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OPTIMIZATION OF PRE-NEUTRALIZED RED PALM OLEIN-CANOLA OIL EMULSION GEL AS ANIMAL FAT REPLACER FOR COMMINUTED MEAT PRODUCT USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Red palm olein, which is rich in β carotene, has the potential as an animal fat replacer for the formulation of healthy processed meat products. However, it is mainly composed of saturated fatty acid, which should be reduced by combining it with unsaturated fatty acid-rich vegetable oil. Canola oil was mixed with red palm olein in order to get the benefits from both. The formulation of an emulsion gel made up of red palm olein and canola oil at 30/70 (w/w) was optimized using a response surface methodology. Beta carotene, cholesterol content, and fatty acid profile of the oil mixture at 30/70 (w/w) resulted in the preferable values. Soy protein isolate (SPI), mono and diglycerides of fatty acids (emulsifier E471, EMS), and inulin (INL) were selected as stabilizer and emulsifier in aqueous phase. SPI was the only one ingredient that was associated with higher cooking yield of the meat emulsion. SPI, EMS, and INL had quadratic effect and positively affected the hardness of meat emulsion model system. Based on formula optimization and validation of gel emulsion ability in the comminuted meat model system, it was concluded that the addition levels of SPI, EMS, and INL required to form gel emulsions with optimal ability to produce comminuted meat products were 5.33%, 0.53% and 3.98% (w/w), respectively.

Key words: β carotene; central composite design; cooking yield; gel firmness; red palm olein.

INTRODUCTION

Animal fats contain a number of medium chain saturated fatty acids (SFAs) and cholesterols which if consumed in excess of daily requirement may increase the occurrence of metabolic disorders. Poor diet and high intake of saturated fat have been associated with the rise of low-density lipoprotein, atherosclerosis, albuminuria and liver fibrosis (Sanders *et al*., 2009; Abbate *et al*., 2021; Jia *et al*., 2021). In contrast, high intake of SFAs is inversely associated with hypertension and oxidative stress in elderly individuals (Nakamura *et al*., 2019). Kang *et al*. (2020) also reported that higher intake of dietary saturated fat is associated with a decreased overall risk of stroke. Despite the direct association between SFA intake and several metabolic disorders in many studies remain controversial, the substitution of SFA by monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in diet promote health benefits for human. Oleic acid intake decreases the risk of type 2 diabetes and enhances protection on the inflammatory response and liver damage (Palomer *et al*., 2018). Substitution of dietary SFAs by n-6 PUFAs decreases the production level of LDL (Drouin-Chartier *et al*., 2018). Although animal fat is rich in SFAs, it is also a source of linoleic acid, a medium chain n6 fatty acid, but it is lack of n3 fatty acids. Daily intake of n-6 PUFAs should be balanced with the intake of n-3 PUFAs as close as possible to unity to prevent chronic inflammatory diseases (Patterson *et al*. 2012; Hammad *et al*. 2016). Recent trend in healthy lifestyle including diet becomes a subject of interest in both academia and food industry. Development of healthy food is skyrocketing, while processed meat products are still considered as unhealthy. Therefore, reformulation of processed meat products could be an alternative strategy to provide healthier processed food.

Processed meat products are linked to fat and preservatives in their ingredients. Replacement of animal fat in meat products using vegetable oils has gained popularity considering its nutritional point of view. Vegetable oils provide dietary MUFA, PUFA with less SFA. Replacement of animal fat containing SFA with PUFA-rich vegetable in comminuted meat products is a technological challenge as PUFAs do not provide plasticity to the meat batter and thus ruins the stability of emulsion and texture attributes of the products (Baek *et al*., 2016). A prospective substitute for solid animal fats is vegetable oil extracted from crude palm oil. Cold-pressed red palm olein is rich in phytonutrients such as antioxidant β-carotene (Tan *et al*., 2021). Red palm olein supplementation in Wistar rats induced by CCl_4 was effective in reducing CCl_4 toxicity by increasing levels of endogenous antioxidant enzymes and reducing free radicals (Emmanuel *et al*., 2021). To optimize the benefits of these oils while retaining the same technological properties as using animal fats in meat products, oil in water emulsion gel is formulated prior to application in comminuted meat product processing. Pre-neutralized oil or emulsion gel has also been previously applied to emulsion-type meat products (Serdaroğlu *et al*., 2017; Pintado *et al*., 2018; Chaijan *et al*., 2021). In this case, the ingredients and their addition level must be optimized to get the

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final comminuted meat product with the expected quality attributes.

Response surface methodology (RSM) is a statistical approach widely used for optimizing various variables. In this regard, a central composite design (CCD) is used for optimization of the selected variables. The use of the designs can effectively minimize the error in measuring the linear, quadratic and interaction effects of the variables (Yolmeh and Jafari, 2017). Formula optimization for improving the quality and nutritional aspects of sausages using CCD was done in previous studies (de Souza Paglarini *et al*., 2019; Souza *et al*., 2019; Utama *et al*., 2018). However, the utilization of red palm olein for making emulsion gel and apply to emulsion-type meat products is still limited. Therefore, the objective of this study was to optimize the formulation of pre-neutralized red palm olein-canola oil emulsion gel using RSM approach and to validate the optimized formula in meat emulsion model system.

MATERIALS AND METHODS

Materials

Cold-pressed red palm olein, canola oil, food-grade beef tallow and chicken breast (5-week-old Ross broiler), mono/diglycerides of fatty acids (emulsifier E471, EMS), soy protein isolate (SPI), refined salt and sodium tripolyphosphate (STPP) were purchased from local market. Inulin (INL) extracted from blue agave (*Agave tequilana*) was purchased from NOW Foods (USA). Chicken breast was cut into cube and ground through 6-mm plate grinder (8A-F, Ramesia, Indonesia). The ground meat was used for meat model system.

