International Journal of Agricultural Technology 2023 Vol. 19(6):2669-2680
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

dApplication of microcrystalline cellulose gel as a fat reduction strategy in phosphate-free emulsified sausage

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Sorapukdee, S., Phengmanee, N., Mongput, W., Pilasombut, K. and Tangwatcharin, P. (2023). Application of microcrystalline cellulose gel as a fat reduction strategy in phosphate-free emulsified sausage. International Journal of Agricultural Technology 19(6):2669-2680.

Abstract Three treatments of phosphate-free pork sausages were formulated by replacing pork backfat with 0% (control), 5%, and 10% microcrystalline cellulose (MCC) gel. MCC gel was prepared by chopping MCC powder with cold water in the ratio of 1:9 prior to use as a fat replacer. The addition of 5% MCC gel showed the similar product textural characteristics and color when compared with control (p>0.05). The 10% MCC gel increased in grilling loss with decrease lightness and yellowness of tested sausages (p<0.05), but could improve firmness with higher hardness, gumminess, and chewiness than control (p<0.05). However, after 4-weeks storage at 4°C, the 10% MCC gel showed the lower lipid oxidation as indicated by thiobarbituric acid reactive substance (TBARS) value than other sausages (p<0.05). Comparing with the control sample, the detrimental impact of MCC gel both 5% and 10% on sensorial characteristics tested by sensory panelist in terms of color, flavor, texture, taste, and overall acceptability were not found (p<0.05). The 10% MCC gel added product had the lowest fat content and total energy value (1.80% and 128.79 kcal/100 g, respectively) together with the highest crude fiber content (4.27%) (p<0.05). While the control sample, high-fat content formulation without added MCC gel, exhibited the highest fat content and total energy value (8.18% and 200.08 kcal/100 g, respectively) with the lowest crude fiber content (0.70%) (p<0.05). These compositions revealed that the 10% MCC gel added sausage could categorize as reduced-fat, reduced-calories, and good source of fiber when compared to the same regular products. The findings pointed out that MCC gel as a fat replacer offered potential advantages in upscaling phosphate-free and reduced-fat sausage.

Keywords: Fat substitution, Healthy sausage, Hydrocolloid, Low-fat sausage, Pork sausage

Introduction

Sausage gains much widespread acceptance in most countries due to favorable taste and flavor, nutrients, convenience, and shelf-life stability. To

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improve consumer acceptance and increase product value, the healthier meat products development is one of the main purposes for the meat industry. Nowadays, the reformulation of meat products drives to replace the health concerning ingredients (phosphate and nitrite), nutritionally more beneficial (low fat, low calorie, low sodium, no cholesterol, and modifying the fatty acid profile) (Asioli *et al.*, 2017).

Among various ingredients mixed in emulsified sausage, the crucial ingredients are both salt (sodium chloride, NaCl) and phosphate. These combinations contribute to the extraction of salt soluble protein, called myofibrillar proteins, then are promoters for emulsification and gelation of final product. The vital function of phosphate in emulsified meat products are increasing pH, increasing ionic strength, and divalent cation binding (Thangavelu *et al*., 2019). However, phosphate-rich diet has some health concerns, it increases the risk of hyperphosphatemia and cardiovascular diseases (Thangavelu *et al.*, 2019). Many researches have been study to substitute phosphates in meat product with suitable ingredients such as starches, hydrocolloids, fibers, and protein (Younis and Ahmad, 2015; Resconi, 2016). Presently, our laboratory can produce phosphate-free emulsified sausage by using the commercial inactive yeast (Sorapukdee, 2022). Inactive yeast produced from natural yeast products from *Saccharomyces cerevisiae* that has been deactivated by heat, roller dried and ground. The resulting ingredient is a beige powder with meaty and creamy flavor notes and can play a role in the substitution of food grade phosphates in emulsified pork sausage (Ferrer, 2022). It could improve protein extractability and contributed favorable textural characteristics as well as flavor and taste of emulsified sausage (Sorapukdee, 2022). In fact, the cell wall or outer shell of the inactive yeast cell is mainly composed of polysaccharides, glucans, and mannans. These compositions allow it to be beneficially used as a phosphate replacer in several meat products, such as emulsified sausages, deli hams, and spreads (Ferrer, 2022).

