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Production and optimisation of used cooking palm oil into protected fat calcium salts by fusion method using response surface methodology (RSM)

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Abstract

Used cooking oil (UCO) is a waste, and creates environmental issues due to its hydrophobic property. UCO, with its high content of fatty acid, can be used as source material for animal feed. However, high unsaturated fatty acid in UCO is harmful to the ruminant's microflora. This can be resolved by transforming UCO into functional product such as ruminant's protected fat (PF). In the present work, the production of used cooking oil protected fat (UCOPF) using fusion method *via* saponification process was investigated. Response surface methodology (RSM) was used to evaluate the effect of calcium oxide concentration (CaO), initial temperature (iTemp.), and percentage of water (H_2O) on the solidification score and free fatty acid (FFA) content of PF. Results showed that all the studied parameters significantly affected the responses. The coefficient of determination (R^2) for solidification score and FFA were high at 0.9433 and 0.9599, respectively. The optimum condition to produced UCOPF by fusion method was CaO (20%), iTemp. (80°C), and percentage of water (30%), which yielded solidification score and FFA of 5.33 ± 0.53 and 0.85 ± 0.07%, respectively. The FFA content of the optimised PF was lower than permitted; thus, it can be used as animal supplement. In conclusion, the UCO can be converted into PF by using calcium fusion method. However, the property and stability of the produced PF should be assessed prior to commercialisation.

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Introduction

The world production of vegetable oil has steadily increased over the years, and reached a peak of 209 million metric tonne (MMT) in 2020/2021 (Shahbandeh, 2021). The high consumption of vegetable oil generates by-product known as used cooking oil (UCO). Every year, the world generates more than 15 MMT of UCO, of which China generates 5 MMT of UCO from culinary oils (Zhang *et al*., 2014), and the European Union generates 1 MMT (Fangfang *et al*., 2021). The unregulated and illegal dumping of untreated UCO into landfills or rivers has a negative effect on the environment, economic, and society (Fangfang *et al*., 2021; Samuel *et al*., 2021). The impact on environment becomes worst when eutrophication occurs. Eutrophication is when a thin layer of oil exists that prevents sunlight from penetrating the top layer of the river, which then disrupts the oxygen availability for aquatic species in the river over time, thus resulting in their death (Refaat, 2010; Wei *et al*., 2011; Azahar *et al*., 2016). Although UCO is increasingly being generated, its utilisation is limited. UCO, with its high content of fatty acid (FA), can be used as source material for animal feed.

However, there are several concerns regarding the utilisation of UCO as an animal supplement, for example unprotected unsaturated acid which is harmful to the rumen microorganisms (Naik, 2013), the presence of foreign materials, and too high FFA and acid value (AV) which are above the permitted limits by the feed industry (Wei *et al*., 2011). Therefore, purification (Wei *et al*., 2011) and

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transformation (Suksombat, 2009) of UCO into protected fat (PF) is required if the material is intended for food or feed applications.

The calcium salts of FA, also known as calcium soap, as a form of PF used for ruminant feed supplements, especially in the dairy industry, is desirable than the raw oil known as unprotected fat (Shelke *et al*., 2012). The PF has an insignificant impact on the ruminants' rumen fermentation, which is different from feeding oil directly to ruminants (Jenkins and Harvatine, 2014). Unprotected fat that is not converted into PF has a negative effect on the rumen fermentation by coating the feed, absorbing the feed particles and microorganisms in the rumen, and decreasing the feed digestibility (Palmquist and Jenkins, 2017). Unsaturated fatty acid (USFA) is more toxic because they attach more to the rumen microorganisms, and negatively affect rumen fermentation (Lounglawan *et al*., 2008). PF has the extra benefit of being dry fats that are easy to transport and combine with other feed components (Jenkins and Harvatine, 2014). Ruminants' intake of calcium salts improves the quality and amount of milk produced. It has been found that consuming 0.45 kg of calcium salts per day improves milk production by 3 - 8% (Naik *et al*., 2009). Additionally, the milk fat content increases by 0.2 - 0.3%, and the cow's first service conception rate increases by 20% (Suksombat, 2009).

There are three methods to convert fat into PF commercially for animal feeds that have been proposed (Suksombat, 2009). The first method is partial hydrogenation of tallow. This method produces the least preferable product, and not often applied for dairy rations. The second method is by prilling partial hydrogenated tallow in a spray-chilling reactor to produce prilled fats (Naik *et al*., 2007). The third method is by reacting liquid vegetable oil with calcium oxide to produce solid and insoluble calcium salts. This is the most suitable method for producing PF (Handojo *et al*., 2018a).

