International Food Research Journal 26(5): 1525-1523 (October 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of hydrolysed collagen and *Man-sao* powder mixture as a fat replacer on quality of Vienna sausages

Olanwanit, W. and *Rojanakorn, T.

Department of Food Technology, Faculty of Technology, Khon Kaen University, 40002, Khon Kaen, Thailand

Article history

Received: 21 February, 2019
Received in revised form: 16 July, 2019
Accepted: 6 August, 2019

Keywords

Hydrolysed collagen
Man-sao powder
Vienna sausage
Fat replacer

Abstract

The present work investigated the quality of reduced-fat Vienna sausages containing a mixture of hydrolysed collagen and *Man-sao* powder (HCMSM). Six treatments of Vienna sausages with 0%, 20%, 40%, 60%, 80% and 100% replacement of added fat with HCMSM were produced, and evaluated for physicochemical properties, microstructure, and sensory quality. The incorporation of HCMSM into the sausages significantly increased emulsion stability, moisture, and protein content and lowered fat content, calorific value, and cooking loss. The amount of HCMSM that was added significantly affected the instrumental texture of the sausages. Variations in microstructure were observed among sausage samples containing increasing concentrations of HCMSM. Greater amounts of HCMSM were associated with fewer honeycomb-like structures, and more heterogeneous and compact microstructures. Most sensory likability scores of sausage samples with up to 40% HCMSM were comparable to those of the full-fat control sample. Incorporation of 40% HCMSM can therefore be used to produce reduced-fat Vienna sausages with high acceptability.

Introduction

Vienna sausages are among the most popular meat products among Thai people because of their unique flavour and taste. Generally, cured and cooked Vienna sausages can be manufactured from lean meat (beef, pork, and a combination of beef and pork), animal fat (pork back fat), and other ingredients, including salt, nitrite, ice, polyphosphate, ascorbate, spices and seasonings. The lean meat, animal fat, ice and other ingredients are chopped in a silent cutter until the cohesive mass or meat batter is obtained. It was reported that all-beef and beef-and-pork blend Vienna sausages contain between 24 and 28% fat and 320 and 325 kcal/100 g (Feiner, 2006). Frequent consumption of this type of meat product may thus be associated with obesity, cardiovascular diseases, and arterial hypertension (Brewer, 2012). Fat, however, provides many satisfactory characteristics to Vienna sausages, including improving tenderness, juiciness and palatability, lowering cooking loss and stabilising meat emulsions (Choi *et al.*, 2009; Kim *et al.*, 2010; Henning *et al.*, 2016). Consequently, a reduction of fat incorporated into meat product formulations may lead to deterioration of texture, flavour and mouth feel and may be associated with lowered consumer satisfaction. The production of reduced-fat meat products with high sensory acceptability can be accomplished by using fat replacers, such as carbohydrates and starches (Aktaş and Gençcelep, 2006), connective tissue protein gel (Osburn *et al.*, 1997), and plant proteins (Kumar *et al.*, 2007). In addition, a combination of fat replacers - *i.e.*, a combination of pig skin and wheat fibre (Choe *et al.*, 2013) or a mixture of green banana flour and pork skin (Alves *et al.*, 2016) was reported to improve the physical and sensory attributes of reduced-fat meat products.

Hydrolysed collagen is a polypeptide complex prepared by a hydrolysis process of denatured collagen or gelatine (Mohammad *et al.*, 2014). Hydrolysed collagen is water-soluble and has no bitter taste caused by the high glycine content of gelatine (Mohammad *et al.*, 2014). Hydrolysed collagen can be manufactured using collagen from bovine, pork skin, and fish as raw material (Ibrahim *et al.*, 2018). Hydrolysed collagen plays a significant role in the human diet because it consists of a significant content of some amino acids, including glycine, proline, and hydroxyproline, helping to prevent joint disease in

*Corresponding author.
Email: thajoj@kku.ac.th

humans (Ferraro *et al.*, 2016; Sousa *et al.*, 2017). The incorporation of hydrolysed collagen may therefore improve the nutritional value and physicochemical, as well as sensory properties of meat products. The work of Sousa *et al.* (2017) revealed that partial proxy of the pork fat with hydrolysed collagen resulted in a better quality of frankfurter-type sausages, in terms of water-binding ability, stability of product after heating, and textural parameters. Ibrahim *et al.* (2018) demonstrated that partial substitution of buffalo fat with fish collagen hydrolysates in buffalo patties from 2.5% to 10% did not affect the cooking loss, water-holding capacity or pH value of the cooked patties. Based on the sensory evaluation results, the buffalo patties with 7.5% fish collagen hydrolysates showed the highest overall acceptability.