Preparation of Emulsion Gel

The oil in water emulsion gel was prepared with 50% of oil. For the oil phase, canola oil was mixed with red palm olein at 70:30 (w/w) ratios. The oil ratio was determined based on the concentration of beta carotene, total cholesterol and the balance of fatty acid profile, particularly the n6 to n3 ratio. For aqueous phase, EMS, SPI, and INL were mixed with 50°C water and the addition level (w/w) was based on the experimental design. Each phase was homogenized separately using hand mixer (HR2533, Philips, Netherlands) and then the mixture was mixed at maximum speed for 30 s. Both the oil and aqueous phase were heated to 50°C in a water bath prior to mix and homogenize according to Mun *et al*. (2010). After homogenization, the mixture was cooled down to room temperature and stored in a chilling room (2 ± 2°C) for 12 h until analysis.

Beta carotene, cholesterol and fatty acid composition analysis

Beta carotene was extracted from the oil using synthetic highly porous resin adsorbent according to Kupan *et al*. (2016). Beta carotene was dissolved in hexane and its concentration was determined using a high-performance liquid chromatography (Water Alliance e2695 XE, Waters, USA). The C18 reversed phase column was used and its temperature was set at 40°C. Acetonitrile/dichloromethane (8:2, v/v) was used as mobile phase at a flow rate of 1 mL/min with the duration of analysis of 45 min (Kupan *et al*., 2016). Beta carotene's peak was identified and its concentration was estimated using its analytical standard (C4582, Sigma-Aldrich, USA) at absorbance of 450 nm.

Cholesterol concentration of the oil was determined using 5α-cholestane (C8003, Sigma-Aldrich, USA) as internal standard. Oil saponification and cholesterol quantification was done according to the procedure by Shukla *et al*. (2002). Cholesterol was quantified using a gas chromatography system (6890N, Agilent Technologies, USA). Injected sample (1 µL), dissolved in n-hexane, was separated with a DB-5MS (J&W Scientific, Folsom, CA, 30 m × 0.25 mm × 0.50 µm film thickness) column. The inlet temperature was set to 250°C with a split ratio of 20:1. Helium was used as carrier gas with a flow

of 1.0 mL/min. The oven was programmed as follows: 285°C for 15 min, then 1°C/min to 300°C, and hold for 1 min. The detector was set to 320°C. The peak of cholesterol was identified, and its concentration was estimated using the analytical standard.

Fatty acid methyl esters (FAME) were prepared by mixing saponified fat with boron trifluoride and then dissolving it in n-hexane. The FAME sample (1 μL) was injected into the GC port using an autosampler (7683, Agilent Technologies, USA). An Agilent gas chromatography system (6890N, Agilent Technologies, USA) was used to determine the fatty acid composition of the oil with the following conditions (Utama *et al*., 2022): The inlet temperature was set to 250°C with a split ratio of 100:1, FAMEs were separated using a WCOT-fused silica capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness; Varian Inc., USA) with a 1.0 mL/min helium flow. The oven was programmed as follows: 150°C/1 min, 150–200°C at 7°C/min, 200°C/5 min, 200–250°C at 5°C/min, and 250°C/10 min and the detector was set to 280°C. The peak of each fatty acid was identified using the retention time of the analytical standards (FAME Mix C4-C24, Supelco, USA). The peak area of each identified fatty acid was used to calculate the proportion (%) of the total identified peak area.

Central Composite Design

Three significant variables (k=3) were selected, and the optimization of the emulsion gel was carried out using a CCD with 2k factorial points, 2k axial points and six replicates at the central point. SPI, EMS, and INL were selected considering their influences on technological properties of the emulsion and nutrition value of the final product and were denoted as X_1, X_2, and X_3, respectively. Each variable was assessed at the following five different addition levels, -1.68, -1, 0, +1, and +1.68. The design consisted of eight factorial points, six axial points and six replicates of central point, totaling 20 runs. The responses were collected from each run and analyzed using a second order polynomial equation. The data were fitted using multiple regression procedure. The statistical relationship between the response (Y) and independent variables (X_1, X_2, and X_3) is given by the quadratic polynomial equation (Utama *et al*., 2018).

pH and instrumental color measurement

The pH value of the sample was recorded in duplicate using a pH meter calibrated with acid (pH 4.01) and neutral (pH 7.00) technical buffer solutions (H1981036, HANNA Instruments, USA) at 25°C according to manufacturer's instruction. For color measurement, sample was poured onto a petri dish. The instrumental surface color was recorded in five repetitions by measuring International Commission on Illumination's system for lightness (CIE L*), redness (CIE a*) and yellowness (CIE b*) using a chromameter (CS-10, CHN Spec., China). The light source of illuminant C (2° observer) with 8 mm aperture and attached-closed cone was calibrated using a white plate (Y = 93.6, X = 0.3134, y = 0.3194).

Gel firmness

The emulsion gel was subjected to gel firmness analysis using a TA-XT2i Plus instrument with a 5 kg load cell (Stable Micro Systems Ltd., UK) at room temperature. A cylindrical 6-mm-diameter probe was used in this study at a compression rate of 50% and with agar gel strength protocol. The sample was placed under the probe that moved downwards at a constant speed of 1.0 mm/s and the firmness (g) was calculated.

Creaming index

Percentage of creaming index (CI%) or oil release of the emulsion gel was determined gravimetrically in triplicate according to Firebaugh and Daubert (2005) with modifications. Sample (10 mL) was filled into 15 mL-centrifuge tube and centrifuged at 1,000 × g for 10 min at 4°C.

The height of the oil released (H_o) and the total of height emulsion (H_e) were recorded. The CI% was reported as follows:

$$Creaming\ index\ (CI\%) = 100 \times \left(\frac{H_o}{H_e}\right)$$

Meat model system

The potential use of the emulsion gel as animal fat replacer was determined in optimization study. Beef tallow was used in control meat model system as the source of fat. The meat batter was made with 20% (w/w) fat (oil/water emulsion or beef tallow), 60% (w/w) ground chicken breast and 20% (w/w) ice. Refined salt (1.5%, w/w) and STPP (0.30%, w/w) were added according to the weight of meat batter. Ground meat, ice and all the salts were homogenized using food processor for 1 min (5KFC3516EER, KitchenAid, USA). The fat/emulsion gel was then added and the batter was homogenized for another 2 min. Sample was vacuum-packed and stored in a chilling room (2 ± 2°C) for 12 h until analysis.

