



Okra cellulose crystals stabilized Pickering emulsion: A practical tool for soybean oil inclusion to improve nutritive profile of sausages

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ABSTRACT

Pickering emulsions stabilized by okara cellulose crystals and the cellulose crystals modified with tannic acid were prepared and used to substitute porcine fat for sausage preparation. Both used cellulosic materials could effectively preserve dispersibility and heat stability of the emulsions. There was improved sausage stability when the emulsions stabilized by the cellulosic materials were used to replace pork backfat in the sausage formulation. The sausages added with the cellulosic material-based emulsions, especially the ones stabilized by the okara cellulose grafted with tannic acid, possessed better oxidative stability during storage than the control added with porcine fat. Moreover, lowered lipolysis degree could be found for the sausages added with the cellulosic materials stabilized emulsions as compared to the control formulation. Therefore, incorporation of the emulsions stabilized by the cellulosic materials might be a feasible way to improve nutritive profile by lowering saturated fatty acid content and energy uptake of the sausages.

1. Introduction

Meat products are popularly consumed owing to their tasty and nutritive value as a good source with high bioavailability of protein, vitamin, and mineral (Zhou, Zhang, & Wang, 2022). Nevertheless, meat products always consist of animal fat with abundant amounts of cholesterol and saturated fatty acids (SFA). Excessive consumption of animal fat may increase the risk of some health threatening effects such as obesity and cardiovascular diseases (Zhou et al., 2022). Owing to a continuously growing trend in healthy food consumption, there is interest in restructuring fat in meat products to lower their SFA and cholesterol contents (Botella-Martínez et al., 2022; Chen et al., 2020). Replacement of animal fat with vegetable oil can improve the nutritive quality of meat products, regarding the presence of polyunsaturated fatty acids (PUFA) and phytochemicals with health-promoting effects such as tocopherols and phytosterols. Replacement of animal fat using wheat germ oil emulsion led to improved PUFA and α -tocopherol contents of the beef burgers (Barros et al., 2021). However, animal fat plays a crucial role in the stability and sensorial property of meat products; thus, the substitution of animal fat with PUFA rich oil might adversely affect these properties in the products. Substitution of animal fat using vegetable oils resulted in inferior quality of emulsified meat products in both

physical—i.e., texture, color, and cooking loss (Chen et al., 2020; Botella-Martínez et al., 2022; Carvalho et al., 2022)—and chemical—i.e., oxidative stability (Carvalho et al., 2020; Domínguez et al., 2019)—aspects.

Pre-emulsification, defined as a preparation of vegetable oil emulsion before incorporating with other ingredients, is a feasible way to maintain the quality of meat products with partial animal fat substitution (Liu, Ji, Zhang, Xue, & Xue, 2019; Prabsangob, 2021; Wang et al., 2018). The emulsifiers used in the pre-emulsification step could enhance meat matrix stability, thereby providing effective enhancement of the quality of meat products (Liu et al., 2019; Prabsangob, 2021; Wang et al., 2018). However, the pre-emulsified oil droplet possesses greater interfacial areas than its native state, making it sensitive to lipid oxidation, particularly those with abundant level of PUFA. Furthermore, meat products always consist of transitional metal ions and heme proteins that are effective pro-oxidants to accelerate oxidation (Tatibaborworntham, Oz, Richards, & Wu, 2022). Lipid oxidation is one of the important chemical deterioration pathways adversely affecting the quality, nutritional profile, and safety of meat products (Zhou et al., 2022). Synthetic antioxidants, such as butylated hydroxyanisole and *tert*-butylhydroquinone, are widely used by the meat industry to restrict lipid oxidation (Almeida et al., 2015). Nevertheless, consumer awareness of the toxicological effects of synthetic agents has driven a growing

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interest in using natural antioxidant compounds.

Use of cellulose in food preparation is promising, involving meat products, because of functional properties of cellulose such as water holding- and emulsifying abilities (Prabsangob, 2023; Zhou et al., 2022). Furthermore, cellulose may improve the nutritive value of meat products by increasing dietary fiber which is always deficient in such products (Pereira et al., 2021). However, the cellulose structure in its native state consists of H-bonds that may hinder its functional property (Prabsangob, 2022). Modification into micro and nano sizes improves the functional properties of cellulose, particularly the interfacial and emulsifying capabilities (Javed, Xu, & Sun, 2024; Ni, Gu, Li, & Fan, 2021; Prabsangob, 2022). Okara, the by-product from the production of soybean-based products, could be used to prepare cellulose crystals with an effective emulsifying property (Prabsangob, 2022). Okara cellulose crystals (OCC) efficiently stabilized eggless mayonnaise with a lowered fat content (Prabsangob & Udomrati, 2024). In addition, the OCC modified by surface grafting with tannic acid (OCCT) possessed the improved functional properties to maintain the colloidal and oxidative stabilities of a Pickering emulsion (Prabsangob, Hangsalad, & Udomrati, 2024). The OCC and OCCT could also retard in vitro lipolysis of the emulsions, making them a potential emulsifier for food preparation with a lower energy uptake (Prabsangob et al., 2024).

Utilization of OCC and OCCT in a real food model have not been reported yet, particularly for their effects on physical and chemical stabilities of food products. The present work aims to elucidate feasible utilization of OCC and OCCT as the dual function additive with emulsifying and antioxidant properties for a preparation of sausages with improved nutritive profile. First, the capability of OCC and OCCT to stabilize emulsion was determined. Then, the pre-emulsified emulsions were substituted for animal fat in sausage preparation, before determining physicochemical stability and in vitro lipolysis degree of the sausages. The output of this work may provide a practical way to prepare meat products with good physical and chemical stabilities and improved nutritive profile by lowering their SFA content and energy uptake.

2. Materials and methods

2.1. Materials and reagents

Okara, the by-product from soymilk production, was kindly provided by the Institute of Food Research and Product Development, Kasetsart University (Bangkok, Thailand). Tannic acid, thiobarbituric acid, and the digestive components, including porcine pancreas α -amylase (≥ 5 U/mg), porcine gastric mucosa pepsins (≥ 250 U/mg), porcine pancreas lipase (100–650 U/mg protein using olive oil as substrate), and porcine bile extract were products of Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Soy protein isolate (SPI) was a product of IMCD Co. (Bangkok, Thailand). Sodium tripolyphosphate was purchased from Aditya Birla Chemicals (Samutprakarn, Thailand). Fresh pork shoulder meat, pork backfat, soybean oil (SBO), and the condiments for sausage preparation were bought from a local market (Bangkok, Thailand).