Man-sao (*Dioscorea alata* L.) is a tuberous root vegetable grown in Thailand where it is used in a variety of foods and desserts. *Man-sao* is an excellent source of β-carotene, carbohydrate, dietary fibre, protein, and minerals (e.g., calcium, phosphorus, and potassium). The water-soluble polysaccharides found in this plant have been reported to lower blood glucose and cholesterol levels (Estiasih *et al.*, 2012).

Although some meat products with better quality produced by the incorporation of hydrolysed collagen and its fractions have been reported (Ham *et al.*, 2016; Sousa *et al.*, 2017; Hjelm *et al.*, 2019), information on the use of a combination of hydrolysed collagen, *Man-sao* powder and water in meat products is limited. The purpose of the present work was therefore to investigate the influence of incorporating hydrolysed collagen, *Man-sao* powder and water mixture (HCMSM) on the quality parameters of reduced-fat Vienna sausage.

Materials and methods

Preparation of Man-sao powder

Tubers of *Man-sao* were purchased from a market in Chaiyaphum Province, Thailand. After washing with chlorinated water (50 ppm) and rinsing several times with tap water, the rind of tubers was removed. The tubers were sliced (~0.5 cm thick) and, steeped in 1.0% citric acid solution for 45 min. After drying in a cabinet drier at 60°C for 15 h, the dried pieces of *Man-sao* were crushed with a hammer mill and screened through a 500-μm sieve. The average moisture, protein, fat, ash, and carbohydrate contents of *Man-sao* powder were 4.26%, 7.53%, 0.77%, 3.36% and 84.08%, respectively. The average fibre content of *Man-sao* powder was 1.79%.

Preparation of a mixture of Man-sao powder, water, and hydrolysed collagen (HCMSM)

HCMSM was prepared by mixing *Man-sao* powder, hydrolysed collagen and distilled water in a 2:1:2 ratio (Alves *et al.*, 2016) using a blender (HR2118, Philips, Indonesia). The average moisture, protein, fat, ash, and carbohydrate contents of the hydrolysed collagen were 3.77%, 92.74%, 0.04%, 0.46% and 2.99%, respectively. The chemical composition of the HCMSM was 46.49% moisture, 23.12% protein, 0.37% fat, 1.48% ash, and 28.54% carbohydrate.

The hydrolysed collagen used in the present work was obtained from Swiss Nutrivalor Company, Switzerland. The hydrolysed collagen was produced from the skin of salmon fish. The average molecular weight, viscosity (20% at 25°C), pH value, isoelectric point, bulk density, and conductivity of hydrolysed collagen were 2,000 Da, 2.2 - 3.7 mPa.s, 5.0 - 6.4, 7.0 - 9.0, 0.25 - 0.35 g/cm^3, ≤ 1.0 mS/cm. Furthermore, the total aerobic count, *Escherichia coli*, anaerobic sulphite-reducing sp. *Salmonella* sp. and *Staphylococcus aureus* of hydrolysed collagen were ≤ 1,000 CFU/g, absence in 10 g, ≤ 10 CFU/g, absence in 25 g and absence in 1 g, respectively.

Table 1. Formulation of Vienna sausages prepared with a mixture of *Man-sao* powder, water and hydrolysed collagen as a fat replacer.

Ingredients (g)	T0	T20	T40	T60	T80	T100
Pork meat	1,500	1,500	1,500	1,500	1,500	1,500
Ice	750	750	750	750	750	750
Pork back fat	750	600	450	300	150	0
HCMSM	0	150	300	450	600	750
Sodium nitrite (125 ppm)	0.375	0.375	0.375	0.375	0.375	0.375
Sodium erythorbate	2.1	2.1	2.1	2.1	2.1	2.1
Sodium tri-polyphosphate	9	9	9	9	9	9
Sodium chloride	64.5	64.5	64.5	64.5	64.5	64.5
Monosodium glutamate	3	3	3	3	3	3
Spice	30	30	30	30	30	30
Smoke powder	3	3	3	3	3	3

Preparation of the sausages

Six treatments of Vienna sausages with different concentrations of a mixture of hydrolysed collagen, *Man-sao* powder and water (HCMSM) as a fat replacer (0%, 20%, 40%, 60%, 80% and 100%) were prepared. All formulations of Vienna sausages in the present work are presented in Table 1.

