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CRYSTALLIZATION TEMPERATURE AND SOLVENT COMPOSITION EFFECT FOR ENHANCEMENT OF UNSATURATED FATTY ACIDS FROM PALM OIL USING UREA COMPLEXATION

Dwi Ardiana Setyawardhani*, Imam Fadhil Ihsani, Sari Ayu Dwi Lestari, Haura Rana Zhafirah, Viana Silvia, Fadilah, Y.C. Danarto

Chemical Engineering Department, Faculty of Engineering, Sebelas Maret University, Jl. Ir. Sutami 36 A, Surakarta, Indonesia

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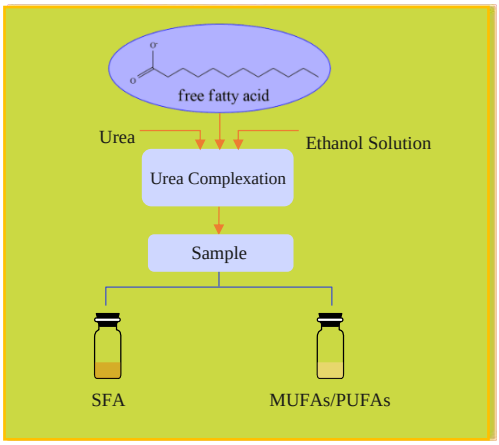
*Corresponding author
dwiardiana@staff.uns.ac.id

Graphical abstract



Abstract

Monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are essential nutritional sources for infants, adults, and elderly. Monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) can be enhanced in vegetable oils or animal fats using several techniques, but the urea complexation is one of the most effective ways. Subsequently, urea was added to form a complex chemical in the form of urea complexed fraction (UCF), leaving the non-urea complexed fraction (NUCF). The fatty acid composition of the UCF and NUCF were then examined using gas chromatography. This study determined the effects of various crystallization temperatures (-4°C, 8°C, 12°C, 22°C, and 30°C) and ethanol concentrations (varying from 0% to 100%) to the yield and fatty acids compositions of UCF and NUCF. They were developed in the predictive equations which are crucial for the design of separation processes in the chemical industry. According to this study, raising the crystallization temperature increased the yield in the NUCF but decreased the concentration of PUFAs and MUFAs. At 30°C, the production of NUCF was 28.79% with a MUFA/PUFA concentration of only 16.51 percent. NUCF achieved the highest MUFAs/PUFAs concentration (89.1%) at the lowest temperature (-4oC). On the other hand, a higher ethanol concentration increased the concentration of MUFAs and PUFAs while decreasing the NUCF production. Pure water produced a 28% yield but only 56.1% MUFAs/PUFAs, whereas pure ethanol produced a 10.3% yield with 94.2% MUFAs/PUFAs. Predictive equations to estimate MUFAs/PUFAs concentration and yield also have been conducted.

Keywords: Crystallization temperature, fatty acids, MUFAs/PUFAs, palm oil, urea complexation

1.0 INTRODUCTION

Indonesia and Malaysia are the largest palm oil producers in the world [1]. The potential of palm oil as an oleochemical material is very diverse, both in the food, renewable energy, pharmaceutical and other chemical industries. One of the potential contents of palm oil is fatty acid, which consists of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs). Unsaturated fatty acids are divided into Mono-unsaturated and Poly-unsaturated fatty acids (MUFAs/PUFAs). Saturated and unsaturated fatty acids each have their own functions and characteristics [2]. As a biodiesel feedstock, SFAs are considered more

profitable due to their oxidation stability. Meanwhile, as an important nutrient for health, MUFAs/PUFAs have more benefits to babies' brain development, curative and/or preventive on cancers and also preventing cardiovascular and other degenerative diseases [3], [4], [5]. Appropriate PUFAs combinations have also been evident to prevent hyperinsulinemia in obese mice [6]. The MUFAs/PUFAs intake as concentrate is more needed than consumed whole oil [2], [7], [8], [9].

Separation of saturated and unsaturated fatty acids can be carried out using various methods, such as solvent extraction, crystallization, distillation, supercritical extraction, enzymatic reactions, chromatography, membrane filtration and urea complexation [10]. In general, urea complexation is the most efficient and viable method to be developed on a commercial scale, due to the moderate processing conditions, environmentally friendly, and also inexpensive renewable materials [11]. To be compared with solvent crystallization, urea complexation is more efficient in energy consumption because it does not require too low temperatures. This is because the crystals which are formed are stable, even at room temperature [12]. Meanwhile, extraction with supercritical fluids as solvents requires high temperatures and pressures which can degrade PUFA. Furthermore, other processes such as membrane filtration, chromatography, and enzymatic reactions require high costs but lower yields, so they are not economical on a large scale. The complexation of urea also provides additional benefits, because saturated fatty acids as a by-product in the UCF fraction can also be recovered and used as raw materials for non-food products, such as biodiesel, surfactants, lubricants and other needs.

