^{a}	0.68±0.05^{a}	9.05±1.12^{abc}	8.01±1.34^{ab}	69.37±0.71^{c}
	8.11	12.16	13.31±2.86^{ab}	0.86±0.08^{a}	0.61±0.03^{a}	8.01±1.39^{bcd}	6.94±1.80^{abc}	66.61±1.08^{d}
	4.05	16.22	8.69±3.20^{cd}	0.87±0.04^{a}	0.67±0.06^{a}	5.66±1.36^{d}	4.92±1.24^{de}	60.47±0.97^{e}
	0	20.27	7.81±2.85^{d}	0.85±0.01^{a}	0.65±0.04^{a}	5.01±1.64^{d}	4.29±1.43^{e}	58.23±1.13^{f}
2.5	20.27	0	14.22±2.88^{ab}	0.88±0.05^{a}	0.67±0.05^{a}	9.44±1.32^{abc}	8.32±1.07^{ab}	74.29±0.33^{a}
	16.22	4.05	15.63±2.07^{a}	0.87±0.03^{a}	0.64±0.03^{a}	10.08±1.52^{a}	8.76±1.31^{a}	73.42±0.86^{a}
	12.16	8.11	15.65±2.59^{a}	0.84±0.04^{a}	0.63±0.06^{a}	9.85±1.82^{ab}	8.36±1.85^{ab}	71.85±0.43^{b}
	8.11	12.16	8.83±2.27^{cd}	0.89±0.06^{a}	0.72±0.05^{a}	6.29±1.59^{de}	5.65±1.69^{cde}	66.82±0.77^{d}
	4.05	16.22	8.64±1.81^{cd}	0.86±0.06^{a}	0.66±0.05^{a}	5.63±0.86^{d}	4.87±1.02^{de}	60.61±0.91^{e}
	0	20.27	8.20±2.07^{d}	0.84±0.07^{a}	0.64±0.08^{a}	5.15±0.86^{d}	4.36±1.02^{e}	57.99±0.80^{f}

WHC = water holding capacity.

Values (mean±SD) within the same column with different lowercase superscripts are significantly different ($p < 0.05$);

impact on these properties than the elevated salt concentration. The fat reduction together with the increase in the NaCl concentration at a certain level is a novel strategy for generating a low fat product. The addition of a fat replacer like HBM can be done in a limited quantity with a high salt concentration (2.5%). The appropriate substitution ratio was BF:HBM at 60:40 which was equivalent to BF at 12.16%and HBM at 8.11%. This model had a firm, rigid gel that was suited to be used in a real meat products system.

Dynamic rheological testing of gel model

The G′and G″values of all samples with and without HBM were obtained from an oscillatory frequency sweep test and a temperature sweep test, respectively (Fig. 3 and Fig. 4). During the frequency sweep, the frequency is varied while the amplitude of the deformation is kept constant. The resulting G′ and G″moduli are plotted against the frequency. The data obtained at low frequencies describe the behavior of the samples at slow changes of stress. On the other hand, the behavior at fast load is expressed in the high frequencies. The results revealed that the G′ value was always higher than the G″value throughout the tested frequency period. There was no crossover point, indicating the elastic property and the viscoelastic behavior of the sample. The magnitudes of G′ and G″ observed in the present study were similar to other research involving the evaluation of protein gels from various meat emulsions (Felisberto et al., 2015; Yasin et al., 2016). Moreover, this type of behavior ($G' > G''$, $G'/G'' < 10$) was expressed as the characteristic of a weak gel, and can be correlated as a measure of density from protein molecules at the oil/water interface which has been associated with the formation of a structural network in the meat emulsion (Franco et al., 1998). The G′ curves of the SSPC with HBM expressed gel with elasticity during the change of frequency (0.2–50 Hz) and the increase in HBM resulted in a shift down of the curve, especially at high concentration (Fig.3). This result indicated the improper interaction of SSPC with HBM. The G′ patterns of all samples tended to increase in the high frequency range, as shown by the rising slope. This result suggested that the structure of the SSPC gel deformed at high frequency. At low frequency, the effect of NaCl and the HBM concentration was clear. The G′ curves at the beginning of the test were chronologically shifted down with increasing HBM concentration. However, the increase in the NaCl concentration affected the G′ value directly which increased as the NaCl increased, which occurred only for SSPC with HBM over 40% substitution (8.11% HBM). This result indicated the synergistic effect of HBM and NaCl in the co-creation of the stable gel matrix which resulted from the change of ionic strength from the addition of NaCl (Hosseini-Parvar et al., 2016). Moreover, it was clear that the G′ patterns of SSPC with BF:HBM ratios of 80:20 and 60:40 were stable over a wide frequency range and were almost similar to the control pattern. This particular pattern implied that the SSPC gel developed a rubbery, elastic property. These experimental results indicated that there was a strong gel network between SSPC, BF and HBM at appropriate concentrations.