Fresh pork shoulder and pork back-fat were obtained from the Khon Kaen University slaughterhouse. After slaughtering, the carcasses were chilled at 4 ± 1°C for 22 h and then divided into different parts, including the shoulder. The shoulder parts were transported from the university slaughterhouse to the food processing laboratory within 5 min by packing them in polystyrene boxes. The shoulder parts within the boxes were covered with plastic bags containing ice cubes. After removing visible fat and connective tissue, the lean meat was ground through a 5-mm and 2.5-mm plate, respectively, using a meat grinder (BIRO®, USA). The partially frozen pork back-fat was also ground using the same conditions. The ground pork plus 25% ice cubes was blended in a silent cutter (cutter M11N Maschinen, Germany) at low speed for 1 min, then sodium chloride, sodium phosphate, sodium nitrite, sodium ascorbate, spices, seasoning, and 50% ice cubes were added to the comminuted meat and further chopped at high speed for 3 min. HCMSM and ground pork fat at test levels, as well as the remaining ice, were incorporated into the meat mixture and additionally chopped for 3 min at high speed. During chopping, the batter temperature was controlled at ~12°C. After chopping, the batter was extruded into a 14-mm-diameter plastic casing (LEM products, Canada) using a TWF-12 stuffer (DICK, Germany) and hand-linked at 10-cm intervals. The raw sausage links were cooked in a water bath at 80°C until the temperature at a geometric centre became 72°C. The cooked sausage samples were immersed in cold water (4°C) for 30 min and refrigerated overnight at 4°C. The casings were removed, the products were vacuum-packed in polyethylene/polyester bags and stored at 4 ± 1°C for one night prior to analyses. The experiment was conducted three times.

Proximate analysis

The chemical composition of all cooked sausage samples was analysed as per AOAC procedures (AOAC, 2012). A bomb calorimeter (CAL3K-AP, Germany) was employed for calorific value determination of all cooked sausage samples.

Physicochemical analyses of Vienna sausage samples

The stability of raw emulsion was evaluated as per Jiménez Colmenero *et al.* (2005). In brief, 20 g of raw batter were filled in pre-weighted plastic tubes (50 mL), centrifuged (5,000 *g*, 30 min at 5°C) and heated at 70°C for 30 min in a water bath. The tubes were cooled to 4°C to assist the severance of fat and water layers. The total amount of fluid separated was collected. The amount of fat and water released was then measured and expressed as a percentage of sample weight.

The viscosity of the raw batter from each treatment was measured using a Brookfield viscometer (Model RVT, Brookfield Engineering, USA). Briefly, 100 g of raw batter was placed in a 100 mL beaker, and spindle number 7 was used for this purpose. After 30 s of spindle rotation in the batter sample, a reading was taken (Shand, 2000). The unit of viscosity was expressed as centipoise (cP). The temperature during viscosity measurement for each sample was also recorded (25 ± 1°C).

The cooking loss was determined as per Andrés *et al.* (2006) by recording the weight of 10 links of sausages from each treatment before and after steeping in hot water (80°C) until 72°C of core temperature was attained.

The surface colour of cooked sausages [*i.e.*, lightness (L^*), redness (a^*) and yellowness (b^*)] was measured at six points on each sausage link using a Hunter colorimeter (Hunter Lab Ultrascan XE, USA) calibrated according to the guideline provided by the instrument supplier. Five links of sausage from each treatment were used for this measurement.

Texture profile analysis of cooked sausages was carried out using a texture analyser (TA-XT2i, Stable Micro System, UK). A total of 15 transversely cut slices (each slice: 14 mm in diameter, 15 mm in height) from each sausage formula were pressed twice to 40% of their original height by using a 25-kg cylindrical probe (P/35). The crosshead speed was set at 2.0 mm/s. The textural parameters (*i.e.*, hardness, springiness, cohesiveness, adhesiveness, and chewiness) were interpreted from the force-time curves using the software provided by the instrument supplier.

Microstructure determination

Microscopic examination of cooked Vienna sausage samples was carried out by scanning electron microscopy (SEM) as per the modified method of Barretto *et al.* (2015). Briefly, sausage samples were treated with a mixture of 0.1 M phosphate buffer (pH 7) and 2.5% glutaraldehyde at 5°C for 24 h, dipped in 0.1 M phosphate buffer (pH 7) for 20 min and then post treated with 0.1 M phosphate buffer (pH 7) containing 1% osmium tetroxide (OsO_4) for 6 h. The prepared samples were washed with 0.1 M phosphate buffer (pH 7) for 25 min, after which the samples were dehydrated in an ethanol series (30, 50, 70, 95, and 100% for 30 min each). The samples were immersed in absolute ethanol for further dehydration, allowed to dry in warm air, sputter-coated with gold and then scanned by miniSEM (SNE-4500M, SEC Co., Ltd., Korea) at 15 kV. Micrographs were taken at 100× magnifications.