Inclusion compounds consist of 2 components which are bonded to each other through hydrogen and Van der Waals bonds [13]. In this bond, the term "host" is commonly known for the crystal molecule, and "guest" is known molecules that enter/bind into the crystal. There are 3 types of inclusion compounds, respectively with the form of the host network, 1) Channel (ex. Urea Inclusion Compounds (UICs), 2) Cage/chlatrate (ex. water (gas hydrate), and 3) Layer/sandwich (ex. clay). Pure urea crystals are tetragonal in shape. When forming inclusion compounds (UICs), the structure turns into hexagonal with the urea molecules interlocking bound to each other by hydrogen bonds in a spiral/helix structure as in DNA chain, which leaves a cavity in the middle in the form of a channel 5.2-5.5 Å in diameter. This cavity is filled with guest molecules in the form of straight-chain compounds with a diameter of less than 5.2 Å [14]. Several previous researches determined that urea complexation was analogous to SFAs adsorption into urea molecules [15], [16]

Urea complexation is affected by several important factors, such as the degree of fatty acid unsaturation, the ratio of urea and fatty acids mixtures, crystallization temperature, type and composition of solvent and the purity of the complexed fatty acids. Separation of unsaturated fatty acids from palm oil has been carried out in previous studies, especially those studying the effect of the urea/fatty acid ratio [17], [18]. Polyunsaturated fatty acids such as ARA (Arachidonic acid), EPA (Eicosapentaenoic Acid) and DHA (Docosahexaenoic acid) were successfully obtained in high concentrates from several fish and microalgae [19], [20]. The effect of crystallization temperature of urea complexation has been studied for corn oil [12], rice bran oil [21], and also for salmon fish oil [22], but no research has been applied to refined palm oil.

The hexagonal geometric structure of UICs is formed due to hydrogen bonds between the nitrogen atom of a urea molecule and the oxygen atom of another urea molecule. The arrangement is such that each side of the hexagonal structure contains 6 urea molecules. Urea complexation requires a straight long-chain carbon ring compound as a guest molecule, at least 6-8 atoms of carbon, because each oxygen atom in the urea molecule will bond to a van der Waals bond towards one methylene group in the alkyl chain [23].

Selective and proper solvents for wetting agents are important for urea complexation [24]. It should make both the complexed compounds completely soluble but does not form inclusion compounds (cannot act as a guest molecule). For the mutual solubility of all components, a less polar solvent is needed. The solvents used in urea complexation generally are short-chain compounds (less than 6 C atoms) like water or short-chain alcohols (methanol or ethanol). The appropriate solvent must be able to dissolve both urea and fatty acids, inexpensive and recoverable. The solubility of urea and saturated fatty acids in short-chain alcohols is also determined by the composition of the solvent. Urea is highly soluble in water but less soluble in short-chain alcohols. Urea solubility decreases with increasing methylenes group in the alcohol. A small impurity of water to ethanol increases the urea solubility significantly [11]. Saturated fatty acids such as Palmitic acid (PA) and stearic acids (SA) solubilities in aqueous alcohols are also dependent on the concentration. High concentration of ethanol and methanol increases fatty acids solubilities [25]. Urea is a highly polar compound whereas long chain fatty acids are slightly polar. It is necessary to apply proper composition which can dissolve both urea and fatty acids without being reacted.

There was no previous research that observed solvent composition effect on the fatty acids composition. This research studied the effect of crystallization temperature and ethanol-water composition as solvent on the separation of unsaturated fatty acids from a mixture of palm oil fatty acids. It also conducted predictive equations to determine the yield and fatty acids composition in the non-urea complexed fraction (NUCF). These data could be recommended for separation process design in chemical industries.

2.0 METHODOLOGY

2.1 Materials

Palm cooking oil from Surakarta's (Indonesia) local market was used as raw material for urea complexation. Analytical grade urea (99.9%) was purchased from Millipore Sigma, and pro-analyzed grade ethanol from Merck was employed as a wetting agent. Supporting materials for the hydrolysis and purification process are potassium hydroxide (85%), sodium sulphate (99.9%), and hydrogen chloride (37%), all of are from Merck. Technical grade n-hexane was used for free fatty acids solvent extraction.