The OCC was prepared according to the method of Prabsangob (2022). Briefly, the dried okara was washed with a mixture of hexane-to-ethanol (2:1, v/v), before mixing with aqueous NaOH solution (100 g/L) at an ambient temperature for 7 h. The treated okara was then washed several times with water and dried (60 °C, 12 h). After that, cellulose separation from the okara was performed using a mixture of acetic acid (80 %) and nitric acid (65 %) at 45 °C for 45 min, and subsequently hydrolyzed using sulfuric acid (64.5 %) at 45 °C for 45 min. Then, the separated cellulose was washed with excess water and dialyzed against deionized water until the pH of the suspension was close to neutral. After drying (60 °C, 12 h), the OCC was received (0.75 μ m diameter; -34.7 mV surface charge; and a crystallinity index of 55 %). The OCCT was prepared according to the method of Prabsangob et al. (2024) by reacting OCC with tannic acid at a mass ratio of 1:2.5 through a simple

impregnation method by heating in a vacuum at 45 °C. Two batches of OCC and OCCT were prepared separately to be used in the present work.

2.2. Emulsifying properties of OCC and OCCT

First, the ability of OCC and OCCT to stabilize a Pickering emulsion was determined. Initially, the OCC and OCCT were dissolved in a phosphate buffer (10 mM, pH 7.0) at varying concentrations (0.1–2 %, w/v), before homogenizing (T25; Ika Instrument Ltd.; Staufen, Germany) with SBO at 20,000 rpm for 3 min to prepare the emulsions with an oil fraction of 0.5. The dispersibility of the emulsions was determined by measurements.

- *Emulsion droplet size*: The oil droplet size was estimated as a volume-weighted diameter ($d_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3$) using a static light scattering particle size analyzer (Zetasizer model nano series; Malvern, UK), where n_i is the number of particles with diameter d_i . The data were also interpreted as a droplet size distribution pattern.
- *Heat stability*: The emulsions were heated (75 ± 2 °C for 30 min); then, the heat stability was quantified as a relative change in $d_{4,3}$ based on the droplet size of the emulsions before and after heating.
- *Creaming rate*: The emulsions were centrifuged (4000 rpm for 5 min); then, the creaming rate was quantified as the Eq. 1 (Cui, Hossain, Wang, & Chang, 2023):

$$\text{creaming rate (\%)} = \left(\frac{H_s}{H_T} \right) \times 100 \quad (1)$$

where H_T and H_s are the heights of the total emulsion before centrifugation and of the serum phase after centrifugation, respectively.

2.3. Sausages preparation

The sausages with 30 % fat content were prepared by incorporating different forms of fat involving pork backfat and pre-emulsified SBO stabilized with OCC and OCCT at the selected concentrations. For comparison, SPI at a corresponding level with the cellulosic materials was also used as an emulsifier to prepare pre-emulsified SBO. The pre-emulsified SBO was prepared according to the procedure in part 2.2. The sausages prepared using porcine fat, pre-emulsified SBO stabilized with SPI, pre-emulsified SBO stabilized with OCC, and pre-emulsified SBO stabilized with OCCT were named Ct, preE-SPI, preE-OCC, and preE-OCCT, respectively.

Fat and connective tissue were removed from the pork meat. The lean meat and backfat were ground and kept at -18 °C for less than 1 month before use. The protein, fat, and moisture contents of the lean meat, pork backfat, and SPI were determined according to the standard methods of AOAC (2000). The protein content of all sausage samples was controlled at 15 % based on the protein present in the lean meat, backfat, and SPI. The moisture content of the sausages was fixed at 70 %, and the amount of added water was subtracted from the moisture available in the lean meat, backfat, and pre-emulsified SBO. The sausage formulations are shown in Table 1.

Preparation of the sausages was performed according to the method described by Prabsangob (2021), with some modification. The frozen lean meat and backfat were thawed at 4 °C on the day before use. The additive and condiments were dissolved in the added water at 4 °C on the day of use. The lean meat was chopped (MK-77; National; Tokyo, Japan) at a low speed for 30 s. One-half of the backfat (or pre-emulsified SBO) and the additive solution were added, before further chopping for 1 min. Then, the rest of the backfat (or pre-emulsified SBO) was added and the mixture was further chopped for 1 min. After standing for 1.5 min, the mixture was finally chopped for 2 min. The temperature was controlled at lower than 12 °C throughout the entire process. The batter was stuffed in a cellulose casing (diameter 15 mm) and heated (75 ± 2 °C for 30 min), before cooling until the core temperature reached

Table 1

Formulation of sausage samples.

Ingredient (g/100 g)	Ct	preE-SPI ^e	preE-OCC	preE-OCCT
lean meat ^a	48.60	46.88	50.40	50.40
pork back fat ^b	29.07	–	–	–
pre-emulsified SBO ^{c,d}	–	48.79	48.79	48.79
water	22.33	4.33	0.81	0.81
others ^d	2.00	2.00	2.00	2.00

^a Protein, fat, and moisture contents of lean meat are 24.2, 1.6, and 74.2 %, respectively.

^b Protein, fat, and moisture contents of pork backfat are 2.1, 85.3, and 8.9 %, respectively.

^c Emulsifier concentration for pre-emulsified SBO preparation is 1.5 %.

^d All samples consist of 0.2 % Na-tripolyphosphate, 1.1 % NaCl, 0.5 % sucrose, and 0.2 % seasoning.

^e Protein and moisture contents of SPI are 80.2 and 6.7 %, respectively.

25 °C. In total, 15 sausages (ca. 300 g of meat batter) were produced in each batch, with two batches prepared separately for each formulation. The sausages were kept at 4 °C in a polyethylene bag packed without vacuum.

2.4. Characteristics and stability of sausages

The characteristics and stability of sausages were determined by measuring.