Table 2. Effect of a mixture of *Man-sao* powder, water and hydrolysed collagen on the physico-chemical properties of Vienna sausages.

	T0 (control)	T20	T40	T60	T80	T100
L^*	64.48 ± 0.53^{a}	64.68 ± 0.85^{a}	63.16 ± 0.73^{ab}	62.32 ± 1.32^{b}	60.42 ± 1.53^{c}	59.45 ± 0.60^{c}
a^*	4.93 ± 0.07^{a}	4.91 ± 0.18^{a}	4.64 ± 0.19^{a}	4.81 ± 0.35^{a}	4.79 ± 0.21^{a}	4.97 ± 0.14^{a}
b^*	9.45 ± 0.51^{a}	10.06 ± 0.30^{a}	10.11 ± 0.30^{a}	10.11 ± 0.61^{a}	10.11 ± 0.90^{a}	9.98 ± 0.30^{a}
Cooking loss (%)	5.91 ± 0.10^{a}	4.26 ± 0.05^{b}	3.24 ± 0.12^{c}	1.73 ± 0.04^{d}	0.56 ± 0.03e	0.36 ± 0.02f
Total fluid release (%)	1.512 ± 0.004^{a}	1.486 ± 0.011^{a}	1.376 ± 0.183^{ab}	1.289 ± 0.049^{b}	1.169 ± 0.046^{b}	0.807 ± 0.093^{c}
Fat release (%)	0.110 ± 0.022^{b}	0.103 ± 0.013^{a}	0.100 ± 0.005^{ab}	0.090 ± 0.011^{b}	0.087 ± 0.015^{b}	0.066 ± 0.013^{c}
Water release (%)	1.402 ± 0.025^{a}	1.383 ± 0.002^{a}	1.270 ± 0.179^{ab}	1.199 ± 0.058^{b}	1.082 ± 0.037^{b}	0.741 ± 0.101^{c}
Viscosity (×10^5 cP)	1.92 ± 0.01^{d}	2.82 ± 0.14^{c}	3.00 ± 0.19^{bc}	3.05 ± 0.14^{bc}	3.16 ± 0.04^{ab}	3.38 ± 0.15^{a}
Hardness (kg)	3.108 ± 0.031^{c}	3.149 ± 0.022^{c}	3.372 ± 0.066^{b}	3.423 ± 0.034^{b}	3.546 ± 0.025^{b}	3.783 ± 0.276^{a}
Adhesiveness (kg.sec)	-0.041 ± 0.007^{b}	-0.055 ± 0.002^{c}	-0.023 ± 0.003^{a}	-0.065 ± 0.000^{d}	-0.382 ± 0.004^{b}	-0.049 ± 0.001^{c}
Springiness (kg.sec)	2.773 ± 0.096^{a}	2.648 ± 0.009^{a}	2.458 ± 0.075^{b}	2.337 ± 0.153^{b}	1.985 ± 0.111^{c}	1.655 ± 0.025^{d}
Cohesiveness	0.691 ± 0.008^{a}	0.691 ± 0.008^{a}	0.749 ± 0.138^{a}	0.800 ± 0.052^{a}	0.809 ± 0.119^{a}	0.817 ± 0.033^{a}
Chewiness (kg)	5.960 ± 0.132^{a}	6.241 ± 1.082^{a}	6.638 ± 0.638^{a}	6.503 ± 1.32^{a}	5.759 ± 0.557^{a}	5.104 ± 0.106^{a}

Means within the same column with different superscript letters were significantly different ($p < 0.05$).

Sensory evaluation of the sausage samples

Sensory analyses of cooked sausage samples were performed only for the first replication using a 9-point hedonic scale test. Thirty untrained panellists who regularly consumed emulsion-type sausages evaluated their likability regarding colour, odour, taste, texture, and overall liking. Before the test, the Vienna sausage samples were steeped in hot water (95 ± 2°C) in individual pans for 2 min. Two slices (2.5 cm long at 35°C) of each sausage formulation coded with 3-digit random number were served in random order to the taste panels in individual booths. The taste panellists were instructed to cleanse their palates between samples (Deda *et al.*, 2007).

Design of experiment and statistical analysis

The experimental designs used in the present work were completely randomised design (CRD) and randomised complete block design (RCBD); the former for chemical and physicochemical qualities, the latter for sensory evaluation. SPSS software version 23 was used to analyse the variance of experimental data (ANOVA) and identified the difference among each pair of treatment means at $p < 0.05$ through Duncan's new Multiple Range Test (DMRT). All experiments were carried out in triplicate ($n = 3$), and the results were expressed as means (± SD).