Used cooking oil can be transformed into PF calcium salts through saponification with a calcium source (Naik *et al*., 2007; Suksombat, 2009; Handojo *et al*., 2018a). There are three common processes used in the production of calcium salts which are double decomposition, direct reaction, and fusion reaction (Handojo *et al*., 2019a). The fusion method is usually selected for calcium salt production because it gives better yield and purer product, and shorter reaction time when compared with other methods (Handojo *et al*., 2018a). The fusion method allows calcium oxide (CaO) to react directly with FFA by melting the fats or lipids using a catalyst in the form of water (Pablos Pérez, 2008). During the saponification of FA, the combined action of CaO concentration, initial temperature (iTemp.) during mixing (Handojo *et al*., 2018a), and volume of water (H_2O) (Pablos Pérez, 2008) significantly affects the physical and chemical properties, in particular fusion characteristics of PF. Increasing CaO concentration and iTemp. during mixing could increase fusion factor such as saponification reaction process, and decrease FFA content in PF. A similar influence was reported on saponification of palm fatty acid distillate (PFAD) to produce animal feed (Handojo *et al*., 2018b).

Therefore, the present work was designed to optimise the fusion process parameters such as concentration of CaO, iTemp., and volume of H_2O on the saponification of UCO to produce PF with acceptable level of FFA using response surface methodology (RSM).

Materials and methods

Materials

Calcium oxide, activated carbon, and monosodium glutamate (MSG) were purchased from Malaysia Fisher Scientific Co. Used cooking oil was purchased from Bangi Mosque UCO Collection Centre, Malaysia.

Production of protected fat calcium salts

UCO purification

Purification was performed by mixing raw UCO with activated carbon and MSG according to Wei *et al*. (2011). After mixing the oil sample and adsorbent, the mixture was heated for 30 min at 70°C. Next, the activated carbon and MSG were removed from the oil using No. 1 Whatman cellulose filter paper.

UCO saponification

Saponification was performed through the fusion method followed by drying and milling as reported by Pablos Pérez (2008). The mixture of UCO, CaO, and water was heated and vigorously agitated to ensure homogeneity. The mixture was dried for approximately 24 h at 80°C in a forced air circulation oven (Memmert, Germany) before chilled to room temperature overnight.

Experimental design and response surface methodology

The RSM with central composite design (CCD) was employed to identify the optimised parameters for producing used cooking oil protected fat (UCOPF). Three independent factors were assessed namely CaO concentration (A), initial temperature (B), and H_2O volume (C). The dependent variable was solidification scores (Y1) and the level of FFA content (Y2). A total of 20 sets of experimental runs were generated using Design Expert Software Version 6.0.4 (State Ease, Inc., Minnesota, United States) with eight cube points, six axial points, and six replications of the central points. After fitting the experimental data using a second-order polynomial equation, the response variable was correlated with the independent variable as shown in Eq. 1:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{k} \sum_{i \neq j=1}^{k} \beta_{ij} X_i X_j + \varepsilon \quad \text{(Eq. 1)}$$

where, Y = predicted response; B_o, B_i, B_{ii}, and B_{ij} = regression constants corresponding to the intercept, linear, quadratic, and interaction coefficients, respectively; n = number of variables analysed; X_i and X_j = variables used as inputs; and Ɛ = random error.

Analysis of variance (ANOVA) was employed to determine the reliability of the mathematical equation generated by RSM as suggested by Tan *et al*. (2008). The ANOVA was used to analyse the statistical fitness of the CCD model by examining numerous factors such as determination coefficient (R^2), adjusted determination coefficient (R^2 adj.), *F*-value, *p*-value, and degree of freedom (DF) to evaluate the adequacy of the second-order polynomial equation. A model that was complementary to the experiments was indicated by a *p*-value of less than 0.05. Besides that, three-dimensional (3-D) graphical plots were used to evaluate the effect of individual and combined independent factors on the solidification scores and FFA of UCOPF.

Solidification score analysis

After UCOPF was produced by saponification, the solidification scores of the samples were evaluated by using scores 1 to 6 based on the observation as shown in Table 1. This solidification score was scored subjectively, and divided from score 1 for liquid, to score 6 for extremely hard solid PF.

Table 1. Solidification score of protected fat produced.