2.4.1. Physical characteristic

- **pH:** The sausages were mixed with deionized water at a ratio of 1:5 at room temperature for 5 min, before measuring the pH using the pH meter (Mettler-Toledo; Viroflay, France) which was previously calibrated using pH standard solutions 4.01 and 7.00.
- **Cooking loss:** The sausages were heated (80 ± 2 °C for 15 min), and the cooking loss was determined based on the change in the sample weight before and after heating (Hur, Jin, & Kin, 2008).
- **Water holding capacity (WHC):** The sausages were centrifuged (4000 rpm for 10 min at 4 °C). The WHC was estimated as the change in the sample weight before and after centrifugation (Hur et al., 2008).
- **Color:** Each sausage was cut freshly and allowed to bloom for 30 min, before observing the color of the cross-section using a HunterLab colorimeter (CR-200b; Minolta; Tokyo, Japan) with a standard illuminant D 65, CIE 10 standard observer, and 11 mm aperture. The parameters of L*, a* and b* values were then determined.
- **Texture profile analysis:** The sausages were cut into a cylindrical shape with the diameter and length of ca. 10 and 250 mm, respectively. The measurements were made using a texture analyzer (TA-XT2; Stable Micro System; Godalming, UK) equipped with a 5 kg load cell and a cylindrical probe (diameter 5 cm). Each sample was compressed to one-half of its original height at a cross speed of 5 mm/s for two cycles at room temperature. The texture attributes (hardness, cohesiveness, springiness, and chewiness) were quantified.
- **Microstructure:** The sausages were cut into cubes ($2.5 \times 2.5 \times 2.5$ mm) and fixed using glutaraldehyde (2 %) for 24 h at 4 °C. The samples were then dehydrated according to the method of Jiang and Xiong (2013) using a series of ethanol solutions with concentrations of 50, 70, 80, and 90 % (once each for 10 min and twice in absolute ethanol for 10 min). After that, each sample was sputter-coated with gold and subjected to scanning electron microscopy (FEI Quanta 450; Hillsboro, OR, USA) at an 15 kV accelerating voltage.

2.4.2. Chemical stability

The chemical stability of the sausages was determined by measuring the degree of lipid oxidation during storage. The sausages that had been kept in a polyethylene bag at 4 °C for 14 days were periodically removed and the peroxide value (PV) and thiobarbituric acid reactive substance

(TBARS) content were determined.

- **PV:** The PV was quantified based on the method described by Shanta and Decker (1994). Briefly, the sausages were ground, added with a mixture of chloroform-to-methanol (1:1) and then passed through a filter paper. Next, the filtrate was reacted with the solutions of ammonium thiocyanate (4.38 M) and Fe₂Cl (18 mM). After incubation at room temperature for 20 min, the absorbance of the mixture was measured at 500 nm and the PV was quantified based on the standard curve of cumene hydroperoxide and reported as milligrams of hydroperoxide equivalent per kilogram of sausage.
- **TBARS content:** The TBARS content was quantified according to the method of Zhang, Xiao, Lee, and Ahn (2011). Briefly, the sausages were mixed with trichloroacetic acid solution (0.75 %) with a presence of 0.1 % ethylenediaminetetraacetic acid to extract the TBARS. Then, the extract was reacted with thiobarbituric acid solution (2 mM) and heated (95 °C, 30 min), before measuring the absorbance at 532 nm. The TBARS content was estimated using a standard curve of malondialdehyde (MDA) and reported as milligrams of MDA equivalent per kilogram of sausage.

2.5. In vitro digestion of sausages

The sausages were subjected to in vitro digestion according to the method described by Le, Loveday, Singh, and Sarkar (2020), with some modifications. The digestive juices consisting of simulated saliva fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were prepared. The SSF (pH 7.0) consisted of KCl (15.1 mM), KH₂PO₄ (3.7 mM), NaHCO₃ (13.6 mM), MgCl₂ (0.15 mM), and (NH₄)₂CO₃ (0.06 mM). The SGF (pH 2.5) comprised KCl (6.9 mM), KH₂PO₄ (0.9 mM), NaHCO₃ (25 mM), NaCl (47.2 mM), MgCl₂(H₂O)₆ (0.1 mM), and (NH₄)₂CO₃ (0.5 mM). The SIF (pH 7.0) contained KCl (6.8 mM), KH₂PO₄ (0.8 mM), NaHCO₃ (85 mM), NaCl (38.4 mM), and MgCl₂(H₂O)₆ (0.33 mM).

The sausage (5 g) was ground and mixed with the SSF (5 mL) in the presence of freshly added α -amylase (750 units/mL) and CaCl₂ (0.1 M, 75 μ L). After making up the total volume to 10 mL using deionized water, the mixture was introduced to a shaking water bath at 37 °C for 2 min. Then, a bolus derived after the simulated oral phase was mixed with the SGF (7.5 mL) and freshly added pepsin (25,000 units/mL) and CaCl₂ (0.1 M, 15 μ L), before adjusting the total volume to 20 mL using deionized water and the pH to 3.0 using a small aliquot of HCl (1 M). The simulated gastric digestion was performed at 37 °C for 120 min to provide a chyme. Next, the chyme was added with the SIF (6 mL) in the presence of pancreatic lipase (5600 units/mL), bile salts (160 mM, 2.5 mL), and CaCl₂ (0.1 M, 120 μ L). The mixture was made up to a total volume of 40 mL with deionized water and to a pH of 7.0 using a small aliquot of NaCl (1 M), before heating at 37 °C for 120 min. Then, the reaction was terminated by placing the mixture in an ice bath. Free fatty acid (FFA) content was then measured based on a titration with NaOH standard solution (0.02 M) and quantified using Eq. 2 (Liu & Kong, 2023):

$$\text{FFA } (\mu\text{mol}) = V_{\text{NaOH}} \times C_{\text{NaOH}} \quad (2)$$

where V_{NaOH} and C_{NaOH} are the end point volume and concentration of the standard NaOH solution, respectively.

2.6. Statistical analysis

The present study was designed based on the Completely randomized design (CRD). For the observation on the colloidal characteristics of the emulsions, the trial was based on 2×2 factorial design in CRD, where type (OCC and OCCT) and concentration (0.1–2 %) of the cellulosic materials were the studied factors. Two batches of the emulsions were prepared separately, and each batch was taken to determine the

characteristics (i.e., droplet size, creaming rate, and heating stability) in triplicate. The data sets from two batches were then reported as means \pm standard error, which were analyzed for difference between the means by Tukey's test at a confidence level of 95 %. The data were analyzed by one-way analysis of variance, and difference between the means were evaluated at a confidence level of 95 %.

For the study on sausage preparation, oil incorporation form was the studied factor for the cooking loss, WHC, and in vitro digestion. For the study on degree of lipid oxidation, the factorial design (2×2) in CRD was used as the experimental model, in which oil incorporation form and storage times were fixed factors. Two batches of the sausages were prepared separately, and each batch was taken to determine the characteristics in triplicate, except for the texture profile analysis in which at least 10 pieces of the fresh cut samples were employed. The data sets from two batches were then reported as means \pm standard error, which were analyzed for difference between the means by Tukey's test at a confidence level of 95 %.