A temperature sweep test was used to study the behavior of the gel upon heating, since this test is useful for determining the denaturation of the protein matrix (Tunick, 2011). The test result (Fig. 4) indicated that the concentration of NaCl significantly affected the G′ value of all samples, especially at 4°C. The highest G′ value resulted from the treatment with 2.5% NaCl without HBM and the G′ curve continually shifted down as the HBM concentration increased. The lowest G′ value was observed in SSPC gel with 100% HBM (0% BF). This result was also observed in samples with 2.0% NaCl. The G′ pattern of the SSPC gel followed a J curve. The G′ value tended to gradually decrease as the temperature increased in the range 4–44°C and then heat set gelation of SSPC began, as shown by the substantial increase in the G′ value. The gelation period of SSPC was continuous in the range 45–69°C. When the system temperature increased to 90°C, the highest G’ value was reached in the treatment with BF:HBM at 60:40 (with either 2.0% or 2.5% NaCl). These treatments had G′ values greater than the control with 100% BF. This result indicated the synergistic effect of BF and HBM which helped to create the rigid heat set gels in the presence of NaCl. It clearly showed that the G′ value of all samples could be divided into three transition temperature ranges, namely 4–44°C, 45–69°C and 70–90°C. In the first period, the G′ value did not change in the treatment with more than 40% HBM substitution. In contrast, SSPC with lower than 40% HBM resulted in a reduction in the G′ value due to the breakage of hydrogen bonds as the temperature increased, as has been reported by other researchers (Ferris et al., 2009; Savadkoohi et al., 2013). In this range (4–44°C), the concentration of NaCl significantly affected the G′ value, especially at 4°C. The shift downwards of the G′ curve occurred because HBM did not tightly bind with SSPC, with only swelling and fragmentation in the system, while NaCl could affect the ionic strength of the protein gel. The second period (45–69°C), could be separated into two groups consisting of low HBM (< 40% substitution) and high HBM (≥40% substitution). The G′ value of SSPC with low HBM had a more rapid increase than with high HBM. This rheological transition is typical of the thermal gelling of myosin. Generally, the initial enhancement of G′ was due to the association of myosin as a consequence of denaturation, indicating the formation of a weak gel network (Sun et al., 2012; Hu et al., 2016). In the last period, the sharp increase in G′ suggested that the conformation of the myosin molecules changed further, with the molecule becoming unfolded into a random coil structure. This led to an increase in the number of cross-links between protein aggregates and a deposition of additional denatured proteins in the protein network to strengthen the gel matrix, giving rise to a firm, irreversible and thermal gel (Chen et al., 2007). When the system temperature increased to 90°C, the highest G′ value was recorded in treatments with BF:HBM at 60:40 (with either 2.0% or 2.5% NaCl). These treatments had G′ values higher than the control. This tendency of G′ was positively correlated to the hardness of the TPA and the WHC. The gel model of SSPC with 2.5% NaCl and a BF:HBM ratio of 60:40 had the highest G′, hardness and WHC. Hence, this model was selected for application in the real meat product system.