Score	Grading
1	Liquid, oil
2	Semi-solid, mixture oil and solid
3	Exceptionally soft, taffy-like material, very sticky
4	Soft, sticky
5	Moderate hard
6	Extremely hard, brittle

Free fatty acid analysis

The acidity of the produced PF was measured following AOCS (1989) standard method. One gram of the UCOPF samples was weighed and placed in a dried Erlenmeyer flask. The samples were then treated with 50 mL of ethyl alcohol. Following that, 500 µL of 1% phenolphthalein indicator was added to the mixture. The Erlenmeyer flask was set on a hot plate, and the temperature was controlled to about 40°C. The mixture was then shaken gently while titrating it against a standard sodium hydroxide solution (0.1 N) until a faint pink colour developed and remained for at least 30 s. The FFA (%) was calculated using Eq. 2:

$$\text{FFA\% as palmitic acid} = \frac{(\text{mL of titrant}) \times (\text{N of titrant}) \times (25.6)}{\text{Sample (g)}} \left(\frac{\text{NaOH (mg)}}{\text{Sample (g)}}\right) \quad \text{(Eq. 2)}$$

where, 25.6 = equivalency factor for palmitic acid.

Chemical analysis

Chemical analyses for dry matter, moisture, ash, and ether extract were described by AOAC (2005) method.

Gas chromatography

FAME analysis was performed by employing an Agilent Technologies gas chromatograph integrated with a flame ionisation detector (FID) and a BPX-70 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 m film thickness). The injector was sustained at 250°C. Helium was employed as the carrier gas at a 1 mL/min flow rate, a volume of injection of 1 L, and a split ratio of 60:1. The temperature was steadily elevated to 240°C at a rate

of 5°C/min, maintaining the oven temperature at 60°C. The final temperature was maintained for 7 min. After classifying and quantifying the FAME peaks, their regions and retention durations were compared against standard FAME (EN 14103:2011).

Statistical analysis

All assessments were carried out in triplicate to ensure statistical significance, and the findings were presented as mean ± standard error (SE). The statistical tools Design Expert Version 12 (State Ease, Inc., Minnesota, United States) and SAS version 9.4 (SAS Institute Inc., Cary, NC) (SAS Institute, 2017) were used for the statistical analysis with *p*-value of less than 0.05 considered statistically significant.

Results and discussion

Statistical analysis

In the present work, the solidification scores and FFA contents of UCOPF from 20 different combinations of CaO, iTemp., and H_2O were determined as shown in Table 2. Analysis of variance, regression, and coefficient of determination (R^2) for solidification scores and FFA were analysed as shown in Tables 3 and 4, respectively. Based on the results, the quadratic model models were found to be unfit to be further explored for optimisation as compared to linear and 2FI models. Aliasing was deployed on the cubic model. Accordingly, the 2FI model was chosen to represent the effect of process factors on UCOPF synthesis. Tables 3 and 4 also show the coefficients of determination (R^2) of the model which were considered high when the value was higher than 95%. The adjusted R^2 and predicted R^2 were in acceptable range. The strength of the model was supported from the lack of fit value. For both responses, the *p*-value of lack of fit was insignificant, thus indicating that the model was valid for optimisation.

For optimisation, the first step was to ensure the data of the experiment were fit to be further explored. This could be evaluated by judging the R^2 and lack-of-fit values. The R^2 is defined as the ratio

Table 2. Experimental design with a response of independent variables using response surface methodology (RSM).

Run	Factor			Response	
	CaO (%)	iTemp. (°C)	H_2O (%)	Solidification Score	Free fatty acid (%)
1	20	70	20	3	1.28
2	3	70	20	1	1.66
3	30	60	30	6	0.77
4	30	60	10	3	1.31
5	20	70	20	3	1.28
6	10	80	30	4	1.02
7	20	70	20	3	1.28
8	20	87	20	4	1.02
9	30	80	30	6	0.67
10	20	70	3	2	1.54
11	20	70	20	2	1.41
12	20	70	37	5	0.90
13	20	53	20	2	1.41
14	10	60	10	1	1.66
15	10	60	30	1	1.54
16	30	80	10	2	1.41
17	37	70	20	6	0.77
18	10	80	10	2	1.54
19	20	70	20	3	1.28
20	20	70	20	3	1.28

Table 3. Regression coefficients, coefficient of determination (R^2), and the *F*-test value of the predicted second order polynomial models for solidification score of used cooking oil protected fats (UCOPF).

Source	Sum of square	DF	Mean square	*F*-value	*p*-value
Model	46.98	6	7.83	36.06	< 0.0001*
A-CaO	22.19	1	22.19	102.20	< 0.0001*
B-iTemp.	2.97	1	2.97	13.66	0.0027*
C-H_2O	14.44	1	14.44	66.52	< 0.0001*
AB	3.13	1	3.13	14.39	0.0022*
AC	3.13	1	3.13	14.39	0.002*
BC	1.13	1	1.13	5.18	0.0404*
Residual	2.82	13	0.2171		
Lack of fit	1.99	8	0.2487	1.49	0.3425
Pure error	0.8333	5	0.1667		
Cor. total	49.80	19			
Std. dev.	0.4660		R^2	0.9433	
Mean	3.10		Adjusted R^2	0.9172	
C.V. %	15.03		Predicted R^2	0.8599	
			Adeq. precision	20.0893	

*Significant at $p < 0.05$.