3. Results and discussion

3.1. Emulsifying properties of OCC and OCCT

First, the ability of OCC and OCCT to stabilize SBO emulsion was evaluated at varying concentrations (0.1–2 %) and the dispersibility of the emulsions is shown in Fig. 1. There was no significant difference in the colloidal stability of the emulsions stabilized using OCC or OCCT at corresponding concentrations. Cellulose based materials could be adsorbed irreversibly to oil-water interfaces (Ikem, Menner, Bismarck, & Norman, 2014), resulting in a formation of interfacial film as a barrier to protect oil-drop aggregation via both electrostatic and steric mechanisms (Javed et al., 2024; Prabsangob, 2022; Prabsangob & Udomrati, 2024). Increasing the concentrations of OCC and OCCT led to improved colloidal stability of the emulsion as implied by the lowered initial droplet sizes (Fig. 1A) and creaming rates (Fig. 1D) of the emulsions. Enhancement on the emulsion stability as suggested by the narrower oil droplet size distribution patterns could be also found for the emulsions stabilized by OCC (Fig. 1B) and OCCT (Fig. 1C) at increased concentrations. Furthermore, raising the OCC and OCCT concentrations promoted heat stability of the emulsions as implied by less relative change

of the droplet size of the emulsions after heating (Fig. 1E). Other studies have also reported a positive relationship between colloidal stability and the concentration of the cellulosic materials used as emulsifier (Javed et al., 2024; Prabsangob et al., 2024). At increased concentration, OCC and OCCT might completely cover the oil drop surfaces where the interaction between the adsorbed cellulosic materials could occur via H-bonding, resulting in the formation of a strong interfacial film (Javed et al., 2024; Ni et al., 2021). The interfacial films of the adsorbed emulsifier play a crucial role as a protective barrier against oil drop aggregation; thus, lowered creaming rate and improved heat stability might be expected for the emulsions stabilized using OCC and OCCT at increased concentrations (Javed et al., 2024; Ni et al., 2021). At higher concentrations, the non-adsorbed cellulosic materials might be available in the aqueous phase, where they could interact and enhance the viscosity of the continuous phase, thereby lowering aggregation and improving heat stability of the emulsions (Ni et al., 2021).

From all these points of view, using OCC and OCCT at 1.5 % provided the emulsions with the lowest initial $d_{4,3}$ and greatest heat stability. Therefore, this concentration level was chosen for further study.

3.2. Characteristics of sausages as affected by pre-emulsified SBO inclusion

The sausages were prepared by incorporating fat in different forms (pork back fat and pre-emulsified SBO stabilized by OCC or OCCT at the selected concentration). For comparison, SPI, the emulsifier popularly used in meat products, was also as an emulsifier to prepare pre-emulsified SBO at a corresponding concentration with the cellulosic materials. Fig. 2 reveals the cooking loss and WHC of the prepared sausages. Cooking loss indicates the water and fat binding capacities of meat products during heating process and crucially relates to juiciness of the products (Chen et al., 2020). Presently, the highest cooking loss was found for the preE-SPI, whereas the others showed comparable levels of cooking loss. Due to the highly unsaturated nature of the composited fatty acids, SBO is a liquid with low viscosity at room temperature; consequently, lesser fluid binding capacity could be expected for the SBO-added cooked meat products than the counterparts incorporated with animal fat (Zhou et al., 2022). Interestingly, the lowered cooking loss of preE-OCC and preE-OCCT compared to preE-SPI suggested the

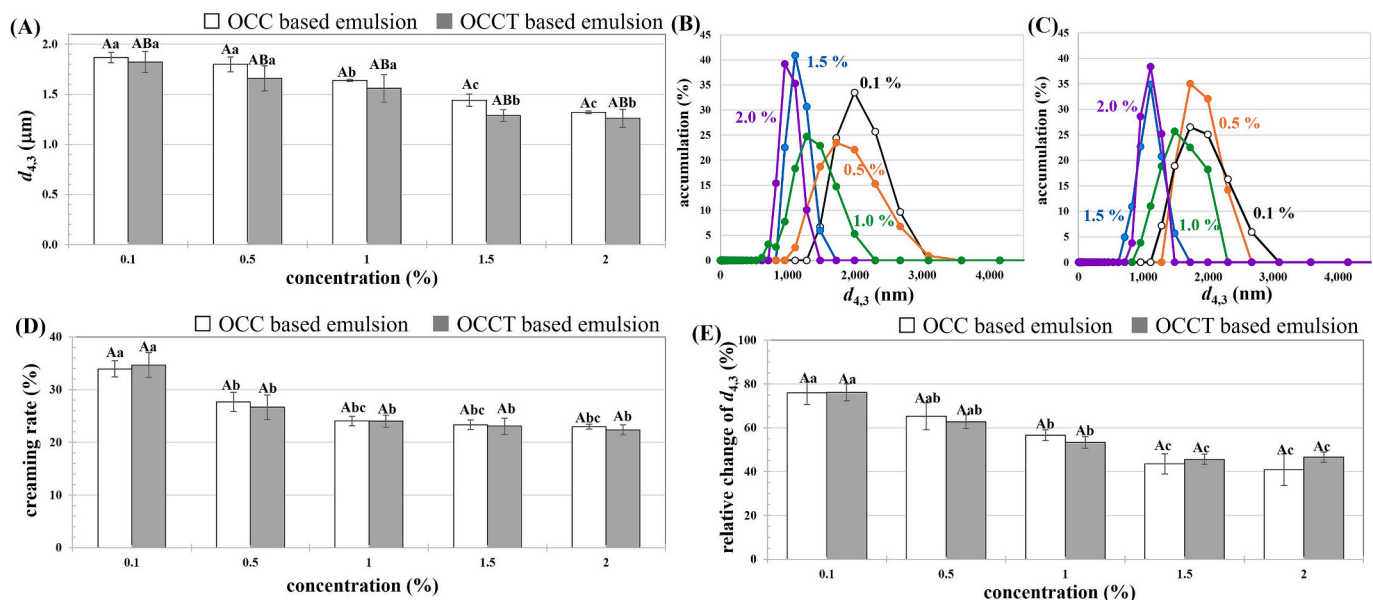


Fig. 1. Characteristics of the emulsions stabilized by OCC and OCCT at different concentrations: (A) initial $d_{4,3}$, (B) oil droplet size distribution patterns of OCC-based emulsions, (C) oil droplet size distribution patterns of OCCT-based emulsions, (D) creaming rate, and (E) relative change of $d_{4,3}$ after heating.