2.2. Fatty Acids Preparation

Fatty acids preparation developed by palm oil hydrolysis reaction according to Wanasundara and Shahidi methods [26] Process flow diagram for this research is shown in Figure 1. Palm oil was reacted with ethanolic potassium hydroxide at 60°C for an hour of saponification. N-hexane was then added to the reaction mixture to extract the unsaponifiable matter and discharge it. The aqueous layer was mixed with 6 N HCl solution until pH 1. The fatty acids were released and re-extracted using n-hexane. The remaining water was then adsorbed using Na_2SO_4. N-hexane was evaporated to obtain pure fatty acid mixtures.

2.3 Urea Complexation

The urea complexation process was conducted at various temperatures, while FFA was mixed with urea and 96% aqueous ethanol, stirring until the mixture formed a homogeneous solution. The urea-FFA inclusion was crystallized at various temperatures (-4 – 30°C). The solid phase (needled-form crystalline) was the urea complexed fraction (UCF), and the remaining solution was the non-urea complexed fraction (NUCF), which is rich in PUFAs. The solid and liquid phases were separated using centrifugation and filtered using a Buchner funnel.

The liquid phase (NUCF) was diluted with water in an equal volume and acidified with HCl to reach pH 4-5. To extract the fatty acids, n-hexane in an equal volume was then added to the mixture with stirring. The mixture was poured into a separation funnel until it formed two layers. The top layer (hexane fraction) contained fatty acids, and the lower layer contained urea. The hexane layer was washed with distilled water to remove any remaining urea and then dried with anhydrous sodium sulphate. The rotary evaporator then needed to evaporate n-hexane from the fatty acids concentrate at room temperature. A similar procedure was developed for the various ethanol composition (0-100%), which was set at room (30°C) crystallization temperature.

The experimental design of this research was designed to study crystallization temperature and solvent composition effects on the fatty acid concentration. The urea: FFA ratio was set at 7:40 w/w according to the optimum condition resulting from our previous research [17], as well as the ethanol volume was set at 400 mL due to urea: solvent ratio was 1:5 w/w. Crystallization temperatures were set from -4 to 30°C to observe below melting point until room temperature. Too low temperature was not suitable for the complexation, due to the solidification of the solvent and complex compound, thereby the complication performance and the content of MUFAs/PUFAs in the product would be reduced [27]. Meanwhile, they were also determined according to the apparatus's capabilities. Solvent compositions were observed in wide range of 0 to 100% ethanol (w/w) to examine all concentrations. Both parameters were evaluated in 5 variations. The crystallization temperature effect was studied under constant solvent composition and urea : FFA ratio, whereas the solvent composition effect was observed under constant urea/FFA ratio and crystallization temperature. The experimental conditions are listed in Table 1.

Table 1 Operating conditions and process variables

Urea:FFA ratio (w/w)	Ethanol volume (mL)	Ethanol: water composition (% w/w)	Crystallization temperature
7 : 40	400	96	-4
7 : 40	400	96	8
7 : 40	400	96	12
7 : 40	400	96	22
7 : 40	400	96	30
7 : 40	400	0	30
7 : 40	400	30	30
7 : 40	400	50	30
7 : 40	400	70	30
7 : 40	400	100	30

2.4 Fatty Acid Analysis

The fatty acid composition was analyzed on the GCMS-QP2010S SHIMADZU equipped with EI 70 Ev ionization detector and a BD 5 MS column (30 m length; 0.25 mm ID and 0.2 µm film thickness). Column flow was 0.5 mL/min. Ultra-high-purity helium was used as the carrier gas at a constant flow rate of 40 mL/min in a split injection mode. The temperatures of the injector and the oven were 300°C and 60°C, respectively. Flow control mode was set at 49.9 kPa in 40.0 mL/min total flow. Identification of fatty acids was based on the retention time of the standards and the proportions were quantified by normalization of the relative area of the chromatogram.

The results were plotted in the FFA percentage diagram versus crystallization temperature and solvent ratio. Linear regression model of various conditions was used to predict the PUFA composition for certain crystallization temperatures and solvent composition.