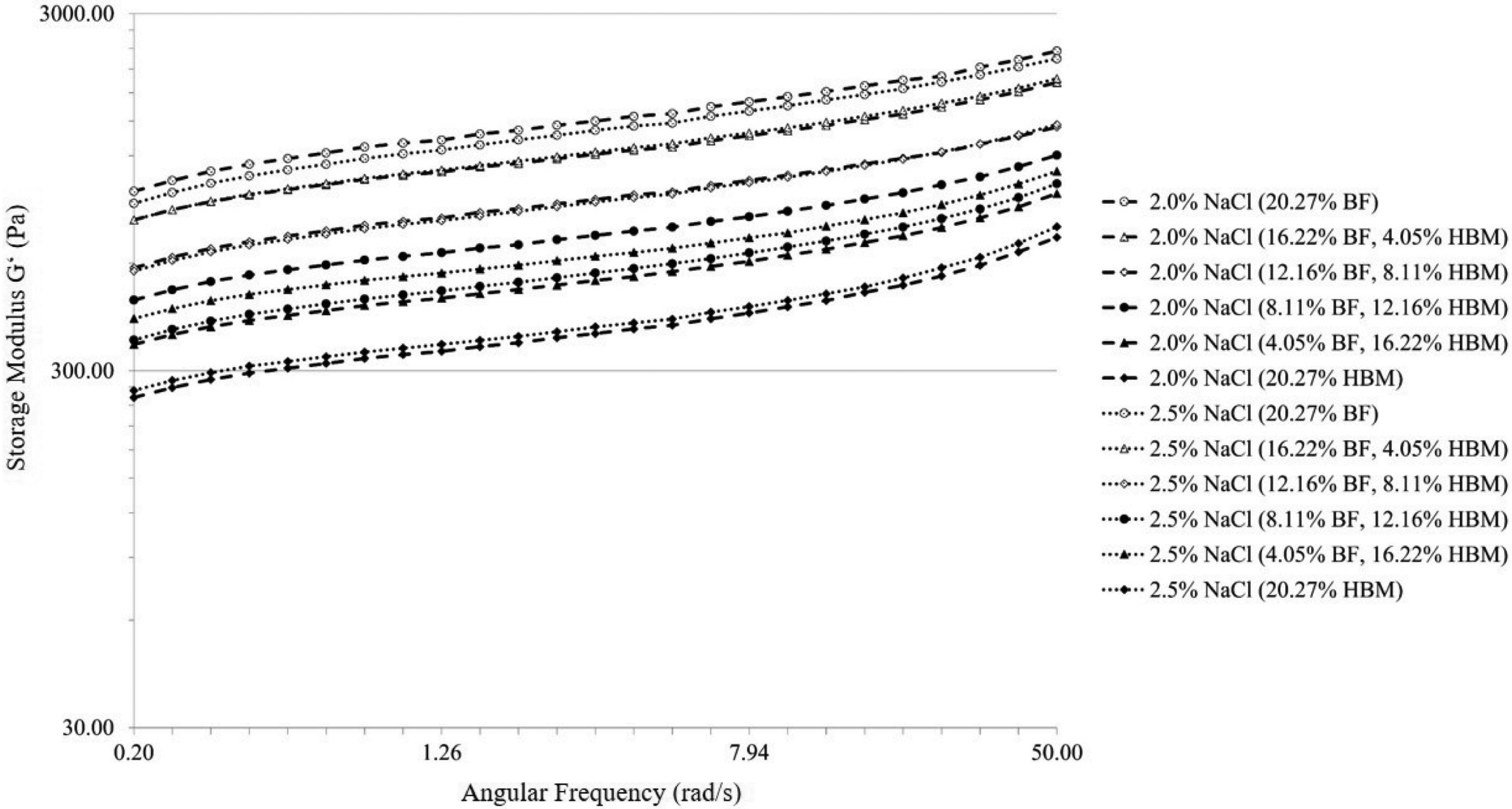

Fig. 3 Frequency sweep (0.20–50.00 rad/s) at 1% amplitude strain of chicken salt soluble protein gel with different NaCl concentrations and amounts of pork back fat (BF) and Thai hoary basil seed mucilage (HBM)