In subfigures A, D, and E, means \pm standard errors were shown. Different capital letters indicate significant ($P \leq 0.05$) differences between means as affected by emulsifier type, and different lowercase letters indicate significant ($P \leq 0.05$) differences between means as affected by emulsifier concentration.

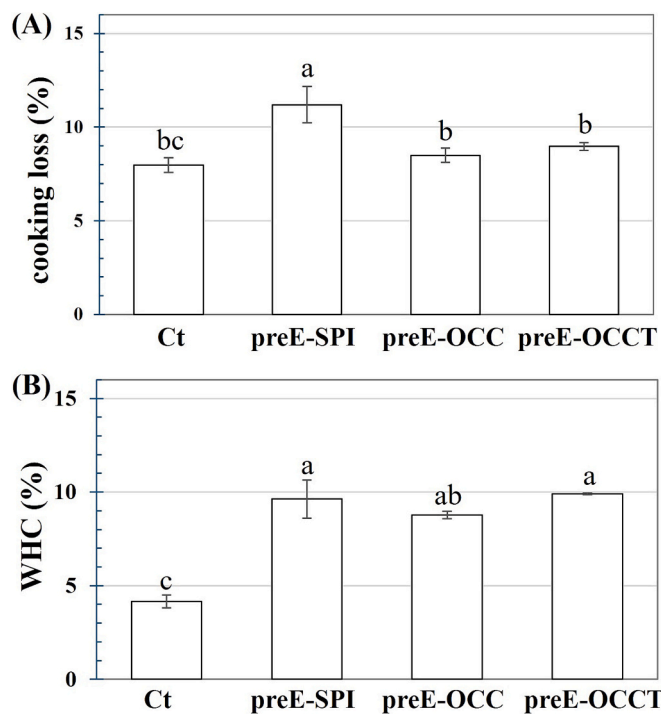


Fig. 2. (A) Cooking loss and (B) WHC of the sausages incorporated with porcine fat and pre-emulsified SBO.

In each subfigure, means \pm standard errors were shown. Different capital letters indicate significant ($P \leq 0.05$) differences between means.

Table 2

pH and color parameters of sausages incorporated with pork backfat and pre-emulsified SBO.

Sample	pH ^{ns}	L*	a*	b*
Ct	6.08 \pm 0.02	67.8 \pm 0.2 ^B	0.12 \pm 0.01 ^B	8.4 \pm 0.1 ^B
preE-SPI	6.11 \pm 0.03	68.7 \pm 0.2 ^A	0.10 \pm 0.02 ^B	9.8 \pm 0.1 ^A
preE-OCC	6.10 \pm 0.02	69.0 \pm 0.1 ^A	0.16 \pm 0.01 ^A	9.9 \pm 0.1 ^A
preE-OCCT	6.08 \pm 0.03	69.2 \pm 0.1 ^A	0.17 \pm 0.02 ^A	9.7 \pm 0.1 ^A

^{ns} Not significantly ($P > 0.05$) different.

improved stability of the sausages by adding pre-emulsified SBO stabilized by the OCC and OCCT. This might be due to the formation of a network structure of the cellulosic materials by crosslinking with meat proteins, thereby restricting migration of fat and water from the matrix. Effective ability of cellulose nanofibrils to retain fluid loss in emulsified meat products has been reported (Wang et al., 2018; Zhuang et al., 2016).

WHC is associated with a well-formed three-dimensional network of the emulsified meat matrix (Liu et al., 2019). The Ct had lower WHC compared to the sausages added with pre-emulsified SBO. The cellulosic materials and SPI contained the hydrophilic parts of hydroxyl groups and hydrophilic amino acids, respectively. Therefore, greater water binding ability of the cellulosic materials and SPI could be supposed, resulting in greater WHC of the sausages added with the pre-emulsified SBO as compared to the Ct added with porcine fat. In addition, the pre-

emulsified oil droplets tended to be smaller in size than the animal fat globules, which would support a superior filling effect for the pre-emulsified SBO, resulting in greater matrix stability of the sausages added with the pre-emulsified SBO (Chen et al., 2020; Liu & Kong, 2019). Similar behavior has been observed in several myofibrillar protein gel matrices, such as sausages added with pre-emulsified palm oil stabilized by cellulose nanofibrils (Wang et al., 2018), meat batters incorporated with cellulose nanomaterial-based SBO emulsion (Qi et al., 2020), and surimi gels mixed with pre-emulsified peanut oil stabilized by glucomannan (Liu et al., 2019).

Next, physical properties of the sausages were determined (pH and color), as shown in Table 2. Substitution of porcine fat with pre-emulsified SBO had no significant effect on pH of the sausages. This behavior was also observed in the sausages incorporated with pre-emulsified palm oil stabilized using cellulose nanofibrils (Wang et al., 2018) and beef burgers added with chia and hemp oil gelled emulsions (Botella-Martínez et al., 2022). However, different forms of the incorporated oils affected the sausage color. The sausages added with pre-emulsified SBO had higher L* and b* values than the Ct added with animal fat. A similar trend was found in other studies reporting on the effects of animal fat substitution with pre-emulsified vegetable oils on the color of emulsified meat products (Qi et al., 2020; Wang et al., 2018; Zhuang et al., 2016). Increasing lightness might be explained by the greater light reflection of the emulsified droplets with greater surface areas than for the animal fat globules (Qi et al., 2020; Wang et al., 2018; Zhuang et al., 2016) and for the emulsified meat matrix with higher WHC (Liu & Kong, 2019). The yellowness of vegetable oils might lead to increased b* values of the sausages added with pre-emulsified SBO. Additionally, the preE-OCC and preE-OCCT had higher a* than the Ct and preE-SPI. This trend coincided with a greater amount of lean meat used to prepare the preE-OCC and preE-OCCT. Positive correlation between the amount of lean meat and the a* value of the sausages might have been due to the natural presence of red pigment (myoglobin) in the pork flesh.

Values (mean \pm standard error) with different capital superscripts in columns are significantly ($P \leq 0.05$) different.