Table 4. Regression coefficients, coefficient of determination (R^2), and the *F*-test value of the predicted second order polynomial models for free fatty acid content in used cooking oil protected fats (UCOPF).

Source	Sum of square	DF	Mean square	*F*-value	*p*-value
Model	1.65	6	0.2719	51.89	< 0.0001*
A-CaO	0.7086	1	0.7086	135.22	< 0.0001*
B-iTemp.	0.1211	1	0.1211	23.10	0.0003*
C-H_2O	0.6574	1	0.6574	125.45	< 0.0001*
AB	0.0512	1	0.0512	9.77	0.0080*
AC	0.0512	1	0.0512	9.77	0.0080*
BC	0.0420	1	0.0420	8.02	0.0141*
Residual	0.0681	13	0.0052		
Lack of fit	0.0545	8	0.0068	2.49	0.1645
Pure error	0.0137	5	0.0027		
Cor. total	1.70	19			
Std. dev.	0.0.724		R^2	0.9599	
Mean	1.25		Adjusted R^2	0.9414	
C.V. %	5.79		Predicted R^2	0.8803	
			Adeq. precision	22.2806	

*Significant at $p < 0.05$.

of explained variation to the total variation. The better the empirical model fits the actual data, the closer the R^2 values are to unity (Ishak *et al*., 2020). In the present work, results showed that the model was valid and could be used for optimisation, and to study the effect of the variables individually and their interaction of the response of UCOPF.

The proposed method in producing PF was by using infuse saponification process. The formation of calcium salt involves chemical reaction in the fusion process as shown in Eq. 3:

$$2RCOOH + CaO \rightarrow Ca(RCOO)2 + H2O$$
Fatty acid + calcium oxide ➔ calcium salt + water (Eq. 3)

Effect of studied variables on solidification score of PF

In the present work, solidification score was used to determine the physical condition of the produced PF. Based on the present model, the studied variables as shown in Table 3 had an effect on the solidification scores of UCOPF. A lower *p*-value supported the statistical significance of the created 2FI model (Xiangli *et al*., 2008). In this case, a high R^2 of 0.9433 indicated a stronger link between observed and predicted response values. The values of the adjusted R^2 was 0.9170, thus indicating that the created RSM model explained 91.7% of the variations in solidification score when CaO/iTemp./H_2O conditions were used. Eq. 4 illustrates the RSM model for the UCOPF solidification scores:

$$\text{Solidification scores} = 3.1000 + 1.2700A + 0.4660B + 1.0300C - 0.6250AB + 0.6250AC + 0.3750BC \quad \text{(Eq. 4)}$$

where A, B, and C = linear expressions; AB, BC, and AC = interaction; A = CaO%; B = iTemp.; and C = H_2O%.

The residual and pure errors between the experimental plot and the repeated experimental design data can be assessed using a lack of fit (LOF) analysis. A large *p*-value indicates that the LOF is insignificant. Therefore, the model was fit to be explored for optimisation. In the present work, the LOF values for the solidification score of the produced UCOPF was 0.3425. The insignificant value of LOF demonstrated that the proposed model had a good fit with the experimental data, and that the independent variables or parameters had a substantial effect on the response.

The advantages of RSM are the effect of variables on the response can be evaluated individually or by interaction from three-dimensional (3-D) image. In the present work, the interactions between independent and dependent variables affecting the solidification scores and FFA content of UCOPF are shown in Figure 1. Figure 1a shows the interaction for solidification scores which implied that CaO and iTemp. interaction at constant percentage of H_2O had a proportional effect on saponification. Figure 1b and Figure 1c show similar behaviour in terms of the interaction pattern between CaO with percentage of H_2O, and iTemp. with percentage of H_2O on the solidification scores of UCOPF, respectively. The interactions between CaO and iTemp., and between CaO and percentage of H_2O, had significant effect on the solidification score with 2 scale increment.

The amount of CaO required as the primary ingredient is critical, and contributes greatly to saponification. It was demonstrated in the present work that the employed CaO concentration had a highly substantial impact on the solidification score of UCOPF. The effect of CaO increased the solidification score of UCOPF in combination with iTemp. The highest solidification scores were achieved at the highest CaO at all iTemp. range. The interaction of CaO with percentage of H_2O also showed significant increment on solidification scores. However, the trend was more prominent when percentage of H_2O was high.