Results and discussion

Physicochemical characteristics of Vienna sausages

The influence of a pork back fat replacement by a mixture of *Man-sao* powder, water, and hydrolysed collagen (HCMSM) on the physicochemical properties of Vienna sausages is shown in Table 2. The incorporation of HCMSM significantly affected the emulsion stability and cooking loss of the sausages ($p < 0.05$). The values of total fluid release, fat release and water release ranged from 0.81 to 1.52, 0.06 to 1.10 and 0.74 to 1.40, respectively. The increase in HCMSM resulted in a reduction of total fluid release, fat release, and water release, indicating higher emulsion stability. The sausage sample containing 100% HCMSM showed the lowest values of fat and water exudation and thus the highest emulsion stability ($p < 0.05$). This result might be due to the high starch and hydrolysed collagen contents of HCMSM, which are able to form a gel network at high temperatures, resulting in greater entrapment of fat and water within the food matrix (Feiner, 2006). In addition, a reduction of fat release with an increase in HCMSH may be due to a reduction in the amount of added fat in sausage formulations. Alves *et al.* (2016) reported that the emulsion stability of bologna-type sausage increased with an increasing content of pork skin and green banana flour gel. de Oliveira Faria *et al.* (2015) demonstrated that higher emulsion constancy was observed when a mixture of pork skin and amorphous cellulose as a fat replacer was added to bologna-type sausage. Similarly, Choe *et al.* (2013) reported that as the level of pig skin and wheat fibre mixture as a fat replacer increased, the emulsion stability of the frankfurters also increased.

The cooking loss of sausage samples with different levels of HCMSM varied between 0.36% and 5.91% (Table 2). The cooking loss decreased ($p < 0.05$) with increasing HCMSM level. The T100 sample (100% HCMSM) showed the lowest cooking loss ($p < 0.05$), which could be attributed to having the lowest fat and water separation during cooking (Serdaroğlu and Değırmencioğlu, 2004). Similarly, Choe *et al.* (2013), de Oliveira Faria *et al.* (2015), and Alves *et al.* (2016) reported lower cooking loss when a mixture of pork skin and dietary fibre were incorporated into the emulsified meat product as a fat substitute.

Viscosity reflects the internal friction of the meat batter (Bourne, 2002). The viscous raw batter can be used to produce high-quality emulsion-type sausages (Choe *et al.*, 2013). In the present work, the viscosity of the raw batter containing different proportions of HCMSM was in the range of 1.92×10^5 cP to 3.38×10^5 cP (Table 2). The addition of HCMSM led to a significantly higher batter viscosity ($p < 0.05$) as compared to the control (no added HCMSM). This result may be due to the high water-holding ability of fibre and protein in HCMSM. The highest viscosity was found in the T100 sample, whereas the control (T0) exhibited the lowest viscosity ($p < 0.05$). Many researchers have found that the addition of plant fibres may increase the batter viscosity of low-fat meat batters (Claus and Hunt, 1991; Shand, 2000; Sariçoban *et al.*, 2008; Choi *et al.*, 2008; 2011). Choe *et al.* (2013) reported that the incorporation of pig skin and wheat fibre mixture significantly increased the viscosity of the meat emulsion. Choe *et al.* (2013) also found a negative correlation between the stability of raw meat emulsion and cooking loss. Aktaş and Gençcelep (2006) reported that the higher the viscosity of a meat batter, the lower the cooking loss of bologna-type sausage. Lee *et al.* (2008) demonstrated that dietary fibre imparted high water-holding ability and improved viscosity of breakfast sausage; thus, the viscosity of sausage batter appeared to be related to emulsion stability.

The impact of HCMSM level on colour parameters of sausage samples is presented in Table 2. The substitution of up to 40% fat with HCMSM did not modify the values of L^*, a^* and b^* of the sausage. In contrast, the sausage samples containing 60% (T60), 80% (T80) and 100% (T100) HCMSM had significantly lower L^* values than the control sample (T0) ($p < 0.05$), whereas the significant difference of b^* and a^* values among all of the samples ($p < 0.05$) was not observed. Alves *et al.* (2016) reported that the addition of a pork skin and green banana flour mixture as a fat replacer for up to 60% did not affect the L^*, a^*, and b^* values of bologna-type sausages ($p < 0.05$). Beyond 60%, however, the L^* value was lower than the full-fat control sample. The level of pork skin and green banana flour mixture did not change the b^* value of bologna-type sausage ($p < 0.05$). de Oliveira Faria *et al.* (2015) likewise found that colour values of bologna-type sausages containing 50% gels prepared with pig skin and amorphous cellulose were comparable to those of the full-fat control. Similarly, Choe *et al.* (2013) observed that replacement of 10% fat with a mixture of pig skin and wheat fibre did not change the L* value of frankfurter-type sausages ($p < 0.05$).