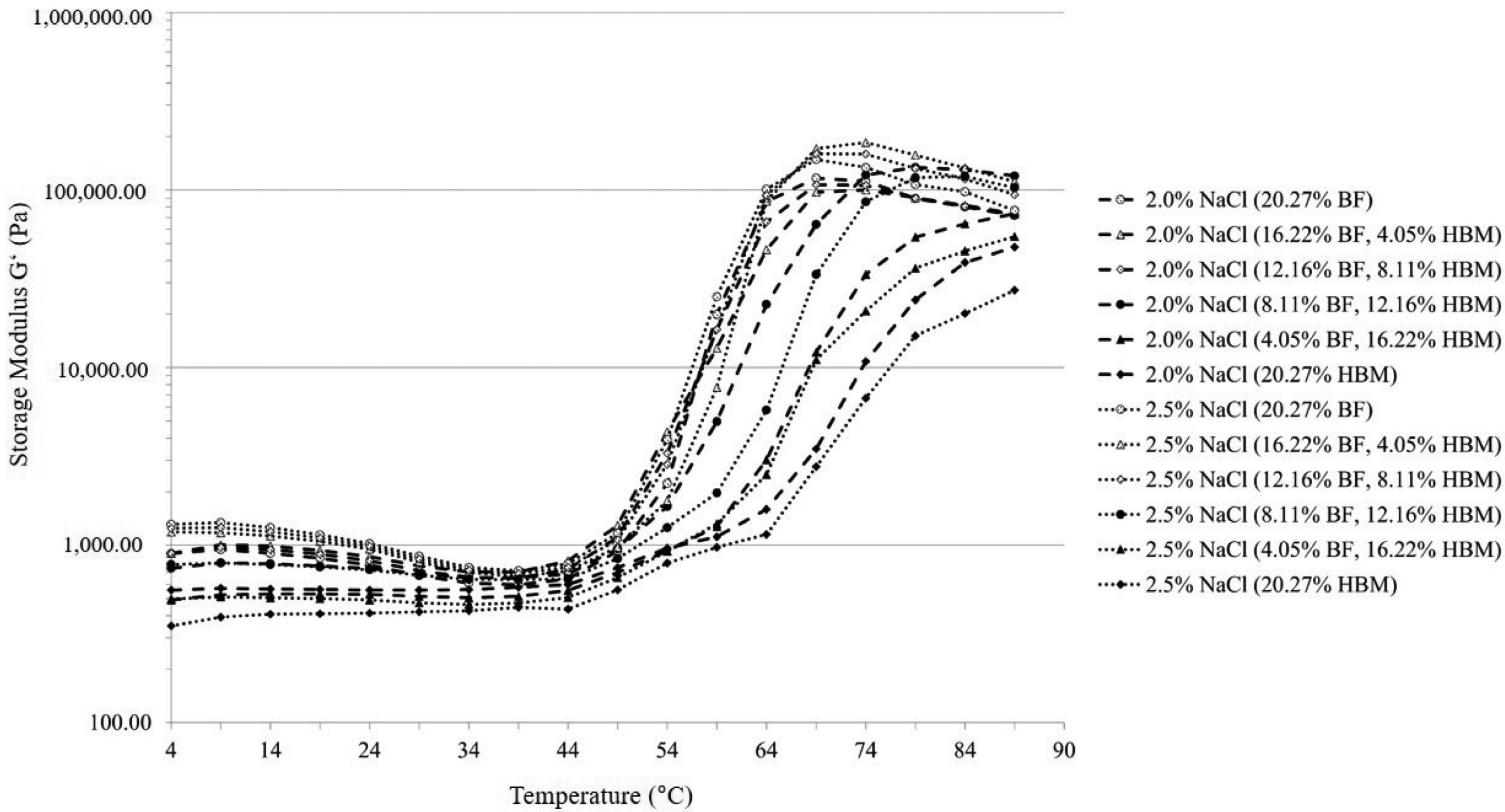

Fig. 4 Temperature sweep (4–90°C) at 5°C/min for chicken salt soluble protein gel with different NaCl concentrations and amounts of pork back fat (BF) and Thai hoary basil seed mucilage (HBM)

Effect of selected pork back fat to Thai hoary basil mucilage ratio and NaCl concentration on properties of Chinese sausage and frankfurter sausage

Chinese sausage and frankfurter sausage were prepared using the gel model of BF and HBM at the ratio of 60:40 (12.16% BF and 8.11% HBM) as fat replacer with 2.5 w/w NaCl.