Emulsion stability and cooking yield

The emulsion stability of meat batter was measured using heating method according to Utama *et al*. (2019) with modifications. A sieve (19 mesh) was put into the middle of a centrifuge tube with conical bottom and with precise volume marks at the bottom (ISOLAB, Germany). Meat batter was weighed into the tube (10 g). The tube was sealed using its cap and heated in a water bath at 80°C for 30 min and cooled down at room temperature for another 30 min. Total fluid loss and oil release were determined gravimetrically and calculated as follows:

$$Total\ fluid\ loss\ (mL/g) = \frac{total\ fluid\ (mL)}{meat\ batter\ (g)}$$

$$Oil\ release\ (mL/g) = \frac{oil\ layer\ (mL)}{meat\ batter\ (g)}$$

Cooking yield was determined according to Utama *et al*. (2018). Sample was immersed in a water bath at 80°C for 30 min. The cooked samples were then immediately removed and cooled down at room temperature for 30 min and weighed. Cooking yield was expressed as the percentage of yield after heating.

Texture profile analysis

The cooked meat emulsion was cut into 8 cubes (1×1×1 cm) and then subjected to texture profile analysis using a TA-XT2i Plus instrument with a 5 kg load cell (Stable Micro Systems Ltd., UK) at room temperature. A cylindrical 35-mm-diameter probe was used in this study at a compression rate of 60%. The sample was placed under the probe that moved downwards at a constant speed of 5.0 mm/s (pre-test), 1.0 mm/s (test), and 5.0 mm/s (post-test) and the following parameters were calculated: Hardness (kg), cohesiveness (%), springiness (%), gumminess (kg.cm) and chewiness (kg.cm).

Validation

The optimized formula of the emulsion gel was validated by applying the mixture in meat model system in triplicate. The firmness of emulsion gel and technological properties (hardness and cooking yield) of cooked meat emulsion were used for validation.

Statistical analysis

The statistically significant difference between the mean values from different oil

ratios was determined using a one-way analysis of variance (ANOVA). The mean values were then separated by Duncan's multiple range test at a 5% significance level using R-version 4.0.5 (R Core Team, 2021) with the "agricolae" package (De Mandiburu, 2017). Minitab® 18.1 (USA) was used for designing the optimization study and data analysis. ANOVA and multiple regressions were performed to verify the goodness of fit of the second order polynomial model. The response surface plots of predicted responses of the model were used to evaluate the interaction between selected variables (EMS, SPI, and INL).

RESULTS AND DISSCUSION

Lipid profile of red palm olein and its mixture with canola oil

Red palm olein was proven to contain beta carotene as much as 341.7 mg/kg, which was not found in canola oil (Table 1) and beef tallow (data are not shown). Red palm olein was also found containing cholesterol with the smallest level compared to canola oil and beef tallow (65.71±0.83 mg/100 g, data were not statistically compared with vegetable oils). Palmitic acid (C16:0) is the predominant saturated fatty acid (SFA) found in red palm olein, while oleic acid (C18:2n9) is the major fatty acid observed in canola oil. Thus, the total proportion of SFAs in red palm olein is higher than canola oil. The physical characteristic of red palm olein is close to that of beef tallow in term of texture. Because of its fatty acid profile, red palm olein has a cream-like texture at room temperature. On the other hand, canola oil is fully liquid at room temperature.

The second most abundant fatty acid found in red palm oil is oleic acid (C18:1n9). Besides it is the major fatty acid of canola oil, this fatty acid is also the dominant fatty acid found in olive oil (Karabagias *et al*., 2019). The third and fourth most abundant fatty acids of red palm olein were an omega 6 polyunsaturated fatty acid (PUFA), linoleic acid (C18:2n6), and stearic acid (C18:0), respectively. Even though red palm olein is rich in SFA, canola oil was found having short chain SFA, caproic acid (C16:0), and other long chain SFAs; heneicosylic acid (C21:0), tricosylic acid (C23:0), and lignoceric acid (C24:0), higher than red palm olein. Besides oleic acid is the major monounsaturated fatty acid (MUFA) found in canola oil, canola oil was also observed having middle chain and long chain MUFAs such as palmitoleic acid (C16:1), ginkgolic acid (C17:1), gondoic acid (C20:1), and erucic (C22:1) which were found at very low level and even absence in red palm olein.

Canola oil is superior in terms of the abundance of PUFAs. Among PUFAs, linoleic acid (C18:2n6) is the most abundant PUFA found in canola oil, followed by α-linolenic acid (C18:3n3), eicosapentaenoic acid (C20:5n3), and docosahexaenoic acid (DHA, C22:6n3).

Red palm olein, however, did not have DHA among its PUFAs. Long chain PUFAs such as EPA and DHA are mostly found in marine products, e.g., algae and fish oil, and this study confirms that canola oil also contain those PUFAs although at smaller amount. Canola oil, thus, excels in terms of the balance of omega 6 and omega 3 PUFAs, which is 2.01, and lower indexes related to heart and blood vessel health than red palm olein (Table 1).

Table 1. The concentration of beta carotene, cholesterol and the composition of fatty acid of red palm olein and canola oil mixture at different ratios