The influence of HCMSM incorporation on textural attributes of sausage samples is presented in Table 2. An increase in HCMSM concentration resulted in an increase in hardness ($p < 0.05$). The sample containing 100% HCMSM (T100) exhibited the highest hardness whereas the control (T0) showed the lowest hardness ($p < 0.05$). This finding may be observed because hydrolysed collagen has a hard texture at low temperatures. The solid content of the sausages may, moreover, increase with increasing *Man-sao* powder, resulting in a harder texture. Choe *et al.* (2013) reported that frankfurter-type sausages containing pig skin and wheat fibre exhibited higher hardness than the control with no pig skin and wheat fibre addition. This finding is observed because pig skin gelatine provides a hard and firm texture at low temperature. Feiner (2006) also reported that the incorporation of pig skin emulsion provides a firm and hard texture. Similarly, Pereira *et al.* (2011) reported that the hardness of emulsion-type sausages depended on the level of collagen fibre incorporated into the meat recipes. Pereira *et al.* (2011) explained that after chemical binding with water in a protein matrix, collagen fibre swelled and provided firmness or hardness to the final product.

The HCMSM concentration also affected the adhesiveness, springiness, and gumminess of the sausages (Table 2). An increase in HCMSM concentration led to a reduction in springiness; thus, sausage samples with a higher HCMSM concentration exhibit a lower spring-back after being deformed. The sausage sample containing 100% HCMSM (T100) had the lowest springiness, whereas the control sample (T0) had the highest value. No significant difference was detected for all textural parameters between the control sample (T0) and

Table 3. Effect of a mixture of *Man-sao* powder, water and hydrolysed collagen on proximate composition and calorific value of Vienna sausages.

	T0	T20	T40	T60	T80	T100
Moisture (%)	60.82 ± 0.03^f	64.49 ± 0.15^e	66.31 ± 0.06^d	68.40 ± 0.44^c	70.10 ± 0.21^b	72.68 ± 0.37^a
Fat (%)	16.64 ± 0.16^a	13.67 ± 0.11^b	10.56 ± 0.31^c	2.82 ± 0.15^d	1.38 ± 0.09e	0.82 ± 0.03^f
Protein (%)	12.76 ± 0.01^e	13.96 ± 0.07^d	14.42 ± 0.07^c	15.35 ± 0.14^b	16.24 ± 0.25^a	16.46 ± 0.19^a
Ash (%)	1.79 ± 0.07^c	2.04 ± 0.07^b	2.06 ± 0.00^b	2.42 ± 0.07^a	2.46 ± 0.01^a	2.46 ± 0.01^a
Calorific value	233.04 ± 0.92^a	202.53 ± 1.31^b	179.58 ± 1.37^c	131.13 ± 0.79^d	116.98 ± 0.72^e	103.86 ± 1.67^f

Means within the same column with different superscript letters were significantly different ($p < 0.05$).

the sample with 20% HCMSM (T20) ($p < 0.05$). In the present work, the sausage sample prepared from 0% pork fat had the highest hardness and the lowest springiness, whereas the sample prepared with 100% fat exhibited the opposite trend.

Even if the sausage sample with 40% HCMSM (T40) exhibited higher instrumental hardness than the control sample (T0) ($p < 0.05$), its sensory likability scores were comparable to those of the control (Table 4). Therefore, it would be accepted by the consumers and would be marketable.

Chemical composition of sausage sample

The results of HCMSH concentration on the chemical composition of Vienna sausages are presented in Table 3. The moisture content of the sausages increased, and the fat content decreased ($p < 0.05$) with increasing levels of HCMSH. The moisture and fat contents of the control sample (T0) were 60.82% and 16.64%, respectively. The respective values reached 72.68% and 0.82% in the T100 sample, possibly due to the high-water absorption and low-fat absorption of the fibre present in the HCMSM. Alves *et al.* (2016) reported that the moisture content of bologna-type sausage increased, and the fat content decreased, with increasing levels of gel prepared from pork skin, green banana flour, and water. As seen in Table 3, an increment of HCMSH as a fat replacer resulted in a reduction of fat content. The incorporation of 20%, 40%, 60%, 80% and 100% HCMSH showed 17.85%, 36.54%, 59.01%, 79.68% and 95.08% fat reduction as compared to the control, respectively. Based on FDA, food products can be classified as "reduced-fat" if their fat content is reduced to at least 25% of the original fat content (US FDA, 2016). Therefore, the Vienna sausage samples with 40% HCMSM or more can be claimed as reduced-fat Vienna sausages.