The formulation of the several types of conventional pork sausages cannot perform without pork backfat. The dietary fat in emulsified sausage benefits the enhancement of texture, juiciness, and flavors (Keeton, 1994). Nevertheless, high fat foods are considered as the negative health implications such as obesity and chronic diseases. Alternative components can use different non-meat protein, and/or other carbohydrate and fiber sources (Varga-Visi and Toxanbayeva, 2017). Among these ingredients, microcrystalline cellulose (MCC) can be used as a potential fat replacer in meat products.

MCC is a cellulose derivative and mostly produced from wood pulp. It is classified as a hydrocolloid with no solubility in water but can absorb water mechanically at the interface. The insoluble MCC is produced by partial hydrolysis of wood pulp in hydrochloric acid, and then by separation of the non-crystalline part of cellulose (Lucca and Tepper, 1994). Prior use as an additive in meat products, it is passed to severe mechanical shearing for physically breaking into colloidal crystallite aggregates. Thereafter, it is co-dried with carboxymethyl cellulose (CMC) and/or other functional ingredients (Lucca and Tepper, 1994). MCC is used for different applications like food products as a thickener and stabilizer, cosmetic, and pharmaceutical due to its wide range of viscoelastic properties (Zhao *et al.*, 2011). MCC is also capable of strong affinity for both the oil and the water. Thus, MCC can stabilize the oil-in-water emulsions via precipitation and some orientation of its particles between the oil-in-water interface (Panda, 2010). There are a few studies reported MCC as a fat replacer in pork sausage including low-fat breakfast sausages (Mittal and Barbut, 1993), low fat frankfurters (Barbut and Mittal, 1996), and emulsified sausages (Schuh *et al.*, 2013). Regarding textural characteristic, low-fat (13% fat) pork breakfast sausages with both MC types (MCC-I and -II) were more elastic and more chewiness as compared to high-fat breakfast sausages (26% fat) (Mittal and Barbut, 1993). Among two types of microcrystalline cellulose (MCC-I and II) which were investigated by Barbut and Mittal, 1996, MCC-II improved the textural properties of the low-fat frankfurters compared with the high fat product. In emulsified sausages as reported by Schuh *et al.* (2013), the increased concentration of MCC ranged from 0.3-2.0% resulted in increased firmness of low-fat with increasing water loss when using higher than 0.7% as compared to control. However, the optimum content of MCC used in these products was concluded at different levels due to their different product formulation and origin of MCC. Moreover, the proximate composition of the final product has not been reported. When selecting the optimal fat replacer for a certain meat product, it is necessary to take into deliberation the meat product type and formulation also. Additionally, composition of developed product should be investigated for the implied nutrient content claim “healthy”. The aim was to assess the impact of MCC gel as a fat replacer on the important quality and proximate composition of low-fat and phosphate-free pork sausages.

Materials and methods

Microcrystalline cellulose (MCC) gel preparation

MCC powder (Vivapur MCG 0018S, J. Rettenmaier & Söhne GmbH + Co KG, Rosenberg, Germany) was kindly received from Rama production Co., Ltd., Bangkok, Thailand. Ten percent of MCC powder was mixed with cold water (mixed cold water and ice in the ratio of 50:50). This hydrated solution was continuously chopped in food processer for 5 mins until gel-like texture was settled with a smooth texture as shown in Figure 1A.