A low level of CaO content may be unable to entirely convert oil to solids. However, too high CaO is not good since excess calcium in the product will disturb the absorption of other trace minerals such as zinc in ruminants (NRC, 2001). Therefore, the produced UCOPF with moderate amount of CaO can be accepted for ruminant supplement since the highest used concentration in this model was 37%.

In the present work, the used water range was between 3 and 37%. Results showed that the solidification scores increased with percentages of water. Water can be added as a reaction catalyst to start the saponification between UCO and CaO. Generally, the fusion process can be accomplished under a high temperature of steam pressure at about 150 - 200°C for around 3 - 5 hours (Scott *et al*., 1974). In the present work, the addition of water as a catalyst accelerated the reaction at low temperatures. This

observation was in alignment with Handojo *et al*. (2019b). The authors suggested that water should be added in the range of 15 to 25% of the weight of oil samples for saponification. Therefore, similar percentage of water was used in the present work, and had significant effect on solidification scores of UCOPF.

Similar to other variables, there was significant effect of iTemp. on the solidification scores by the reaction mixture as shown in Table 2 and Figure 1. Results also showed that the solidification scores of UCOPF increased with iTemp. The trend was prominent when iTemp. positively interacted with CaO as shown in Figure 1a. However, iTemp. did not have strong interaction with percentage of H_2O when the solidification score of UCOPF increased with iTemp. at all studied percentages of H_2O (Figure 1c).

Initial temperature is important for chemical reaction. Since saponification is an exothermic reaction, heating the mixture may speed it up. That could explain the present observation when iTemp. significantly affected the solidification scores. This observation was in alignment with Handojo *et al*. (2018b), in which the authors mentioned that temperature as low as 60°C was enough to initiate saponification.