The protein content of sausage samples increased significantly ($p < 0.05$) as the substitution of animal fat by HCMSH increased, presumably due to the greater protein content of the HCMSM (23.12%) when compared with the pork back fat (8.26%). Choe *et al.* (2013) reported that the protein content of frankfurter-type sausages increased with increasing amounts of fat replacer - a mixture of pork skin, water, and wheat fibre in a ratio of 2:2:1.

The reduced-fat samples containing HCMSM exhibited higher ash content than the control sample ($p < 0.05$), probably due to the high ash content in the HCMSM (1.48%). The calorific value of Vienna sausages was affected by the HCMSM concentration ($p < 0.05$) (Table 3): it was 233.04 kcal/100 g for the full-fat sample (T0) and ranged between 103.86 kcal/100 g and 202.53 kcal/100 g for reduced-fat samples containing HCMSM. The calorific value was 13.09% to 55.43% lower in the Vienna samples containing HCMSM as compared to that in the full-fat control sample. Choe *et al.* (2013) reported that the caloric content of frankfurter-type sausages decreased ($p < 0.05$) with increasing concentrations of the gel prepared from pork skin, water, and wheat fibre in a 2:2:1 ratio. Namhong and Rojanakorn (2016) reported that the addition of fat replacer (a mixture of sweet potato powder, gelatine powder, and water in a ratio of 1:0.25:9) decreased the calorific value of Vienna sausages ($p < 0.05$). Verma *et al.* (2015) demonstrated that reduced-fat pork patties containing sweet potato exhibited a 23% lowering in calorific value. Aslinah *et al.* (2018) demonstrated that increasing adzuki beans (*Vigna angularis*) as a meat extender and fat replacer in beef meatballs from 0% to 100% resulted in a significant reduction of fat content and calorie value of the reduced-fat beef meatball samples. Prasad *et al.* (2011) reported that the replacement of fat in chicken kofta with 8% oat flour and 2.5% casein led to a reduction of fat content from 14.2% to 8.5%.

Scanning electron microscopy

Generally, the surface morphology of emulsion-type sausages observed under a scanning electron microscope relates to their functional properties (*i.e.*, water-holding capacity) and texture (Rather *et al.*, 2016). Figure 1 shows micrographs of cooked Vienna sausage samples. The microstructure of the

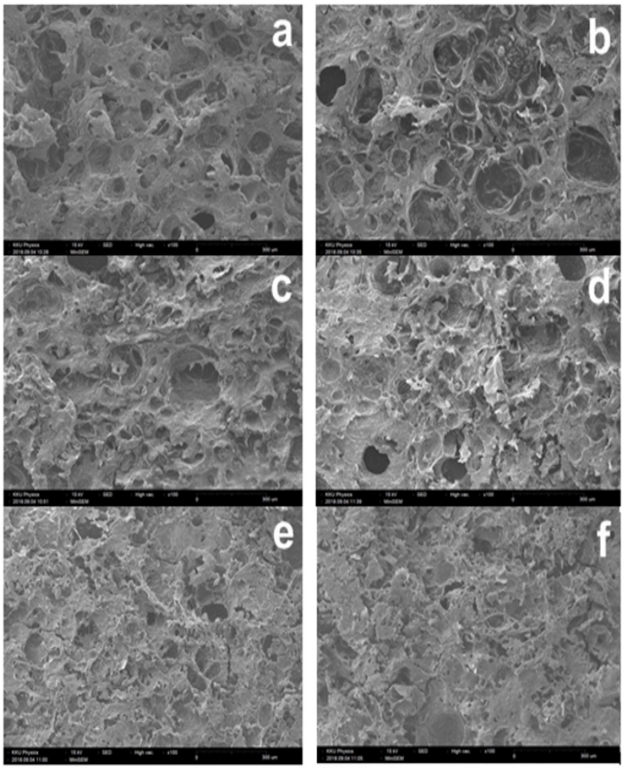

Figure 1. Scanning electron microscopy (SEM) images of Vienna sausages with different amounts of HCMSM: (a) control or 0% HCMSM; (b) 20% HCMSM; (c) 40% HCMSM; (d) 60% HCMSM; (e) 80% HCMSM; (f) 100% HCMSM.