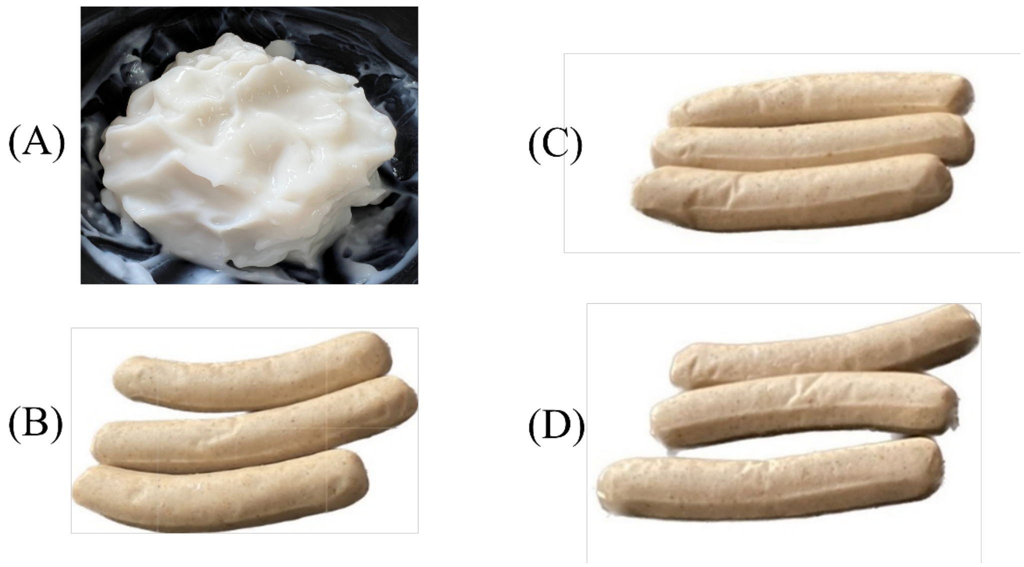

Figure 1. MCC gel after chopping (A) and emulsified pork sausage from control (B), 5% MCC gel (C), and 10% MCC gel (D)

Manufacturing of phosphate-free emulsified sausage

Pork from ham portion and pork backfat were purchased from modern trade, Ladkrabang, Thailand. Lean materials and backfat were separately ground through an 8 mm plate. The Lalvita 2190 (Lallemand Bio-Ingredients, Montréal, Canada) used as phosphate replacer obtained from Rama production Co., Ltd. Three formulation frankfurters, phosphate-free emulsified sausage, shown in Table 1, include a control (no added MCC), 5% MCC gel, and 10% MCC gel, with varying fat contents. The sausage manufacture followed Sorapukdee *et al.* (2013), with some modification. Briefly, the salts (sodium chloride), Lalvita 2190, and half the ice were added to the ground meat. The mixture was chopped in a 5-liter stainless steel cutter mixer (Universal Fritter-brane QS-505A, CKI Family Co., Ltd, China) for 120 s. Ground pork backfat with or without the hydrated MCC gel and the remaining ice were added to the batter and chopped for 90 s. The other ingredient was added to the meat batter and continuously chopped for 90 s. Finally, the meat batter was stuffed into a 21 mm diameter collagen casing, hand linked at 150 mm intervals and boiled until a core temperature of 70°C was reached. Thereafter, sausages were chilled overnight at

4°C. Three batches (n=3) were practically carried out. For each batch, samples for determination of pH, grilling loss, texture profile analysis, color, and sensory evaluation were tested within 2 days after manufacturing. Samples for monitoring lipid oxidation were stored at 4°C for 4 weeks and then assessed the thiobarbituric acid reactive substance (TBARS) values. Regarding proximate composition, sausages were frozen at −20°C and analyzed within 2 months.

Table 1. Phosphate-free emulsified pork sausage formulations (units: g/kg)

Ingredients	Control	5% MCC gel	10% MCC gel
Ground pork	480	480	480
Ground pork backfat	250	200	150
MCC gel	-	50	100
Ice	189	189	189
Salt (sodium chloride)	15	15	15
Lalvita 2190	6	6	6
Monosodium glutamate	10	10	10
Sugar	5	5	5
Frankfurter premix	45	45	45

Physical and chemical analysis

pH values

pH was measured with the electrode inserted directly into sausage after overnight chilling (Mettler Toledo, Greifensee, Switzerland). The pH value was measured in triplicate for each sample.

Grilling loss

Samples were weighed and grilled in a pan until the core temperature reached 70°C, as monitored by probes of Type-K thermocouple from a digital thermometer (52 Series II, Fluke Corp., Everett, WA, USA). Grilling loss was calculated from the weight loss after grilling and expressed as the percentage (%) of initial sample weight.

Texture profile analysis (TPA)

Texture was tested using a texture analyzer (Instron Model 3344, lllinois Tool Works lnc., USA) with cylindrical probe. Seven pieces of each treatment (21 mm diameter, 20 mm height) were prepared and placed on the instrument base. TPA parameters, following Bourne (1978), included hardness (N), cohesiveness (ratio), gumminess (N), springiness (ratio), and chewiness (N) were derived from the force-time curves generated by the Bluehill 2 software.

Color

The central part of the internal surface color of six cylindrical samples (21 mm diameter, 20 mm height) as CIE L*a*b* (lightness, redness, and yellowness) values using a colorimeter (Hunter Associates Laboratory lnc., USA).

Thiobarbituricacid reactive substances (TBARS)

TBARS were determined following Buege and Aust (1978). Five grams of sausage was added to a 50 mL centrifuge tube. Thereafter, 25 mLof thiobarbituric acid (TBA) solution (0.0375% (w/v) TBA, 15% (w/v) TCA, 0.25 M HCl) was added and then was homogenized for 1 min. The homogenate was boiled (100ºC) for 10 min, cooled and centrifuged. The absorbance of the supernatant was read at 532 nm. The TBARS value was estimated against a standard solution of 0-10 ppm malondialdehyde (MDA). The TBARS concentration was expressed as mg MDA/kg sample.