	Red palm olein-canola oil mixture (w/w)					SEM
	100/0	20/80	30/70	50/50	0/100	
Beta carotene (mg/kg)	341.7^{a}	68.3^{d}	102.5^{c}	170.8^{b}	0.00^{e}	4.37
Cholesterol (mg/100 g)	1.16^{d}	4.23^{b}	4.11^{b}	3.00^{c}	4.85^{a}	0.21
Fatty acid composition (%)						
C4:0	n.d.	n.d.	n.d.	n.d.	n.d.	
C6:0	0.00^{b}	0.01^{a}	0.01^{a}	0.01^{a}	0.01^{a}	0.00
C8:0	n.d.	n.d.	n.d.	n.d.	n.d.	
C10:0	n.d.	n.d.	n.d.	n.d.	n.d.	
C11:0	n.d.	n.d.	n.d.	n.d.	n.d.	
C12:0	0.03^{a}	0.01^{c}	0.02^{b}	0.02^{b}	0.01^{c}	0.00
C13:0	n.d.	n.d.	n.d.	n.d.	n.d.	
C14:0	0.64^{a}	0.18^{d}	0.24^{c}	0.35^{b}	0.06^{e}	0.19
C15:0	0.05^{a}	0.03^{b}	0.03^{b}	0.04^{b}	0.02^{c}	0.00
C16:0	44.2^{a}	12.3^{d}	16.7^{c}	24.3^{b}	4.35^{e}	4.59
C17:0	0.08	0.06	0.07	0.07	0.06	0.01
C18:0	4.51^{a}	2.56^{c}	3.01^{b}	3.29^{b}	2.07^{c}	1.32
C20:0	0.34^{c}	0.52^{a}	0.55^{a}	0.45^{b}	0.56^{a}	0.21
C21:0	0.00^{b}	0.01^{a}	0.01^{a}	0.01^{a}	0.01^{a}	0.00
C22:0	n.d.	n.d.	n.d.	n.d.	n.d.	
C23:0	0.00^{b}	0.02^{a}	0.02^{a}	0.01^{a}	0.02^{a}	0.00
C24:0	0.05^{b}	0.10^{a}	0.11^{a}	0.08^{a}	0.11^{a}	0.01
C14:1	n.d.	n.d.	n.d.	n.d.	n.d.	
C15:1	n.d.	n.d.	n.d.	n.d.	n.d.	
C16:1	0.14^{b}	0.20^{a}	0.21^{a}	0.18^{a}	0.21^{a}	0.11
C17:1	0.02^{b}	0.05^{a}	0.05^{a}	0.04^{a}	0.05^{a}	0.01
C18:1n9	39.96^{c}	57.18^{a}	61.18^{a}	50.72^{b}	61.49^{a}	6.59
C18:2n6	9.26^{c}	17.71^{ab}	18.64^{a}	14.54^{b}	19.83^{a}	2.34
C18:3n6	n.d.	n.d.	n.d.	n.d.	n.d.	
C18:3n3	0.25^{c}	7.62^{a}	7.64^{a}	4.86^{b}	9.46^{a}	1.63
C20:1	0.12^{d}	0.84^{b}	0.86^{b}	0.57^{c}	1.02^{a}	0.18
C20:2	0.00^{c}	0.04^{a}	0.04^{a}	0.02^{b}	0.04^{a}	0.01
C20:3n6	n.d.	n.d.	n.d.	n.d.	n.d.	
C20:3n3	n.d.	n.d.	n.d.	n.d.	n.d.	
C20:5n3	0.07^{c}	0.22^{a}	0.23^{a}	0.16^{b}	0.26^{a}	0.12
C20:4n6	n.d.	n.d.	n.d.	n.d.	n.d.	
C22:1	0.00^{b}	0.01^{a}	0.01^{a}	0.01^{a}	0.02^{a}	0.00
C22:2	0.12	0.13	0.14	0.13	0.13	0.01
C22:6n3	0.00^{c}	0.11^{a}	0.11^{a}	0.07^{b}	0.14^{a}	0.01
C24:1n9	n.d.	n.d.	n.d.	n.d.	n.d.	
SFA	49.91^{a}	15.82^{c}	20.81^{c}	28.60^{b}	7.30^{d}	4.57
MUFA	40.22^{c}	58.23^{a}	62.26^{a}	51.48^{b}	62.74^{a}	2.13
PUFA	9.70^{d}	25.83^{b}	26.80^{b}	19.78^{c}	29.86^{a}	3.52
MUFA/SFA	0.81^{d}	3.68^{b}	2.99^{b}	1.80^{c}	8.60^{a}	1.12
PUFA/SFA	0.19^{d}	1.63^{b}	1.29^{b}	0.69^{c}	4.09^{a}	0.12
UFA/SFA	1.00^{d}	5.31^{b}	4.28^{b}	2.49^{c}	12.69^{a}	0.23
n9	39.96^{c}	57.18^{a}	61.18^{a}	50.72^{b}	61.49^{a}	2.65
n6	9.26^{c}	17.71^{a}	18.64^{a}	14.54^{b}	19.83^{a}	2.87
n3	0.32^{d}	7.95^{b}	7.98^{b}	5.09^{c}	9.85^{a}	1.28
n6:n3	28.90^{a}	2.23^{c}	2.34^{bc}	2.86^{b}	2.01^{c}	1.44
Atherogenic index	0.94^{a}	0.16^{c}	0.20^{c}	0.36^{b}	0.05^{d}	0.12
Thrombogenic index	1.74^{a}	0.20^{c}	0.26^{c}	0.51^{b}	0.06^{d}	0.11

SEM, standard error for means; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid.

The omega 6 to omega 3 PUFAs ratio of red palm olein is above 10, while the recommended ratio of omega 6 and omega 3 in food is 4:1 (Simopoulos, 2016). Canola oil can be concluded as a healthy oil based on its fatty acid profile. Even though red palm olein is rich in SFAs, it contains large amounts of beta-carotene as antioxidant and low level of cholesterol. According to the technological properties of the oil when it is used in manufacturing emulsion type meat products, canola oil has been proved reducing cooking loss and maintaining emulsion stability (Baek *et al*., 2016; Monteiro *et al*., 2017; Utama *et al*., 2019). Thus, mixing both oils at proper ratio would result in an oil mixture having advantages from both sides. Among the mixture ratios, the mixture of red palm olein and canola oil at 70/30 (w/w) is shown having beta carotene of 102.5 mg/kg, cholesterol of 4.11 mg/100 g, oleic acid (C18:1n9) of 61.18%, PUFA of 26.80%, n6 to n3 PUFAs ratio of 2.34, atherogenic index of 0.20 and thrombogenic index of 0.26, which are considered as proper ratio in terms of nutritional value and technological properties.

Formula optimization and characteristics of the emulsion gel

Three ingredients (SPI, EMS, INL) were used as emulsion stabilizer in aqueous phase along with water. SPI and INL have been proved in previous study as functional ingredients playing a role in stabilizing pre-emulsified perilla-canola oil, adding health benefit as prebiotic without affecting the techno-functional properties of emulsion meat product (Utama *et al*., 2018). EMS (powder form) was used in this study as it is considered lower in cost and easier to handle and store compared to polyglycerol polyricinoleate (PGPR, gel form). In addition, EMS is widely used in the manufacturing of whipping cream and ice cream to maintain its emulsion and foam stability, while PGPR is found in chocolate making to prevent it from blooming and affect positively on its flow behavior (Blankart *et al*., 2020; Toker *et al*., 2021).