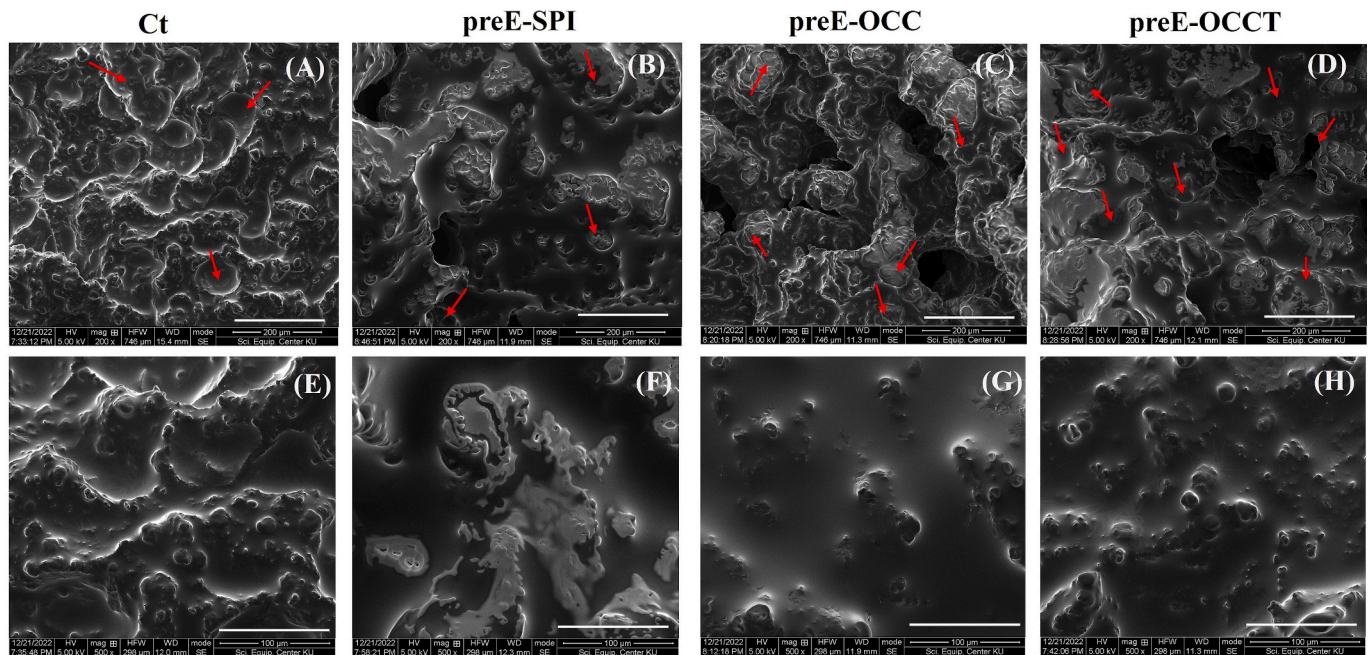
The results of texture profiling of the sausages are presented in Table 3. Inclusion of fats in different forms affected textural characteristics of the sausages. Generally, decreased hardness and increased springiness were observed for the sausages in which porcine fat had been substituted using pre-emulsified SBO. The lowered hardness due to adding pre-emulsified SBO might be expected, since a liquid state of the oil has lower consistency than a solid state of the porcine fat. Diminished hardness in restructured ham was observed when animal fat was added in a pre-emulsified form compared to the native state, with this effect presumed to be due to the smaller-sized, pre-emulsified fat particles with lowered hardness than the larger-sized ones (Shim et al., 2018). The higher springiness of the sausages added with pre-emulsified SBO compared to the Ct corresponded with another study using palm oil emulsion to replace porcine fat in the sausage formulation (Wang et al., 2018). However, the present result suggested greater retained textural attributes of the preE-OCC and preE-OCCT than the preE-SPI as indicated by the lowest hardness and chewiness of the preE-SPI compared to the others. This trend implied better ability of the OCC and OCCT than SPI to reinforce the meat matrix structure incorporated with pre-emulsified SBO, thereby resulting in the sausages with better

Table 3

Textural attributes of sausages incorporated with pork backfat and pre-emulsified SBO.

sample	hardness (N)	cohesiveness (ratio) ^{ns}	springiness (%)	chewiness
Ct	13.23 \pm 0.21 ^A	0.46 \pm 0.02	19.96 \pm 0.85 ^B	5.27 \pm 0.33 ^A
preE-SPI	9.33 \pm 0.19 ^D	0.44 \pm 0.03	22.63 \pm 0.32 ^A	4.17 \pm 0.34 ^{BCE}
preE-OCC	11.93 \pm 0.24 ^B	0.44 \pm 0.02	22.70 \pm 0.27 ^A	4.98 \pm 0.32 ^A
preE-OCCT	11.48 \pm 0.14 ^C	0.43 \pm 0.04	22.37 \pm 1.07 ^A	4.73 \pm 0.34 ^{AB}

^{ns} Not significantly ($P > 0.05$) different. Values (mean \pm standard error) with different capital superscripts in columns are significantly ($P \leq 0.05$) different.



White scale bars in subfigures (A)–(D) and (E)–(H) represent 200 and 100 μm, respectively
Grey: protein matrix; Dark grey: fat droplets, as marked by red arrows

Fig. 3. Microstructure of the sausages incorporated with porcine fat and pre-emulsified SBO observed at 200× (A–D) and 500× (E–H) magnifying.

maintained textural characteristics (Wang et al., 2018).

The microstructure of the studied sausages was further explored as shown in Fig. 3. Incorporation of pre-emulsified SBO led to a smoother texture, with improved continuous microstructure in the sausages as compared to the counterparts added with porcine fat. This result suggested a filling effect of the pre-emulsified SBO into the emulsified meat matrix, resulting in a denser structural network of the sausages. This trend was also found for the meat products incorporated with pre-emulsified palm oil (Wang et al., 2018) and sesame oil (Zhuang et al., 2016). Entanglement between the emulsifier used during the pre-emulsification step with the muscle protein might occur via several interactions involving disulfide-, hydrogen-, hydrophobic-, and electrostatic-interactions, with the subsequent formation of a gel-like network that promoted the continuity of the emulsified meat matrix (Zhuang et al., 2016).