Proximate composition and energy value

The proximate compositions (moisture, ash, crude protein, crude fat, carbohydrate, and crude fiber) of samples were determined in triplicate using the methods described by the AOAC (2012). Energy values from fat content (×9 kcal/g), protein (×4 kcal/g), and carbohydrate (×4 kcal/g) were used to calculate for total energy content as kcal/100 g. The percentage of calories from fat was reported. Total fat and total energy reductions were also calculated.

Sensory evaluation

Sensory evaluation for the cooked sausages followed the method of Wheeler *et al.* (2015). The tested attributes of cooked sausage including appearance, flavor, taste, texture, and overall acceptability were examined. All samples were coded with a 3-digit random number. All sensory evaluation tests used 30 semi-trained panelists. Sensory attributes were evaluated using a night points hedonic scale from the following nine-point hedonic scale was carried out: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely.

Statistical analyses

The experiment was performed by a Randomized Complete Block Design, in which three independent batches were blocked. Significant of main effects (treatments) were carried out by One-way ANOVA. Means compared by

Duncan's multiple range test. Data was analyzed using SPSS (v.28, IBM SPSS Inc.).

Results

Physical and chemical analysis

There were no significant differences among treatments for pH of cooked sausage (*p*>0.05) (Table 2). The pH of the sausages ranged from 6.25 to 6.30. For grilling loss, the replacement of pork backfat by 10% MCC gel could increase grilling loss in phosphate-free sausage (*p*<0.05), while 5% MCC exhibited a similar result with control (*p*>0.05).

Table 2. Impact of MCC gel on some physical and chemical properties of phosphate-free emulsified sausage

Parameters	Control	5% MCC gel	10% MCC gel
pH of cooked sausage	6.30±0.06$^{a,/1,2}$	6.20±0.02^{a}	6.25±0.05^{a}
Grilling loss (%)	2.22±0.15^{a}	2.44±2.23^{a}	3.40±0.16^{b}
Texture Profile Analysis			
- Hardness (N)	19.96±2.94^{b}	18.61±1.36^{b}	24.28±1.99^{a}
- Cohesiveness (ratio)	0.66±0.04^{a}	0.72±0.06^{a}	0.68±0.02^{a}
- Gumminess (N)	13.09±1.33^{b}	13.40±1.51^{b}	16.71±1.61^{a}
- Springiness (ratio)	0.86±0.02^{a}	0.88±0.06^{a}	0.84±0.04^{a}
- Chewiness (N)	11.27±0.95^{b}	11.81±1.07^{b}	14.18±1.85^{a}
Color of cooked sausage			
- Lightness (L*)	66.25±0.39^{a}	64.52±1.38^{ab}	63.60±0.37^{b}
- Redness (a*)	4.43±0.18^{a}	4.78±0.39^{a}	4.21±0.34^{a}
- Yellowness (b*)	18.15±0.69^{a}	17.18±0.69^{ab}	16.29±0.35^{b}
TBRARS (mg MDA/ kg sample)	2.33±0.44^{b}	1.46±0.01^{b}	0.90±0.02^{a}

[1]/ Different superscripts in the same row indicate significant differences (***p***<0.05), [2]/ Values are given as means±standard deviation of each processing batch (n = 3).

The instrumental TPA is shown in Table 2. The use of MCC gel had a significant impact on textural characteristic of sausage in various terms (*p*<0.05). The treatment 10% MCC gel showed the highest hardness, gumminess, and chewiness. However, the treatment 5% MCC gel had a similar hardness, gumminess, and chewiness as compared with control. Cohesiveness and springiness for each treatment did not show significant differences (*p*>0.05).

The partial substitution of pork backfat by MCC gel impacted the sausage color (Table 2). The replacement of pork backfat by 10% MCC gels decreased lightness and yellowness as compared with control (*p*<0.05). All treatments did not exhibit significant differences in redness (*p*>0.05).

In case of lipid oxidation, the TBARS values of sausages stored at 4 weeks at 4ºC is shown in Table 2. The reduction of fat in sausage by adding 10% MCC gels exhibited the lower lipid oxidation than control and 5% MCC gels (*p*<0.05).