SPI, EMS, and INL were independent variables used in central composite design and their addition level was optimized. SPI, EMS, and INL were representing protein, fat and carbohydrate, respectively. The responses for pH, instrumental color, gel firmness and creaming index of the red palm olein-canola oil emulsion gel are shown in Table 2, while the responses for emulsion stability, cooking yield and texture profile of the meat emulsion are shown in Table 3. The second order polynomial equations for the selected responses (gel firmness, meat model system cooking yield and hardness) as affected by the addition levels of SPI, EMS and INL are presented in Table 5. The surface plot for the selected responses as affected of SPI and EMS addition levels are shown in Figure 1.

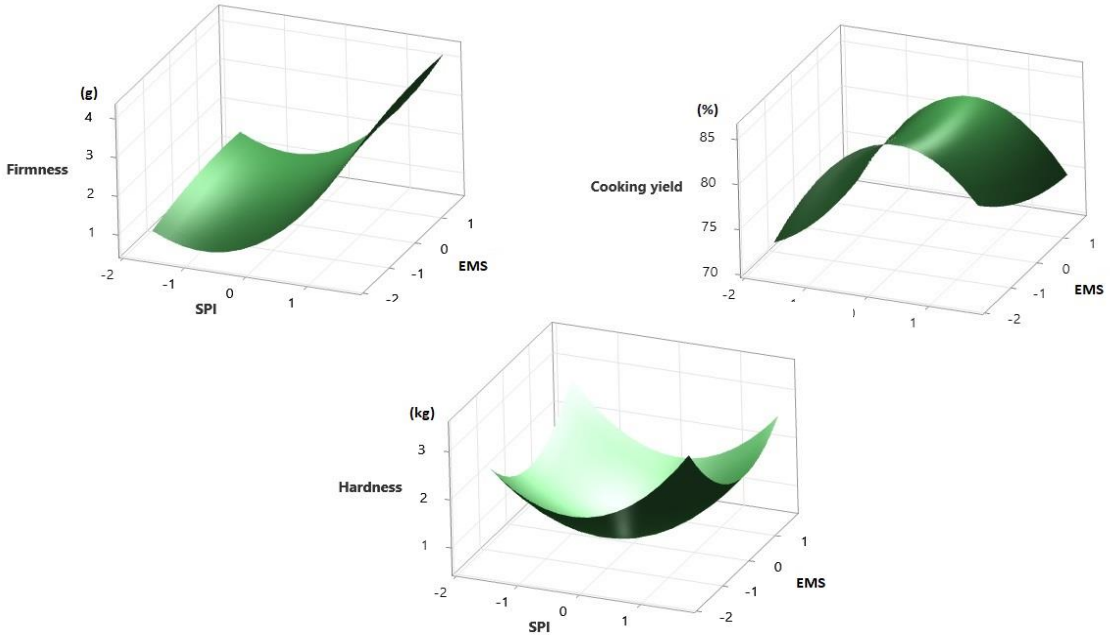

Figure 1. Response surface plots for gel firmness of emulsion gel, cooking yield and hardness of meat model system formulated with red palm olein-canola oil emulsion gel as affected by soy protein isolate (SPI) and emulsifier E471 (EMS) addition level.

Table 2. Central composite design for the optimization of selected variables on the formulation of a red palm olein-canola oil emulsion and the responses in the emulsion gel

Run	Variables[a]			Responses					
	SPI (%)	EMS (%)	INL (%)	pH	L*	a*	b*	Firmness (g)	Creaming index (%)
1	-1 (1.5)	-1 (0.5)	-1 (3)	6.30	46.5	52.7	18.5	1.09	0
2	1 (4.5)	-1 (0.5)	-1 (3)	6.41	47.8	51.2	11.6	2.13	0
3	-1 (1.5)	1 (1.5)	-1 (3)	6.54	46.4	52.2	19.0	1.05	0
4	1 (4.5)	1 (1.5)	-1 (3)	6.43	48.1	51.7	10.5	2.29	0
5	-1 (1.5)	-1 (0.5)	1 (5)	6.50	45.8	52.3	18.5	0.98	0
6	1 (4.5)	-1 (0.5)	1 (5)	6.54	47.3	51.5	12.5	2.33	0
7	-1 (1.5)	1 (1.5)	1 (5)	6.55	45.6	52.6	19.3	1.13	0
8	1 (4.5)	1 (1.5)	1 (5)	6.54	47.8	51.5	12.6	2.24	0
9	-1.68 (0)	0 (1)	0 (4)	7.02	20.4	32.4	4.40	0.66	6.67
10	1.68 (6)	0 (1)	0 (4)	6.35	48.0	51.1	9.40	4.72	0
11	0 (3)	-1.68 (0)	0 (4)	6.57	45.4	51.3	17.1	0.78	0
12	0 (3)	1.68 (2)	0 (4)	6.48	47.7	51.7	14.0	1.52	0
13	0 (3)	0 (1)	-1.68 (2)	6.58	47.6	51.5	14.7	0.98	0
14	0 (3)	0 (1)	1.68 (6)	6.44	46.3	52.2	15.7	1.01	0
15-20[b]	0 (3)	0 (1)	0 (4)	6.39±0.07	46.1±0.21	50.5±0.37	13.1±0.24	1.30±0.13	0±0.00

[a] SPI, soy protein isolate; EMS, emulsifier E471; INL, inulin.
[b] Responses are presented as mean±standard deviation.