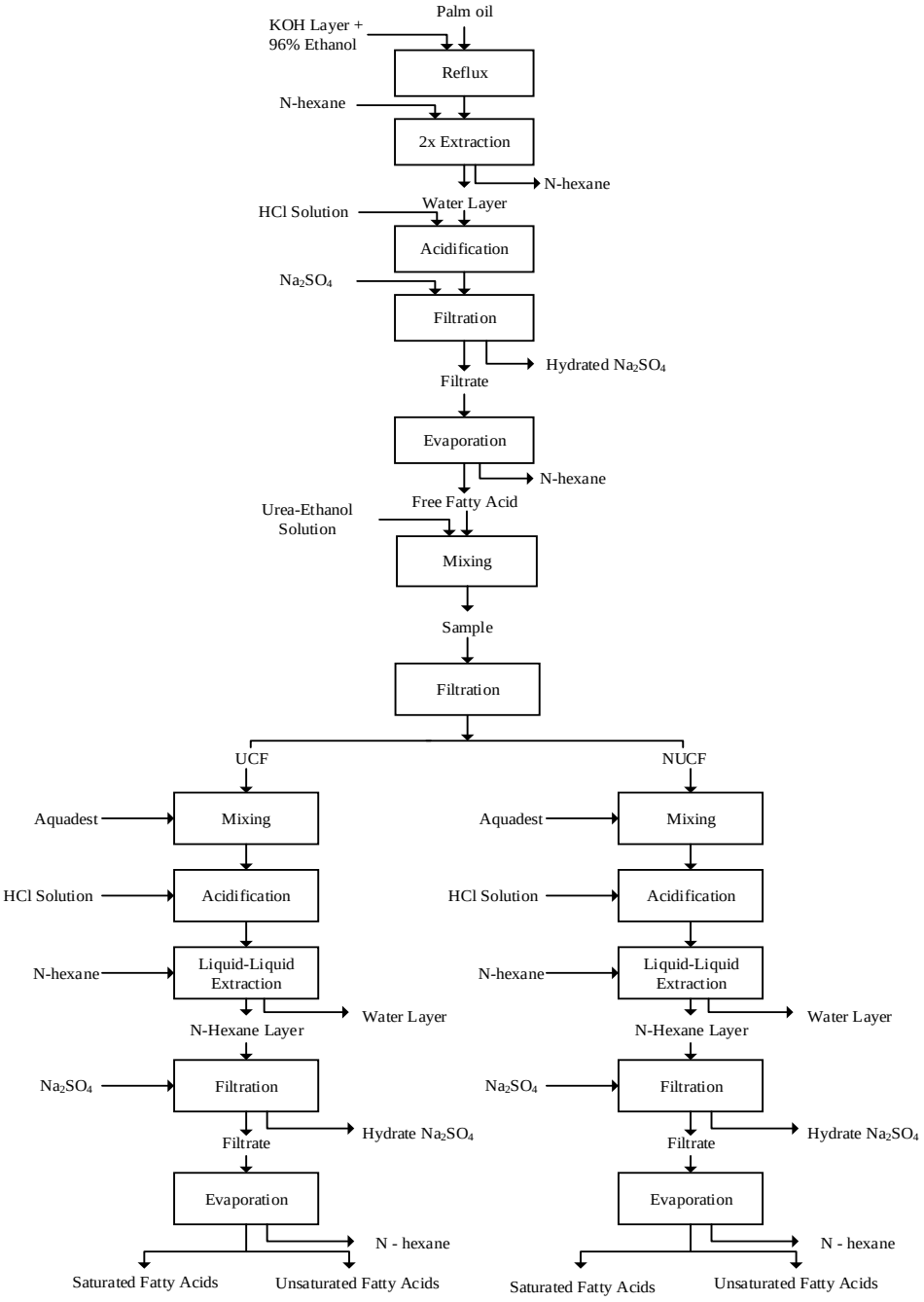


Figure 1 Process flow diagram of urea complexation

3.0 RESULTS AND DISCUSSION

This research studied crystallization temperature and solvent composition effects. The ratio of urea and fatty acids was set at 25.9: 1 moles ratio, which is in accordance with 7: 40 (w/w), by choosing the best conditions from previous research [17], [28]. In this ratio, the number of saturated fatty acid molecules adsorbed in urea crystals is maximum.

Urea complexation generates 2 products : highly concentrated of SAFs in UCF and highly concentrated of MUFAs/PUFAs in NUCF. The UCF which is rich in SFAs preferred to be used as raw materials for good quality biodiesel. Saturated fatty acid methyl esters (SFAME) tend to have high oxidation stability, high cloud point and high cetane number. Whereas the MUFAs/PUFAs concentrated in NUCF tend to be useful for health and pharmaceutical fields. When used as a raw material for biodiesel, it tends to produce biodiesel with favorable low-temperature performance but a lower cetane number.