full-fat control revealed the development of cavities of different sizes, which generated structures with a spongy or honeycomb-like appearance (a three-dimensional gel network) (Figure 1a), similar to those described by Ayadi *et al.* (2009), Jiménez-Colmenero *et al.* (2010), Barretto *et al.* (2015) and Rather *et al.* (2016). The microstructure of the sample with 10% HCMSM exhibited increased disorganisation and a less honeycomb-like structure with fewer and larger holes (Figure 1b). The substitution of pork fat with HCMSM content higher than 20% led to an unrecognisable, more heterogeneous and compact microstructure (Figure 1c-f). Greater heterogeneity and compactness of the microstructure occurred with increasing HCMSM concentration (Figure 1c-f). This result might be observed because *Man-sao* powder in HCMSM is embedded in the protein gel matrix of low-fat emulsion meat systems, resulting in the development of a firmer gel/emulsion network with greater fat- and water-binding properties (Table 2). Jiménez-Colmenero *et al.* (2010) reported that a combination of seaweed and konjac gel added to reduced-fat frankfurters was distributed in the meat protein matrix, resulting in a more heterogeneous and compact microstructure with better water- and fat-binding abilities. Thus, large differences in microstructure among sausage samples in the present work may reflect the differences in texture and emulsion stability (Table 2) of the product.

Sensory evaluation

The sensory likability scores of different sausage samples are presented in Table 4. There were no significant differences in appearance, colour, and odour likability scores among all sausage samples ($p < 0.05$). Nevertheless, the full-fat control sample (T0) showed higher likability scores for texture, taste, and overall liking than the samples containing 60%, 80%, and 100% HCMSM. The incorporation of HCMSM into the sausage samples at 60% or more led to a reduction of texture and taste likability scores as compared to the control ($p < 0.05$). This result may be observed because the panellists could detect the flavour and odour of *Man-sao* and hydrolysed collagen - the two main components in HCMSM. In addition, the texture of these samples was too hard for the panellists to accept. The overall liking scores of the control sample (T0) and the samples with 20% HCMSM (T20) and 40% HCMSM (T40) ranged between 6.67 and 7.00, indicating that the taste panels moderately accepted these three samples. The substitution of up to 40% pork fat by HCMSM can be used to produce acceptable Vienna sausages. This outcome is comparable to the findings of the previous study of Alves *et al.* (2016), who found no significant differences for all sensory attributes tested with up to 60% substitution of pork back fat with gel prepared from pork skin, green banana flour, and water.

Table 4. Effect of a mixture of *Man-sao* powder, water and hydrolysed collagen on sensory likability scores of Vienna sausages.

	T0	T20	T40	T60	T80	T100
Appearance	6.23 ± 1.41^{a}	6.13 ± 1.38^{a}	6.70 ± 1.21^{a}	6.57 ± 1.30^{a}	6.67 ± 1.21^{a}	6.43 ± 1.43^{a}
Colour	6.23 ± 1.36^{a}	6.30 ± 1.29^{a}	6.43 ± 1.28^{a}	6.43 ± 1.19^{a}	6.63 ± 0.93^{a}	6.23 ± 1.36^{a}
Odour	5.70 ± 1.95^{a}	5.77 ± 1.79^{a}	5.67 ± 1.75^{a}	6.00 ± 1.74^{a}	5.50 ± 1.74^{a}	5.77 ± 1.50^{a}
Texture	7.00 ± 1.31^{a}	6.93 ± 1.17^{a}	6.80 ± 1.00^{a}	5.83 ± 1.44^{b}	5.87 ± 1.59^{b}	5.40 ± 1.45^{b}
Taste	7.07 ± 1.44^{a}	6.27 ± 1.64^{ab}	6.43 ± 1.28^{ab}	5.93 ± 1.57^{bc}	5.60 ± 1.81^{bc}	5.13 ± 1.55^{c}
Overall liking	7.00 ± 1.26^{a}	6.67 ± 1.24^{a}	6.83 ± 1.12^{a}	5.96 ± 1.24^{b}	5.93 ± 1.68^{b}	5.40 ± 1.43^{b}

Means within the same column with different superscript letters were significantly different ($p < 0.05$).

Conclusion

The addition of hydrolysed collagen, *Man-sao* powder and water mixture (HCMSM) as a fat replacer increased the moisture and protein contents, emulsion stability, and hardness of the sausage samples. The fat content, calorific value, cooking loss, and lightness (L^*) decreased with increasing HCMSM concentration. The SEM showed unrecognisable, more heterogeneous, and compact microstructure in treatments containing 60%, 80% and 100% HCMSM, resulting in the development of a firmer gel/emulsion network with greater fat- and water-binding abilities. The results of the sensory evaluation indicated that up to 40% HCMSM can be employed as a fat substitute to produce Vienna sausages with high quality and acceptability.

Acknowledgement

The authors thank (a) Khon Kaen University for the financial support received for the completion of the present work, and (b) Mr. Bryan Roderick Hamman for assistance with the English language presentation of the manuscript.

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