Table 3. Regression coefficients of selected responses representing technological properties of red palm olein-canola oil emulsion according to central composite design

	Gel firmness		Cooking yield		Hardness	
Model term	Coefficient	*p* value	Coefficient	*p* value	Coefficient	*p* value
Constant	1.30	<0.001	82.30	<0.001	0.61	0.003
Linear effect						
SPI	0.85	<0.001	1.64	0.026	0.10	0.349
EMS	0.10	0.329	–1.14	0.098	–0.15	0.196
INL	0.01	0.905	0.73	0.271	0.01	0.909
Quadratic effect						
SPI	0.50	0.001	–3.10	<0.001	0.44	0.002
EMS	-0.05	0.638	0.45	0.477	0.34	0.008
INL	-0.01	0.323	–0.14	0.823	0.38	0.004
Interaction effect						
SPI.EMS	-0.01	0.971	0.370	0.662	–0.08	0.584
SPI.INL	0.02	0.869	–0.63	0.460	–0.08	0.597
EMS.INL	–0.01	0.956	–0.03	0.972	–0.37	0.023

SPI, soy protein isolate; EMS, emulsifier E471; INL, inulin.

In this study, SPI played a significant role in preventing emulsion break in emulsion gel. SPI affected pH by maintaining it under 7.0 and maintained color stability and gel firmness. The emulsion break was found in run 9, where SPI was not present. Other than run 9, the yellowness (b*) was affected by different addition levels of each independent variable. EMS and SPI are significantly influenced the yellowness of emulsion gel ranged from 9.40 (highest SPI level) to 19.3. The absence of SPI in run 9 resulted in the lowest b* value, which means the oil phase and aqueous phase were separated. The highest level of SPI in run 10 also lowered b* value as the appearance became pale. Run 1, 3, 5, 7, and 11 had bright yellow appearance as the level of SPI and EMS is low, thus the oil droplets were not bound into gel matrix, but existed on the surface. Even though INL was added at slightly higher amount than SPI, SPI influenced more linearly and quadratically on gel firmness (linear $p<0.001$, quadratic $p=0.001$) than INL (linear $p=0.905$, quadratic $p=0.323$) as shown in Table 4.

SPI is 90% of protein that is used as structure building component in gel matrix development (Chen *et al*., 2019). On the other hand, INL is an oligosaccharide that is soluble in water and can lower the hydrophobic interaction between protein and fat when combined with lecithin (emulsifier) in reduced-fat cheese making (Li *et al*., 2019). INL, however, provides ability as protein binder and improves the uniformity of tofu (Stanojevic *et al*., 2020). In nutritional point of view, INL is highly recommended to take daily as it has inherent therapeutic effects by stimulating the growth of colon bacteria (Gupta *et al*., 2019). SPI plays the role as both emulsifier and stabilizer. Its role is stronger than EMS. Neither EMS nor INL significantly affected the firmness of the emulsion gel. EMS was used at a level less than INL and SPI as it is derived from fat. The quadratic effect of EMS ($p=0.008$), however, shows a positive influence on the texture (hardness) of cooked meat model system. Thus, EMS and INL are still considered important for manufacturing red palm olein-canola oil emulsion gel.

The techno-functional characteristics of meat batter and cooked meat model system formulated with red palm olein-canola oil emulsion gel and beef tallow as control are comparable (Table 3). There were no significant differences found on meat model system emulsion stability, cooking yield and all texture attributes among control group and treatment group. SPI, EMS, and INL did not show any linear

effect on the hardness of cooked meat model system. However, SPI was identified having positive linear and quadratic influences (linear p=0.026, quadratic p<0.001) on cooking yield. Interestingly, the quadratic effect of SPI, EMS, and INL positively affected the hardness of cooked meat model system. In addition, there was a negative interaction effect (p=0.023) between the use of EMS and INL. This is in accordance with Li *et al*. (2019) who found that INL is able to lower the hydrophobic interaction between protein and fat when combined with fat-derived emulsifier, e.g., lecithin, and thus lowering the firmness/hardness of the emulsion.

Table 4. Central composite design for the optimization of selected variables on the formulation of red palm olein-canola oil emulsion and the responses in cooked meat model system

Run	Variables[a]			Responses							
				Emulsion stability			Texture profile				
	SPI (%)	EMS (%)	INL (%)	Total fluid loss (mL/g)	Oil release (mL/g)	Cooking yield (%)	Hardness (kg)	Cohesiveness	Springiness (cm)	Gumminess (kg.cm)	Chewiness (kg.cm)
1	-1 (1.5)	-1 (0.5)	-1 (3)	0.06	0.03	78.0	1.73	0.66	0.89	1.14	1.01
2	1 (4.5)	-1 (0.5)	-1 (3)	0.03	0.01	84.9	1.83	0.75	0.93	1.34	1.20
3	-1 (1.5)	1 (1.5)	-1 (3)	0.06	0.04	73.6	1.99	0.72	0.89	1.47	1.32
4	1 (4.5)	1 (1.5)	-1 (3)	0.04	0.02	78.8	2.11	0.71	0.87	1.50	1.31
5	-1 (1.5)	-1 (0.5)	1 (5)	0.04	0.02	80.4	2.62	0.74	0.90	1.92	1.56
6	1 (4.5)	-1 (0.5)	1 (5)	0.03	0.01	84.6	2.75	0.74	0.89	2.02	1.81
7	-1 (1.5)	1 (1.5)	1 (5)	0.04	0.03	75.9	1.72	0.77	0.89	1.32	1.17
8	1 (4.5)	1 (1.5)	1 (5)	0.04	0.03	78.8	1.20	0.74	0.91	0.89	0.81
9	-1.68 (0)	0 (1)	0 (4)	0.08	0.05	72.7	2.16	0.75	0.87	1.62	1.38
10	1.68 (6)	0 (1)	0 (4)	0.06	0.04	74.5	2.01	0.69	0.86	1.39	1.19
11	0 (3)	-1.68 (0)	0 (4)	0.04	0.02	82.1	1.31	0.79	0.93	1.03	0.96
12	0 (3)	1.68 (2)	0 (4)	0.03	0.01	85.2	1.24	0.78	0.92	0.97	0.89
13	0 (3)	0 (1)	-1.68 (2)	0.04	0.02	80.4	1.53	0.79	0.92	1.20	1.11
14	0 (3)	0 (1)	1.68 (6)	0.03	0.01	83.6	1.25	0.80	0.93	0.99	0.92
15-20[b]	0 (3)	0 (1)	0 (4)	0.03±0.01	0.01±0.00	82.7±0.12	0.65±0.08	0.81±0.03	0.97±0.02	0.52±0.01	0.51±0.01
Overall mean [b]				0.04±0.01	0.02±0.01	79.8±4.17	1.74±0.56	0.75±0.04	0.90±0.03	1.29±0.39	1.14±0.32
Control meat emulsion[b]				0.05±0.01	0.02±0.01	78.0±1.64	1.66±0.43	0.73±0.03	0.89±0.04	1.23±0.35	1.10±0.33

[a] SPI, soy protein isolate; EMS, emulsifier E471; INL, inulin.