Proximate composition of Chinese sausage and frankfurter sausage

The proximate compositions of the sausages are shown in Table 3. The addition of HBM significantly increased the moisture content of the sausage because of the water retention of HBM (Choi et al., 2009). These results agreed with some studies, in which the moisture content increased with the addition of different levels of substitute fat in meat products (Cengiz and Gokoglu, 2007).

The sausage with HBM had a lower fat content compared to the control. The fat contents of the control of Chinese sausage, Chinese sausage, control of frankfurter and frankfurter were 16.77±0.35, 12.56±0.30, 11.53±0.60 and 8.18±0.40, respectively (Table 3). The percentages of fat reduction were 25.10% and 29.05% for the Chinese sausage and frankfurters, respectively. Some reports also supported these findings regarding the replacement of pork back fat in meat product with other ingredients such as yam (Tan et al., 2007), vegetable oil (Choi et al., 2009) and konjac (Jiménez-Colmenero et al., 2013) which resulted in reduced fat in the finished products. Moreover, the protein and ash contents of the developed sausage were significantly reduced.

Physical properties

The redness of the developed sausage was higher than the control. The reduction in the fat content caused a significant increase in redness values (Cengiz and Gokoglu, 2007). Changes in the textural properties were detected in hardness, cohesiveness, gumminess and chewiness. Chinese sausage with HBM had a hard texture after drying due to the reduction in the fat content in the formulation (Mugueza et al., 2002; Liaros et al., 2009). As the fat content decreases, there is an increase in hardness and chewiness, while cohesiveness is reduced (Ruiz-Capillas et al., 2012). The high water content in HBM was able to increase the water content in the meat batter system. After drying the Chinese sausage, a packed and toughened protein matrix resulted (Jiménez-Colmenero et al., 2013). In contrast, the textural properties of the frankfurter sausage with HBM were significantly different from the control, having a soft texture. The increase in the water content in the finished product tended to affect the heat set gelation of protein during the formation of the emulsion batter and hence resulted in batter with a high water content and a product with a soft texture after boiling. Moreover, the reduction of fat resulted in a significant decrease in the hardness, gumminess, and chewiness of the resulting frankfurters, similar to results reported by Crehan et al. (2000). An increase in a_w occurred when HBM was added into the frankfurter sausage. This result could be explained by similar reasons to those regarding the texture. The WHC and cooking loss in the frankfurter were significantly different from the control as the WHC decreased and the cooking loss increased (Table 3). The water content of the meat batter system with HBM was higher than that of the control, while the presence of HBM in the batter also changed the structure of the meat protein gel as a result of filling, weak binding and interfering, depending on the concentration. As a result, a decrease in the WHC occurred. However, the increase in the water content in the raw batter could not be retained after re-heating and the cooking loss increased in this case.