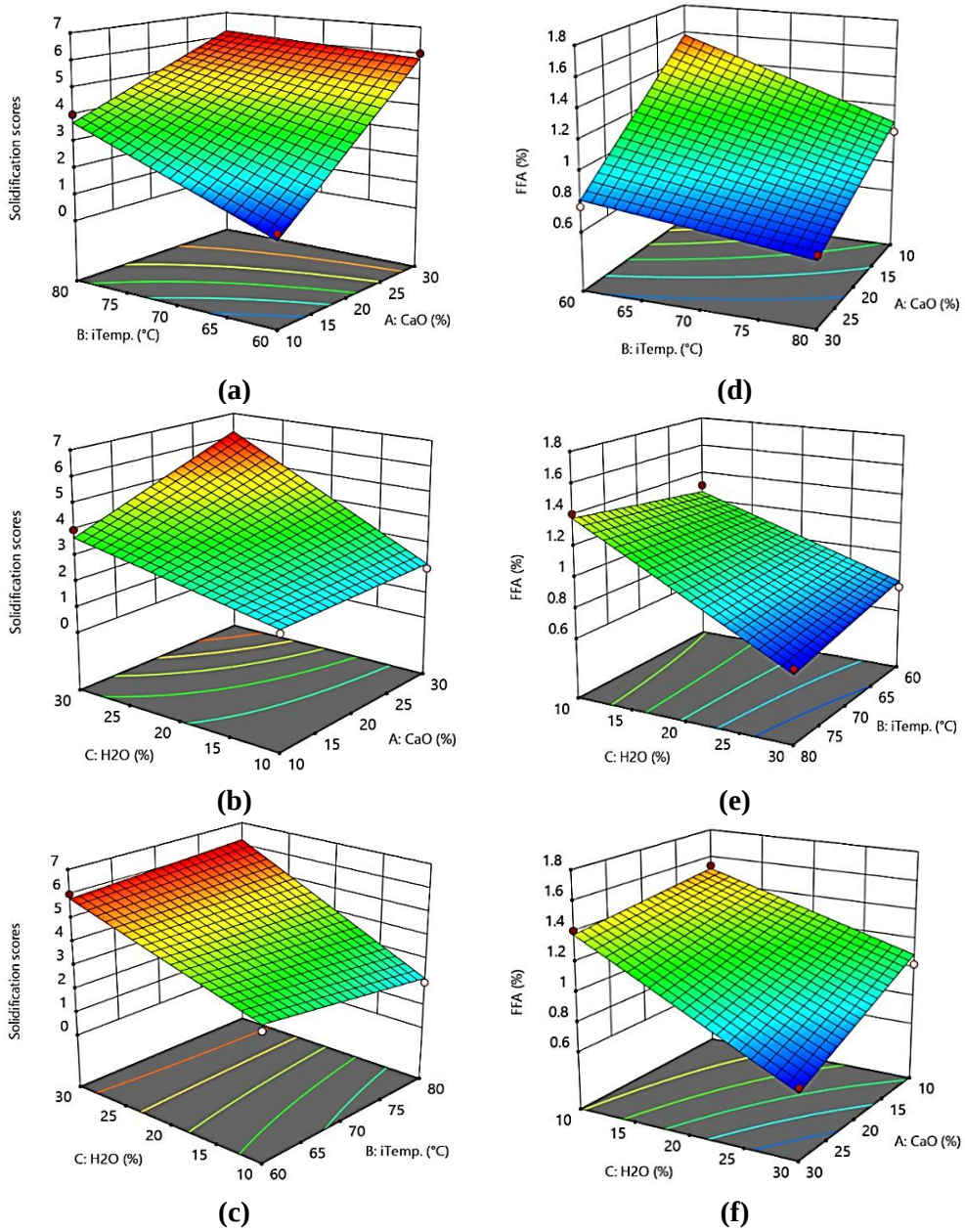

Figure 1. Three-dimensional response surface plots for the interaction of studied variables on solidification score **(a - c)**, and free fatty acid content **(d - f)** of used cooking oil protected fat (UCOPF).

Solidification is one of the important scores for PF. Solid PF is more stable and easy for handling when compared with liquid PF. Besides that, the solidification score can be used to evaluate the completeness of the saponification process (Handojo *et al*., 2019a). The solid state of final PF means that all liquid lipids have been converted into PF. Lipid in the form of oil usually contains unsaturated FA (Suksombat, 2009). The solidification of oil happens due to the ionic bond between FA and calcium (Gönen *et al*., 2010). Therefore, in the present work, the solidification score indicated that the FA and FFA in UCO were combined with calcium during saponification, and formed a solid PF. The unsolidified FA when combined with calcium will be not considered as PF since the unsaturated FFA without binding to calcium will be fermented in ruminant's rumen. This condition is harmful to the ruminants (Naik *et al*., 2010).

Effect of process variables on free fatty acid of UCOPF

In the present work, the effect of saponification variables on the FFA content of UCOPF was measured as shown in Table 4. FFA can be used to determine the suitability of the PF to be used as ruminant supplement. The ANOVA of the data showed that the selected variables had significant effect on the FFA content of the produced UCOPF. The developed model had a high *F*-value (51.89) and significant *p*-value, thus indicating that the selected variables significantly affected the FFA content in UCOPF. Besides that, the validity of the model could also be evaluated from R^2.

In the present work, high R^2 value of 0.9599 showed a strong correlation between observed and predicted values. The value of the adjusted coefficients of determination (Adj. R^2) was 0.9414, thus indicating that 94.1% of total variations for FFA in UCOPF could be explained by the studied variables. In addition, the adequate precision of the model was 22.28, thus demonstrating that the developed model was significant. Similarly, the adequate precision for solidification score was 20.08 as shown in Table 3, and was considered high. According to Asghar *et al*. (2014), the adequate precision more than 4 shows that the model is strong to be used for optimisation. Therefore, for both responses, the developed model had a strong characteristic to be used for optimisation. Besides that, the acceptance of the model depends on LOF values.

The LOF test can be used to finalise the validation of the experimental CCD model. In the present work, LOF for FA content was insignificant with the *p*-value of 0.1645. This indicated that the LOF was in proportion to the pure error. Therefore, the recommended model using second order polynomials was found to be compatible with the experimental data. The selected variables had significant effect on the FFA content in PF. The predictive model equation for FFA content is shown in Eq. 5:

$$FFA\% = 1.2500 - 0.2278A - 0.0942B - 0.2194C + 0.0800AB - 0.0800AC - 0.0725BC \quad \text{(Eq. 5)}$$

where, A, B, and C = linear expressions; AB, BC, and AC = interaction; A = CaO%; B = iTemp.; and C = H_2O%.

Results also showed that there were interactions of the studied variables on FFA content of UCOPF as shown in response surface plot (Figures 1d - 1f). Figures 1d and 1e showed that the iTemp. had the interaction with percentage of CaO and H_2O, respectively. The FFA content in UCOPF decreased with iTemp. This could be explained by the fact that the tested temperature range was unable to degrade the lipids since the maximum was only at 87°C. The selected temperature was too low to degrade the oil and produce FFA. Palm olein is heat-stable due to high percentage of saturated FA. Handojo *et al*. (2019b) reported the temperature above 60°C is required to degrade the triacyglceride, and produce FFA in lipid sources.