[b] Responses are presented as mean±standard deviation.

The mean value of the response in cooked meat model system formulated with red palm olein-canola oil emulsion was not significantly different with that of control cooked meat model system formulated with beef tallow.

Experimental validation of the model

The regression models were validated using selected optimal conditions and the optimized addition levels of SPI, EMS, and INL are presented in Table 5.

The optimum conditions were obtained by maximizing the gel firmness and cooking yield of meat model system, also by adjusting the hardness to 2.0 kg using response surface predictions. Under these optimized conditions, the observed experimental values (in triplicate) of the gel firmness (3.45±0.68 g), cooking yield (81.2±0.17%) and hardness (1.91±0.14 kg) were close to the predicted model response (3.26 g for gel firmness; 80.4% for cooking yield; 2.00 kg for hardness) with low relative deviation.

Table 5. Second order polynomial equations for selected responses representing major technological properties of the red palm olein-canola oil emulsion and the optimized addition level of selected ingredients

Second order polynomial equations for selected responses representing major technological properties	
Gel firmness	1.299+ 0.847 SPI + 0.104 EMS + 0.012 INL + 0.4975 SPI*SPI – 0.0481 EMS*EMS - 0.1030 INL*INL – 0.005 SPI*EMS + 0,023 SPI*INL - 0,007 EMS*INL
Cooking yield	82,269 + 1.639 SPI – 1.143 EMS + 0.729 INL – 3.102 SPI*SPI + 0.451 EMS*EMS – 0.140 INL*INL – 0.368 SPI*EMS – 0.628 SPI*INL + 0.030 EMS*INL
Hardness	0.612 + 0.105 SPI – 0.148 EMS + 0.013 INL + 0.436 SPI*SPI + 0.345 EMS*EMS + 0.384 INL*INL – 0.079 SPI*EMS – 0.076 SPI*INL – 0.374 EMS*INL
Optimized addition level of selected ingredients (%, w/w)	
SPI	1.36 (5.33%)
EMS	-0.82 (0.53%)
INL	-0.02 (3.98%)

CONCLUSION

Red palm olein-canola oil mixture stabilized with soy protein isolate, emulsifier E471 and inulin is an effective ingredient that can be utilized as a substitute for animal fat in meat emulsion model system. The use of red palm olein and canola oil at 70 to 30 ratio (w/w) in 50% of oil phase can reduce the final fat content of the product and add beta carotene with balanced n6 to omega3 ratio. In addition, the use of inulin in this emulsion can fulfill the needs of dietary fiber. Further studies are needed to validate the effect of the replacement of animal fat with the optimized pre-neutralized red palm olein emulsion on the quality properties of emulsion-based processed meat products such as sausages.

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REFERENCES

Abbate, M., Mascaró, C. M., Montemayor, S., Barbería-Latasa, M., Casares, M., Gómez, C., Ugarriza, L., Tejada, S., Abete, I., Zulet, M. Á. & Sureda, A. (2021). Animal fat intake is associated with albuminuria in patients with non-alcoholic fatty liver disease and metabolic syndrome. *Nutrients*, *13*(5), 1548. https://doi.org/10.3390/nu13051548

Baek, K. H., Utama, D. T., Lee, S. G., An, B. K., & Lee, S. K. (2016). Effects of replacing pork back fat with canola and flaxseed oils on physicochemical properties of emulsion sausages from spent layer meat. *Asian-Australasian Journal of Animal Sciences*, *29*(6), 865-871.

Blankart, M., Kratzner, C., Link, K., Oellig, C., Schwack, W., & Hinrichs, J. (2020). Technical emulsifiers in aerosol whipping cream–Compositional variations in the

emulsifier affecting emulsion and foam properties. *International Dairy Journal*, *102*, 104578. https://doi.org/10.1016/j.idairyj.2019.104578

Chaijan, M., & Panpipat, W. (2021). Pre-neutralized crude palm oil as natural colorant and bioactive ingredient in fish sausage prepared from tilapia (*Oreochromis niloticus*). *LWT*, *135*, 110289. https://doi.org/10.1016/j.lwt.2020.110289

Chen, H., Gan, J., Ji, A., Song, S., & Yin, L. (2019). Development of double network gels based on soy protein isolate and sugar beet pectin induced by thermal treatment and laccase catalysis. *Food Chemistry*, *292*, 188-196.

De Mandiburu, F. (2017). *Agricolae: Statistical procedures for agricultural research*. [https://CRAN.R-project.org/package=agricolae]. Accessed date: 1 November 2021.

Drouin-Chartier, J. P., Tremblay, A. J., Lépine, M. C., Lemelin, V., Lamarche, B., & Couture, P. (2018). Substitution of dietary ω-6 polyunsaturated fatty acids for saturated fatty acids decreases LDL apolipoprotein B-100 production rate in men with dyslipidemia associated with insulin resistance: a randomized controlled trial. *The American Journal of Clinical Nutrition*, *107*(1), 26-34.

Emmanuel, O., Okezie, U. M., Iweala, E. J., & Ugbogu, E. A. (2021). Pretreatment of red palm oil extracted from palm fruit (*Elaeis guineensis*) attenuates carbon tetrachloride induced toxicity in Wistar rats. *Phytomedicine Plus*, *1*(4), 100079. https://doi.org/10.1016/j.phyplu.2021.100079

Firebaugh, J. D., & Daubert, C. R. (2005). Emulsifying and foaming properties of a derivatized whey protein ingredient. *International Journal of Food Properties*, *8*(2), 243-253.

Gupta, N., Jangid, A. K., Pooja, D., & Kulhari, H. (2019). Inulin: A novel and stretchy polysaccharide tool for biomedical and nutritional applications. *International Journal of Biological Macromolecules*, *132*, 852-863.