Proximate composition and energy value

The content of moisture, protein, fat, ash, carbohydrate, and crude fiber of sausages is shown in Table 3. The product with 10% of MCC gel provided higher moisture and crude fiber than control ($p<0.05$). Whereas, the control sausage had the highest fat content, followed by 5% and 10% MCC gel treatments, respectively ($p<0.05$). The 10% MCC gel had a lower ash content than the 5% MC and control ($p<0.05$). There were no significant differences in protein and carbohydrate contents among treatments ($p>0.05$).

Table 3. Impact of MCC gel on Proximate composition and energy value of phosphate-free emulsified sausage

Parameters	Control	5% MCC gel	10% MCC gel
Moisture (%)	57.56±0.03[b/1,2]	59.31±2.52[ab]	64.40±0.06[a]
Protein (%)	12.95±0.24[a]	12.67±0.55[a]	13.45±0.27[a]
Fat (%)	8.18±0.02[a]	3.01±0.35[b]	1.80±0.08[c]
Ash (%)	1.64±0.01[a]	1.72±0.01[a]	1.36±0.02[b]
Carbohydrates (%)	18.84±0.35[a]	19.63±3.14[a]	14.69±1.20[a]
Crude fiber (%)	0.70±0.02[b]	3.68±1.47[ab]	4.27±1.10[a]
Total energy value (kcal/100 g)	200.80±0.60[a]	156.29±17.91[ab]	128.79±4.52[b]
Energy from fat (kcal/100 g)	73.62±0.01[a]	27.07±2.21[b]	16.24±2.56[c]
Energy from fat (%)	36.66±1.12[a]	17.32±2.41[b]	12.61±1.15[c]
Total fat reduction (%)[/3]	-	63.20	78.00
Total energy value reduction (%)[/3]	-	22.16	35.86

[1]/ Different superscripts in the same row indicate significant differences (***p***<0.05), [2]/ Values are given as means±standard deviation of each processing batch (n = 3) and [3]/ Values are presented as percentage of reduction when compared with control sample.

The total energy content of control sausage, as estimated by calculation from protein, fat, and carbohydrate content, was 200.80 kcal/100 g (around 37% of total energy originated from fat). The reformulated sample with 5% and 10% MCC gel were 156.29 kcal/100 g (around 17% from fat) and 128.79 kcal/100 g (around 13 % from fat). These changes resulted in 5% and 10 % MCC gels could reduce total energy to around 22% and 36%, respectively, as compared with control sample.

Sensory analysis

The color, flavor, texture, and overall acceptability evaluated by panelists demonstrated that there were no significant differences between treatments ($p>0.05$) (Table 4). Although the taste score of 10% MCC gel were lower than 5% gel ($p<0.05$), there were no significant differences in taste scores among 10% MCC gel and control sample ($p>0.05$).

Table 4. Impact of MCC gel on sensorial quality of phosphate-free emulsified sausage

Parameters	Control	5% MCC gel	10% MCC gel
Color	7.00±1.41[a/1,2]	6.93±1.58[a]	6.60±1.30[a]
Flavor	7.40±1.50[a]	7.46±1.30[a]	6.73±1.10[a]
Texture	7.66±1.40[a]	8.00±1.07[a]	7.33±1.29[a]
Taste	7.40±0.99[ab]	8.00±0.93[a]	6.93±0.96[b]
Overall acceptability	7.53±0.99[a]	7.93±1.16[a]	7.33±1.05[a]

[1]/ Different superscripts in the same row indicate significant differences (***p***<0.05), [2]/ Values are given as means±standard deviation of each processing batch (n = 3).

Discussion

Although the pH of phosphate-free sausages was not affected by MCC gel addition, the 10% MCC gel inclusion sausage contributed to the greater grilling loss. Moreover, the 10% MCC gel adding product also showed a higher moisture content than other samples. In the detail of the 10% MCC gel added sausage production, the MCC gel was pre-produced from 10% of MCC powder mixed with 90% water, corresponding to 1% of MCC powder in final sausage. This formulation corresponded to 40% replacing pork backfat as compared to control sample. Thus, the higher adding MCC gel meant increase the amount of free water to product formulation, leading to the higher grilling loss. The effect of MCC increased cooking loss of low-fat sausage was also reported by Barbut and Mittal (1996). Sausages tested by Schuh *et al.* (2013) that contained 1.0%-2.0% of MCC powder in emulsified sausage with similar fat content as control had also higher water loss than control.