Similar trend was observed on the effect of CaO on FFA content in UCOPF as shown in Figures 1d and 1f. Results showed that the FFA content in UCOPF decreased with concentration of CaO. There was an interaction between CaO with iTemp. and percentage of H_2O on the FFA content of UCOPF. This can be explained by the fact that water is required to react with CaO to from $Ca(OH)_2$. The energy released from the reaction boosts the combination of FA and calcium ion (Galván-Ruiz *et al*., 2009). Similar observations have been reported where CaO reduced FFA in palm FA distillated PF (Handojo *et al*., 2019c).

Water is one of the components required for saponification. Results showed that the FFA in UCOPF decreased with amount of water as shown in Figures 1e and 1f. The effect was more prominent when percentage of water interacted with CaO as compared to the interaction with iTemp. This could have been due to the fact that water was required as catalyst for the reaction to occur rapidly at low temperature, and FFA to be bound with calcium ion (Pablos Pérez, 2008). However, inadequate amount of water will not complete the saponification reaction, and the addition of excess water will disturb the saponification reaction (Handojo *et al*., 2019a). In this experiment, 25 to 30% of water was required to provide appropriate amount for the reaction catalyst, and lower FFA indicated higher conversion intensity. This finding was also in alignment with a report by Handojo *et al*. (2019a).

The FFA is among the metrics used to determine the amount of the saponification process, and to reflect the proportion of conversion reaction that has taken place. The lower the FFA% of calcium soap products the greater the proportion of unsaturated FA that have been synthesised for PF (Handojo *et al*., 2019c). The level of FFA of the calcium salts for animal feed should be as low as possible (Handojo *et al*., 2019a). The rancidity status of the product can be determined from the FFA content. Based on the standard prescribed by the Nutrient Requirement of Dairy Cattle (NRC), the permitted FFA content in PF as ruminant supplement is 1%. Higher value of FFA may cause the PF to have short shelf life (Handojo *et al*., 2018b), and disturb the fibre digestion in the rumen of ruminants (Jenkins and Harvatine, 2014).

Calcium is required by ruminants as microcomponent. It helps in skeletal tissue, muscular contraction, nerve impulse transmission, and blood clotting (Liu *et al*., 2020). Insufficient calcium balance early in lactation leads to additional metabolic disorders and milk decrease that cannot be recovered afterwards (Jenkins and Harvatine, 2014). However, when the animals are fed with excessive calcium content, the absorption of other minerals such as zinc and phosphorus might be hindered (Jenkins and Palmquist, 1984). The proportion of calcium to phosphorus is one of the variables that influence the effectiveness of phosphorus uptake. Based on previous findings, cattle should get less than 50.7 g calcium daily (NRC, 2001). Too much calcium can block the absorption of phosphorus, in particular when the ratio of calcium to phosphorus is greater than 1.75 (Handojo *et al*., 2018a). Therefore, the calcium oxide to UCO ratio in calcium salt production should not be too high. Selection of low or moderate amount of calcium should be considered during optimisation.

Optimised process of parameters for production of UCOPF

The advantage of using RSM is that the optimisation of independent variables can be chosen from the developed model. In the present work, the assessment of the experimental findings was carried out to optimise the production of UCOPF within the ranges of studied variables. The best condition to produce UCOPF was at CaO (20%), iTemp. (80°C), and percentage of H_2O (30%) with predicted solidification score and FFA content of 4.94 and 0.86%, respectively, as shown in Table 5. Based on the present results, the solidification score and FFA content of optimised PF were 5.33 ± 0.58 and 0.85 ± 0.07%, respectively. This showed insignificant difference with predicted values. This in turn suggested that the experimental and predicted findings were in excellent agreement for prediction of optimum fusion conditions. Hence, the generated RSM 2FI model could be used to effectively optimise the synthesis of PF from UCO. At this optimum condition, the FFA% was considered within the safe range of animal feed supplements (Handojo *et al*., 2018a). Previous study also showed similar parameters to produce PF. For example, Naik *et al*. (2007) reported the use of CaO at 25% to produce PF from palm oil, rice bran oil, and mixed oil.

Table 5. Experimental data of the validation of predicted values at optimal parameters for production of used cooking oil protected fat (UCOPF).

Factor	Response	
	Solidification score	**Free fatty acid (%)**
Predicted response	4.94	0.86
Experimental value*	5.33 ± 0.58**	0.85 ± 0.07**

*Values are from three independent experiments. The optimised condition to produce protected fat from used cooking oil was at CaO (20%), iTemp. (80°C), and percentage of H_2O (30%). **No significant difference as analysed using Student's *t*-test at $p > 0.05$ as compared to predicted response.