Hammad, S., Pu, S., & Jones, P. J. (2016). Current evidence supporting the link between dietary fatty acids and cardiovascular disease. *Lipids*, *51*(5), 507-517.

Jia, F., Hu, X., Kimura, T., & Tanaka, N. (2021). Impact of dietary fat on the progression of liver fibrosis: lessons from animal and cell studies. *International Journal of Molecular Sciences*, *22*(19), 10303. https://doi.org/10.3390/ijms221910303

Kang, Z. Q., Yang, Y., & Xiao, B. (2020). Dietary saturated fat intake and risk of stroke: systematic review and dose–response meta-analysis of prospective cohort studies. *Nutrition, Metabolism and Cardiovascular Diseases*, *30*(2), 179-189.

Karabagias, I. K., Badeka, A., Casiello, G., Longobardi, F., & Kontominas, M. G. (2019). Rapid screening of olive oil cultivar differentiation based on selected physicochemical parameters, pigment content and fatty acid composition using advanced chemometrics. *European Food Research and Technology*, *245*(9), 2027-2038.

Kupan, S., Hamid, H., Kulkarni, A., & Yusoff, M. (2016). Extraction and analysis of beta-carotene recovery in CPO and oil palm waste by using HPLC. *ARPN Journal of Engineering and Applied Sciences*, *11*(4), 2184-2188.

Li, H., Yu, H., Liu, Y., Wang, Y., Li, H., & Yu, J. (2019). The use of of inulin, maltitol and lecithin as fat replacers and plasticizers in a model reduced-fat mozzarella cheese-like product. *Journal of the Science of Food and Agriculture*, *99*(12), 5586-5593.

Monteiro, G. M., Souza, X. R., Costa, D. P. B., Faria, P. B., & Vicente, J. (2017). Partial substitution of pork fat with

canola oil in Toscana sausage. *Innovative Food Science & Emerging Technologies*, *44*, 2-8.

Mun, S., Choi, Y., Rho, S. J., Kang, C. G., Park, C. H., & Kim, Y. R. (2010). Preparation and characterization of water/oil/water emulsions stabilized by polyglycerol polyricinoleate and whey protein isolate. *Journal of Food Science*, *75*(2), E116-E125.

Nakamura, H., Tsujiguchi, H., Kambayashi, Y., Hara, A., Miyagi, S., Yamada, Y., Nguyen, T.T., Shimizu, Y., Hori, D., & Nakamura, H. (2019). Relationship between saturated fatty acid intake and hypertension and oxidative stress. *Nutrition*, *61*, 8-15.

Palomer, X., Pizarro-Delgado, J., Barroso, E., & Vázquez-Carrera, M. (2018). Palmitic and oleic acid: the yin and yang of fatty acids in type 2 diabetes mellitus. *Trends in Endocrinology & Metabolism*, *29*(3), 178-190.

Patterson, E., Wall, R., Fitzgerald, G. F., Ross, R. P., & Stanton, C. (2012). Health implications of high dietary omega-6 polyunsaturated fatty acids. *Journal of Nutrition and Metabolism*, *2012*. https://doi.org/10.1155 /2012/539426

Pintado, T., Herrero, A. M., Jiménez-Colmenero, F., Cavalheiro, C. P., & Ruiz-Capillas, C. (2018). Chia and oat emulsion gels as new animal fat replacers and healthy bioactive sources in fresh sausage formulation. *Meat Science*, *135*, 6-13.

R Core Team. (2021). *R: A language and environment for statistical computing*. [http://www.R-project.org]. Accessed date: 1 December 2021.

Sanders, T. A. B. (2009). Fat and fatty acid intake and metabolic effects in the human body. *Annals of Nutrition & Metabolism*, *55*(1/3), 162-172.

Serdaroğlu, M., Nacak, B., & Karabıyıkoğlu, M. (2017). Effects of beef fat replacement with gelled emulsion prepared with olive oil on quality parameters of chicken patties. *Korean Journal for Food Science of Animal Resources*, *37*(3), 376-384.

Stanojevic, S. P., Barać, M. B., Pešić, M. B., & Vucelic-Radovic, B. V. (2020). Protein composition and textural properties of inulin-enriched tofu produced by hydrothermal process. *LWT*, *126*, 109309. https:// doi.org/10.1016/j.lwt.2020.109309

Shukla, V. K. S., Dutta, P. C., & Artz, W. E. (2002). Camelina oil and its unusual cholesterol content. *Journal of the American Oil Chemists' Society*, *79*(10), 965-969.

Simopoulos, A. P. (2016). An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*, *8*(3), 128. https:// doi.org/10.3390/nu8030128

Tan, C. H., Lee, C. J., Tan, S. N., Poon, D. T. S., Chong, C. Y. E., & Pui, L. P. (2021). Red palm oil: A review on processing, health benefits and its application in food. *Journal of Oleo Science*, ess21108.

Toker, O. S., Pirouzian, H. R., Palabiyik, I., & Konar, N. (2021). Chocolate flow behavior: Composition and process effects. *Critical Reviews in Food Science and Nutrition*, 1-15.

Utama, D. T., Jang, A., Kim, G. Y., Kang, S. M., & Lee, S. K. (2022). Distinguishing aroma profile of highly-marbled beef according to quality grade using electronic nose sensors data and chemometrics approach. *Food Science of Animal Resources*, *42*(2), 240-251.

Utama, D. T., Jeong, H., Kim, J., & Lee, S. K. (2018). Formula optimization of a perilla-canola oil (O/W) emulsion and its potential application as an animal fat replacer in meat emulsion. *Korean Journal for Food Science of Animal Resources*, *38*(3), 580-592.

Utama, D. T., Jeong, H. S., Kim, J., Barido, F. H., & Lee, S. K. (2019). Fatty acid composition and quality properties of chicken sausage formulated with pre-

emulsified perilla-canola oil as an animal fat replacer. *Poultry Science*, *98*(7), 3059-3066.

Yolmeh, M., & Jafari, S. M. (2017). Applications of response surface methodology in the food industry processes. *Food and Bioprocess Technology*, *10*(3), 413-433.