Among the sausages added with pre-emulsified SBO, there was a smoother microstructure in the preE-OCC and preE-OCCT than in the preE-SPI. This result was coincidental with the lowered cooking loss (Fig. 2A) and better retained textural attributes (Table 3) of the preE-OCC and preE-OCCT. These behaviors implied greater ability of the cellulosic materials to reinforce the emulsified meat matrix than could the SPI, which might be due to the preferable interaction of the cellulosic materials with myofibrillar proteins via several bonding such as the van der Waals force and H-bonds (Ahmad, Khalid, & Younis, 2020). The cellulose-based substances could form a gel network, resulting in strengthening the emulsified meat matrix (Qi et al., 2020). Furthermore, the cellulosic materials could enhance unfolding of the proteins, thereby promoting hydrophobic interaction between the myofibrillar proteins themselves (Zhuang, Jiang, Zhou, et al., 2020). Unfolding of myosin proteins and then their cross-linking importantly strengthened meat matrix stability (Liu & Kong, 2019). Denser and more compact microstructure could be observed for the surimi gel added with pre-emulsified peanut oil stabilized by konjac glucomannan as compared to the ones stabilized by SPI (Liu & Kong, 2019). This result could be explained by

greater extent of the interaction between the polysaccharide and myofibrillar proteins, resulting in a three-dimensional gel-like network formation to reinforce meat matrix stability. On the other hand, for the SPI-based emulsion, there was lowered gelling and structuring capability observed, resulting in reduced matrix stability, as indicated by the lowered hardness and greater fluid loss of the surimi gel (Liu & Kong, 2019). Strengthen effect of cellulose nanofibers on the microstructure of heated protein matrix has been reported elsewhere, resulting in improved stability and textural characteristic of the emulsified meat products (Wang et al., 2018).

Next, the effect of oil inclusion in different forms on the chemical stability of the sausages was evaluated by monitoring the change of PV and TBARS during cold storage, as shown in Fig. 4. Progressive lipid oxidation of the sausages was observed as indicated by the increases in PV and TBARS along the storage period: The degree of oxidation in descending order was preE-SPI > Ct ≈ preE-OCC > preE-OCCT. Emulsified meat products are prone to lipid oxidation due to the presence of large contact areas between oxygen molecules and the comminuted lipid phase (Almeida et al., 2015; Zhao et al., 2018). Owing to unsaturation of the composited fatty acids of vegetable oils, replacing animal fats with such vegetable oils affected to accelerate lipid oxidation of the comminuted meat products (Carvalho et al., 2020). Therefore, higher lipid oxidation could be expected for preE-SPI than for Ct.

However, inclusion of pre-emulsified SBO stabilized by the cellulosic materials could improve oxidative stability of the sausages as suggested by the comparable (preE-OCC) and lower (preE-OCCT) lipid oxidation indices compared to the Ct. The dissimilar oxidative stability trend of the preE-SPI compared to the preE-OCC and preE-OCCT might have been due to different structures of the pre-emulsified SBO incorporated in the sausages. The cellulosic materials could be adsorbed irreversibly at the oil-drop surfaces by forming interfacial films as a barrier to protect the oil drops from prooxidants (Bai et al., 2019). Furthermore, the cellulosic materials might enhance viscosity of the meat batter, resulting in improved oxidative stability of the emulsified meat matrix by restricting mobility of reactive compounds (Asyurul-Izhar, Bakar, Sazili, Goh, & Isamil-Firty, 2023). Improvement on viscosity and oxidative stability of

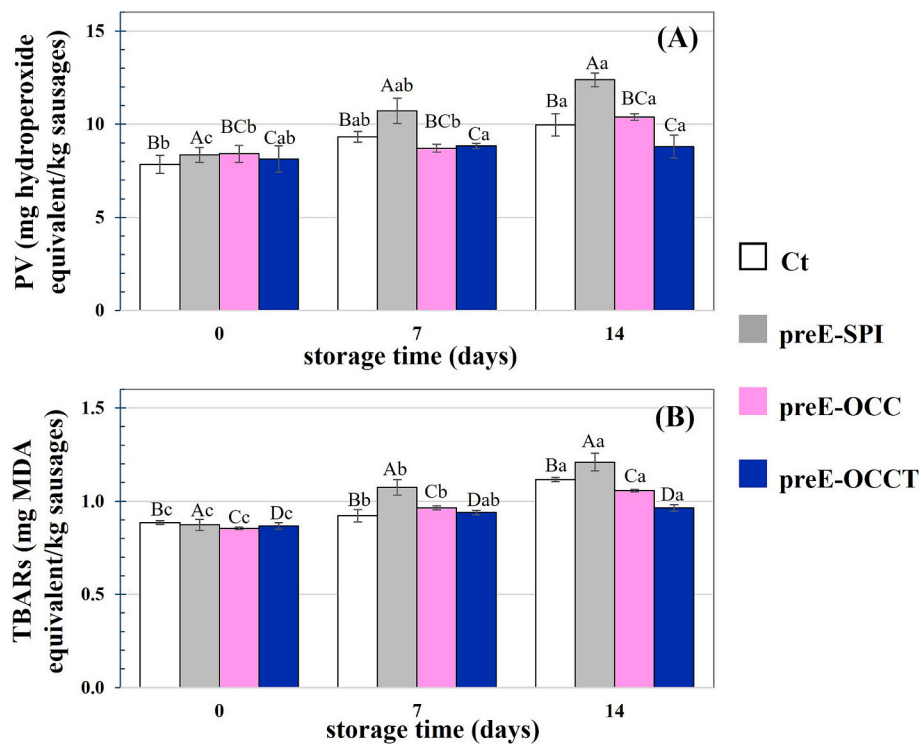


Fig. 4. Storage time dependence on (A) PV and (B) TBARs of the sausages incorporated with porcine fat and pre-emulsified SBO.

In each subfigure, means \pm standard errors were shown. Different capital letters indicate significant ($P \leq 0.05$) differences between means as affected by oil inclusion form and different small letters indicate significant ($P \leq 0.05$) differences between means as affected by storage time.

palm oil emulsion could be observed by employing xanthan gum as the emulsifier, resulting in enhanced oxidative stability of the meat patties incorporated with the xanthan gum-based emulsion (Keum et al., 2024). In this work, the highest oxidative stability could be found for the preE-OCCT, which might be explained by the antioxidant activity of the grafted tannic acid residues of OCCT (Prabsangob et al., 2024). Improvement on oxidative stability of the emulsion could be also achieved by using the cellulose nanocrystals modified by grafting with gallic acid (Javed et al., 2024). Moreover, the OCC-gallic acid complex could enhance oxidative stability of the mayonnaise as compared to the counterparts stabilized using unmodified OCC (Prabsangob & Udomrati, 2024). Development on oxidative stability of the sausages could be accomplished when the samples were incorporated with whey protein-tannic acid conjugate than did the native protein (Aewsiri, Ganesan, & Thongzai, 2023). Due to the improved oxidative stability, the sausages added with the whey protein modified using tannic acid had a greater protein network stability with lowered cooking loss than the counterparts added with unmodified whey protein (Aewsiri et al., 2023). Phenolic compounds possessed electron acceptability and donatability to stabilize free radicals, thereby retarding lipid oxidation effectively (Almeida et al., 2015). Due to a rich presence of galloyl groups, tannic acid could chelate ferrous ions effectively (Andrade Jr. et al., 2005). Therefore, potent ability of tannic acid to retard the prooxidant effect of ferrous ions available in meat products could be expected (Andrade et al., 2005).