The inclusion of 10% MCC gel in phosphate-free sausage influenced hardness, gumminess, and chewiness. The impact of MCC gel on textural characteristic of sausage were also reported by Mittal and Barbut (1993) and Barbut and Mittal (1996). MCC gel mixed in low-fat breakfast sausage impacted on higher chewiness and shear force compared to high fat product (Mittal and Barbut, 1993). For low-fat frankfurter, a higher shear force value of MCC gel than control was found, but no differences in TPA parameters were reported (Barbut and Mittal, 1996). In emulsified sausage, the MCC positively affected on a mechanical property of a product by increasing the firmness of a finish product because of its high compatibility in the meat matrix (Schuh *et al.*, 2013). Since MCC is largely crystalline with no net charge, it could form gel network and filled the gaps between a meat-gel matrix without any disruption of the protein network during heating (Schuh *et al.*, 2013). However, the use of 5% MCC gel in present study could mimic the effect the fat reduction. The 5%MCC gel did not change any of the textural characteristics of the phosphate-free and reduced fat emulsified sausage.

The addition of 10% MCC gel caused decreased lightness and yellowness values of product. The lower lightness might relate with more excessive water loss from sample than control, producing darker product. In terms of yellowness, the higher intensity of yellowness in sausage usually agrees with higher concentrations of fat presented in the product (Schuh *et al.*, 2013). Nevertheless, the changes in color were too small to influence consumer color liking score by hedonic test.

After storage product for 4 weeks at 4°C, the product with higher fat reduction showed lower lipid oxidation as measured by TBARS value. The significant reduction in TBARS values of low-fat products using konjac gel as fat replacer in merguez sausage (Triki *et al.*, 2013) and Northeastern Thai fermented sausage (Sorapukdee *et al.*, 2019) were also reported. Pork backfat which composes of high amounts of unsaturated and polyunsaturated fatty acids are susceptible to oxidation. When it was replaced by MCC gel, the lipid oxidation generally would be minimized.

Crude fiber and moisture contents in sausages increased proportionally with amount of MCC gel added. MCC powders are composed of glucose units linked with β-1, 4-glycosidic linkages. Each glucose unit contains three free hydroxyl groups on C2, C3 and C6 (Nsor-Atindana *et al.*, 2017). Because β-glycosidic linkages cannot be digested by human carbohydrate digestive enzymes, MCC is classified as a dietary fiber (Nsor-Atindana *et al.*, 2017). Not surprisingly, when MCC gel incorporated in our sausage, it could reduce fat content and then reduce total energy of product depending on the use level. The regulations in Thailand specify that the reduced-fat and reduced-total energy products are identified as a minimum of a 25% reduction in fat and 25% reduction in total energy, respectively, as compared to the conventional product (Food and Drug Administration of Thailand, 2014). Additionally, in terms of fiber, this regulation also states that food containing more than 3 grams of fiber per 100 grams of food is a good source of fiber. Therefore, our developed phosphate-free and low-fat sausage products using 10% MCC gel as fat replacer could be categorized as reduced-fat, reduced-calorie, and a good source of fiber. This formulation could reduce fat and total energy than control accounted for 78% and 35%, respectively, together with more than 3% of fiber. While the addition of 5% MCC gel claimed for the reduced-fat and good source of fiber in which provided approximately 63% lower fat than control and contained 3% fiber.

The sensory panelist did not significantly differentiate in all terms of sensory attributes including color, flavor, texture, taste, and overall acceptability between low-fat MCC gel and control sausages. Thus, both 5% and 10% MCC gel, replacing pork backfat as 20% and 40% of fat in control, were not detrimental impact on sensory quality of low-fat sausages. In fried beef patties, Gibis *et al.* (2015) found that MCC could effectively substitute up to 50% fat compared to standard product based on sensory properties. The sensory results also summarized that the MCC-formulated beef patties provided fat-like mouthfeel and were specifically acceptable to the panelists.

The finding demonstrated that the MCC gel had a potential use as a fat replacer in phosphate-free and low-fat emulsified sausage. The MCC gel must be prepared separately prior to mixing with sausage recipe. The 5% and 10% MCC gel effectively replacing pork backfat in sausage formulation accounted for 20% and 40% of high-fat products. The higher hardness, gumminess, and chewiness of 10% MCC gel added sausage indicated more product firmness than the high-fat sausage. Without the detrimental effects on sensory quality, the finding demonstrated that the 10% MCC gel could produce healthier pork sausage, which accounted for reduced-fat, reduced-calories, and a source of fiber than the same regular product.

Acknowledgements

The author would like to offer particular thanks to the School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang for financial and laboratory support.

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(Received: 26 September 2023, Revised: 2 November 2023, Accepted: 14 November 2023)