Table 3 Properties of Chinese sausage and frankfurter sausage

Property		Chinese sausage		Frankfurter sausage	
		Basic formula	Developed formula	Basic formula	Developed formula
Chemical properties					
Proximate (%weight basis)	Moisture	29.10±0.50^a	29.06±0.35^a	63.61±0.49^y	69.31±0.31^x
	Protein	23.78±0.67^a	22.81±0.32^b	19.00±0.64^x	18.76±0.57^x
	Fat	16.77±0.35^a	12.56±0.30^b	11.53±0.60^x	8.18±0.40^y
	Ash	3.21±0.12^b	3.65±0.05^a	1.88±0.01^x	1.85±0.01^y
Physical properties					
Color	L*	41.34±1.58^a	40.74±1.18^a	73.87±0.92^x	73.43±0.41^x
	a*	3.86±0.30^b	4.41±0.32^a	1.90±0.23^y	2.58±0.18^x
	b*	12.23±0.95^a	12.23±0.90^a	13.09±0.31^x	13.22±0.45^x
TPA	Hardness	21.04±2.37^b	26.91±2.46^a	30.54±1.22^x	26.00±1.50^y
	Springiness	0.83±0.04^a	0.82±0.02^a	0.92±0.01^x	0.91±0.02^x
	Cohesiveness	0.77±0.03^a	0.73±0.01^b	0.31±0.04^y	0.41±0.15^x
	Gumminess	16.29±2.02^b	19.54±1.70^a	10.72±0.84^x	9.43±1.10^y
	Chewiness	13.46±1.54^b	15.99±1.69^a	9.83±0.76^x	8.69±1.09^y
a_w		0.82±0.00^a	0.81±0.00^b	0.94±0.01^y	0.97±0.01^x
WHC (%)		n.d.	n.d.	94.76±0.31^x	91.03±1.65^y
Cooking loss (%)		n.d.	n.d.	5.14±0.48^y	7.47±0.16^x
Microbial property					
Total Plate Count (cfu/g)	Day 1	3.20×10^3	3.50×10^3	1.74×10^3	1.70×10^3
Yeast/Mold (cfu/g)	Day 1	<10 ESPC	<10 ESPC	<10 ESPC	<10 ESPC

TPA = texture profile analysis; WHC = water holding capacity; a_w = water activity; cfu = colony forming units; n.d.= not determined; ESPC = estimated standard plate count. Values (mean±SD) for the same products within the same row superscripted with different lowercase letters are significantly different ($p < 0.05$).

Table 4 Sensory evaluation of Chinese sausage and frankfurter sausage

Attribute	Chinese sausage		Frankfurter sausage	
	Basic formula	Developed formula	Basic formula	Developed formula
Sensory evaluation of Chinese sausage				
Color	6.26±1.19[a]	5.76±1.22[a]	-	-
Aroma	6.04±1.12[a]	5.64±1.22[a]	-	-
Oily mouth feel	6.34±0.85[a]	6.22±1.15[a]	-	-
Texture	5.74±1.23[a]	5.96±1.24[a]	-	-
Overall liking	6.08±0.90[a]	6.00±1.03[a]	-	-
Sensory evaluation of Frankfurter sausage				
Appearance	-	-	6.86±0.70[b]	6.80±0.76[b]
Color	-	-	6.72±0.76[b]	6.62±0.64[b]
Aroma	-	-	6.54±0.95[b]	6.44±1.03[b]
Texture	-	-	6.74±0.63[b]	6.62±0.73[b]
Overall liking	-	-	6.70±0.71[b]	6.66±0.56[b]

There was no difference ($p > 0.05$) between means of the basic and developed formula of the same product. ±SD) within same product in the same row are not significantly different.

Microbiological properties

The microbial properties of the developed sausage were not significantly different from the control. The TPC numbers of all samples were in the range 1.70×10^3–3.5×10^3 colony forming units (cfu)/g, while yeast and mold were less than 10 estimated standard plate counts (ESPC)/g (Table 3). This result indicated the safety of the product as it did not exceed the Thai standard regulation NO. TIS2299-2549 (Thai Industrial Standard Institute, 2007).

Sensory evaluation

The developed meat products were acceptable by consumers in all sensory characteristics and had similar sensory characteristics to those of the control sausages (Table 4). The overall liking score of the developed Chinese sausage and frankfurter sausages were 6.00 and 6.66, respectively. The developed products were accepted by over 90% of the target consumers (results not shown). This result suggested that the reduction of BF by replacement with HBM was an alternative strategy which yielded a product similar to that of the control. The substitution of BF with HBM at 40% did not significantly affect the detectable properties of the meat product by the consumers. These results implied that the property change of these developed products were minor and might not affect consumer perception. Moreover, these results were congruent with the findings of Kim et al. (2011). Hence, HBM could be used as pork back fat replacer in meat product at 40% BF substitution.

In conclusion, HBM could be successfully used as pork back fat replacer in meat product at limited (40% BF substitution) levels without any perceptible changes and the final product was accepted by consumers with a like-moderately score.

Conflict of Interest

The authors declare that there is no conflict of interest.

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