Physicochemical characteristics and fatty acid composition of used cooking oil (UCO) and optimised used cooking oil protected fat (UCOPF)

In the present work, the physicochemical characteristics and FA composition of used cooking oil (UCO) and optimised used cooking oil protected fat (UCOPF) were measured as shown in Table 6. This measurement was conducted to ensure the authenticity of sample and safety of the produced PF. There was significant characteristic on physicochemical properties of UCO and UCOPF in term of colour, appearance, odour, crude fat, moisture content, ash content, FFA, iodine value, and gross energy where the UCOPF showed better quality attributes when compared with UCO. However, relative comparison for FA between UCO and UCOPF was not significantly different with saturated FA was the highest followed with palmitic acid, monounsaturated FA, and oleic acid. Polyunsaturated FA, linoleic acid, lauric acid, myristic acid, and stearic acid occurred lower than 10% in both samples.

Palmitic acid is predominant FA in palm olein (Maclellan, 1983). Analysis of FA of UCO showed that palmitic acid was the highest in the sample followed by oleic acid. Results also showed balance between saturated and unsaturated FA as it is the common physicochemical for palm olein (Gee, 2007). The ash content of UCOPF suggested the level of calcium salts to be 24.11% since the only mineral added in the saponification process originated from CaO.

In the present work, RSM was utilised to develop the model for optimisation of saponification to convert UCO into valuable PF. The RSM has a variety of advantages over conventional approaches, including a reduction in the number of experiments, and optimisation of condition gained from experimental data, as well as time and resource savings. The CCD provides two distinct configurations, namely central points and axial points. Typically, the experimental set's central points were replicated to maintain the experiment's precision. Moreover, central points fit with the axial points above and below of two factorial levels (Razali *et al*., 2013).

Table 6. Physicochemical characteristics and fatty acid composition of used cooking oil (UCO) and optimised used cooking oil protected fat (UCOPF).

Characteristic	Parameter	UCO	UCOPF
Physico-chemical	Colour	Dark yellow	Dark yellow
	Appearance	Liquid	Flake
	Odour	Unpleasant	Pleasant
	Crude fat (%)	97.69 ± 1.03	72.63 ± 1.00**
	Moisture content (%)	0.50 ± 0.15	3.05 ± 0.26**
	Ash (%)	ND	24.11 ± 0.61
	FFAs (as palmitic acid) (%)	1.45 ± 0.09	0.85 ± 0.85**
Fatty acids (%)*	C12; lauric acid	2.96 ± 0.02	2.81 ± 0.09
	C14; myristic acid	2.33 ± 0.06	2.30 ± 0.12
	C16; palmitic acid	44.21 ± 0.17	44.27 ± 0.20
	C18; stearic acid	1.11 ± 0.06	1.63 ± 0.41
	C18:1; oleic acid	39.38 ± 0.20	39.46 ± 0.22
	C18:2; linoleic acid	8.25 ± 0.03	8.21 ± 0.05
	SFA	50.61 ± 0.29	51.00 ± 0.77
	MUFA	39.38 ± 0.20	39.46 ± 0.22
	PUFA	8.25 ± 0.03	8.21 ± 0.05

Values are mean ± standard error. *Relative composition of fatty acid. The optimised condition to produce protected fat from used cooking oil was at CaO (20%), iTemp. (80°C), and percentage of H_2O (30%). **Means in the same row were significantly different at $p < 0.05$. UCO = used cooking oil; UCOPF = used cooking oil protected fat. ND = not detected. SFA = saturated fatty acid, MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid.

The concentration of CaO, the iTemp. of mixing, and volume of H_2O needed were optimised to produce PF with high solidification scores and low FFA. It was found that the solidification scores increased for the production of UCOPF upon the addition of CaO with lower FFA% content. Also, depending on the amount of water in the saponification process, water can act as a promoter or retarder of the saponification process. Consequently, CaO concentration and H_2O volume had a positive effect on the saponification process (solidification scores and FFA%), but this was highly dependent on the amount of CaO in the prepared formulations. The best saponification factor was finally proposed for the formulation of PF using UCO by using the contour plots of response surface analysis. The solidification score indicated the degree of fusion completeness of a PF from UCO. Meanwhile the low FFA (%) indicated the product's safety. The experimental responses (solidification scores and FFA%) were utilised to optimise the independent variables.

Conclusion

The present work found that the optimum CaO concentration, initial temperature, and percentage of water to produce PF from UCO by fusion method were 20%, 80°C, and 30%, respectively. The optimised UCOPF for solidification scores and FFA% were 5.33 ± 0.58 and 0.85 ± 0.07%, respectively. The produced UCOPF was safe for use as animal feed, and easy to handle since it existed in solid form. For future improvement, *in vitro* analysis of UCOPF should be carried out prior to commercialisation, in particular on bioavailability and its function as PF.

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Patent

A part of the data has been patented in Malaysia with patent number PI 2022004648.

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