3.3. Lipolysis of sausages as affected by pre-emulsified SBO inclusion

Degree of lipolysis of the sausages after passing through the simulated gastrointestinal tract was evaluated by measuring the released FFA content, with the results depicted in Fig. 5. Inclusion of lipids in pre-emulsified form could diminish FFA content, suggesting a lowered degree of lipolysis than the Ct added with porcine fat. Lipolysis extension in consumed foods might differ depending on both intrinsic (physical

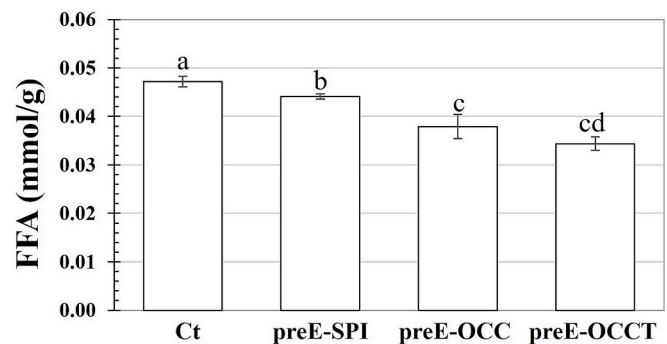


Fig. 5. In vitro lipolysis of the sausages incorporated with porcine fat and pre-emulsified SBO.

Means \pm standard errors were shown. Different letters indicate significant ($P \leq 0.05$) differences between means.

state of lipids, the profile, and the *sn*-position of the composited fatty acids) and extrinsic (structural and rheological) factors of food matrix (Guo, Ye, Bellissimo, Singh, & Rousseau, 2017). It has been suggested that lipolysis degree of the edible oils depended on the composited fatty acids in the descending order of palm oil rich in SFA of palmitic acid (C16:0) > rapeseed oil rich in oleic acid (C18:1) > linseed oil rich in linolenic acid (C18:3), respectively (Ye et al., 2019). The greater lipolysis rate of palm oil than the others might be expected due to its higher hydrophobicity of the composited fatty acids, thereby preferably interacting with bile salts (Ye et al., 2019). This effect led to greater displacement of the adsorbed emulsifiers from the surfaces of palm oil droplets, thereby allowing lipase to attack the oil phase easily (Ye et al., 2019). This trend was consistent with the present result, where the higher released FFA content was recorded for the Ct consisting of porcine fat with higher SFA than the counterparts added with pre-emulsified SBO rich in PUFA of linoleic acid (C18:2).

The preE-SPI had greater lipolysis than the preE-OCC and preE-OCCT. Fabrication of the interfacial layers could be a practical means to control lipolysis of the emulsion, owing to a crucial role of the adsorbed emulsifier films to hinder contact of lipases and bile salts to the oil phase (Ni et al., 2021). Furthermore, dissimilar characteristics of the interfacial films might respond differently to environmental factors, such as the different values of pH and ionic strength in the gastrointestinal tract, resulting in unlike physicochemical properties of the emulsions, and thereby their digestibility (Prabsangob, 2023). It has been suggested that the interfacial films of emulsifiers with negative charges could form a three-dimensional gel network in the gastric environment (Ni et al., 2021). With the formed gel network, the contact areas between lipases and bile salts to the emulsified oil phase was lowered, thereby diminishing lipolysis in the emulsion (Ni et al., 2021). Notably, the OCC had negative surface charges (Prabsangob et al., 2024), whereas SPI with an isoelectric point typically around pH 4.5 showed positive charges in the gastric environment. Furthermore, the cellulosic materials could effectively form a steric interfacial film to protect oil droplets from digestion by lipases and diminish the displacement effect of bile salts, thereby restricting lipolysis of the emulsion (Guo et al., 2017; Bai et al., 2019; Loveday, Singh, & Sarkar, 2020). Good tolerance of the emulsions against in vitro lipolysis has been reported by employing some cellulose substances as the emulsifier such as cellulose nanofibrils (Ni et al., 2021) and cellulose crystals (Prabsangob et al., 2024). In contrast, with the protein-based emulsion, the harsh acidic condition of the simulated gastric phase might induce denaturation of the protein, leading to a collapse of the emulsion (Sjöö, Emek, Hall, Rayner, & Wahlgren, 2015). Therefore, lipases and bile salts could attack the oil droplets directly, resulting in greater lipolysis degree of the protein-based emulsions (Sjöö et al., 2015). Gastric digestion in the presence of pepsin and acidic pH greatly affected the coagulation of soy proteins, resulting in a destabilization of the soymilk emulsion (Wang, Ye, Dave, & Singh, 2021). In addition, it has been suggested that the interfacial films of proteins tended to have high porosity, so the attack of bile salts on the oil drop surfaces occurred preferably, thereby allowing lipases to digest the oil phase effectively (Ye et al., 2019).

4. Conclusion

Cellulosic materials (OCC and OCCT) could stabilize Pickering SBO emulsion with good colloidal and heating stability. Moreover, the pre-emulsified SBO stabilized using OCC and OCCT could be a practical vehicle for vegetable oil incorporation into the sausages. Compared to the sausages added with porcine fat, improved meat matrix stability could be achieved by incorporating the OCC- and OCCT stabilized SBO emulsion. The sausages added with the pre-emulsified SBO stabilized by the cellulosic materials, particularly with OCCT, showed the improved oxidative stability during storage. Interestingly, the sausages incorporated with OCC- and OCCT stabilized SBO emulsions possessed lower lipolysis degree than the control sausages added with porcine fat. The present work indicated that fabrication of the interfacial layers of the emulsion using OCC and OCCT as emulsifiers could successfully improve stability and functional property of the emulsions. Incorporation of the OCC- and OCCT-based emulsions could be a promising means to produce the sausages with improved physicochemical stability and nutritive profile by elevating PUFA content and lowering lipolysis extension of the product. Nevertheless, structural change and kinetic lipolysis of the cellulosic materials stabilized emulsions in the simulated gastrointestinal tract should be examined to elucidate further utilization of the OCC and OCCT in other food products with lowering energy uptake effect.

Studies in humans and animals

There is no human and live animal studies in this paper.

CRediT authorship contribution statement

Nopparat Prabsangob: Writing – original draft, Visualization, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Sasitorn Hangsalad:** Formal analysis. **Sunsaanee Udomrati:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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