The fatty acids composition of each fraction was studied in varied crystallization temperatures and solvent (ethanol : water) ratios. The yield of fatty acid

in both fractions was also determined. Based on the data, mathematical equations were developed to show the effect of crystallization temperature and solvent composition on fatty acids content, along with the yield in each fraction. From this equation, fatty acids composition and yield of the concentrates can be determined/predicted.

3.1 Crystallization Temperature Effect

To study the effect of crystallization temperature on the urea complexation of palm oil fatty acids, temperature variations were taken in a range between unsaturated fatty acids melting point to room temperature. This is intended to be able to observe the extent of the influence of cooling on the formation of complex crystals. Theoretically, crystallization can occur by exceeding supersaturated solution conditions, through cooling, evaporation, or the addition of crystal seeds (seeding).

From the results of this research, crystallization temperature greatly influences the PUFA concentration in the liquid (NUCF) fraction. Lower crystallization temperature caused the fatty acids and urea solubility in the solvent (ethanol solution) decrease, making it easier for saturated fatty acid molecules to be included in the urea crystals. On the other hand, higher temperatures increased the solubility of fatty acids and urea in the solvent mixture. MUFAs/PUFAs concentrations increased from 16.51% at room temperature to 89.01% at -4°C in the NUCF. However, in general, urea complexation still took place even at room temperature (30°C). This can be seen in Figure 2.

This experiment's results are in accordance with some previous research developed for corn oil, rice bran oil, and also seal oil. Urea complexation of corn oil resulted in 52.36% PUFAs at -15°C but only 48.74°C at room temperature (30°C) [12]. As well for rice bran oil, PUFAs could increase from 40.36% at higher temperatures (10°C) to 60.6% at lower temperature (-10°C)[21]. The same tendency also resulted from urea complexation for seal oil [29]. PUFAs were enriched from 54.51% at 20°C to 66.83% at 10°C.

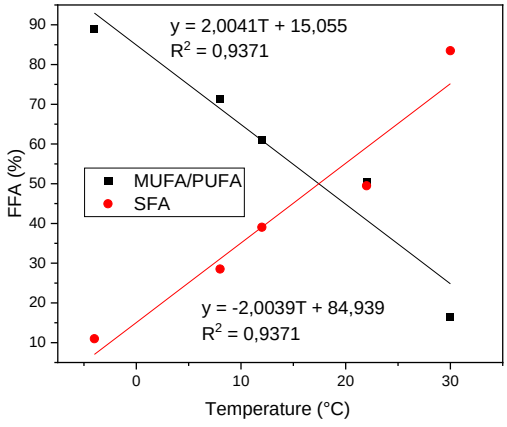


Figure 2 Fatty acids composition of NUCF in various crystallization temperature

Meanwhile, the concentration of MUFAs/PUFAs (in %) and SFAs in NUCF can be predicted using a linear equation according to Figure 2:

$$[UFAs]_{NUCF} = 2.0041\ T + 15.055 \quad (1)$$
$$[SFAs]_{NUCF} = -2.0039\ T + 84.939 \quad (2)$$

T is crystallization temperature in °C.

As seen in Figure 3, at higher temperatures, the MUFAs/PUFAs contents in UCF are still high, because the number of SFA molecules adsorbed in the urea crystals are few. As the temperature decreases, the SFA content in the crystals increases and the MUFAs/PUFAs content decreases.

The approach equation for predicting FFA concentrations in UCF is written in Eq. (3) and Eq. (4):

$$[UFAs]_{UCF} = 0.3605\ T + 76.658 \quad (3)$$
$$[SFAs]_{UCF} = -0.3605\ T + 23.342 \quad (4)$$

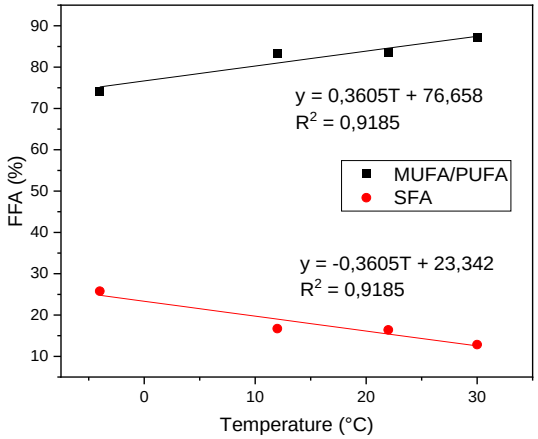


Figure 3 Fatty acids composition of UCF in various crystallization temperatures

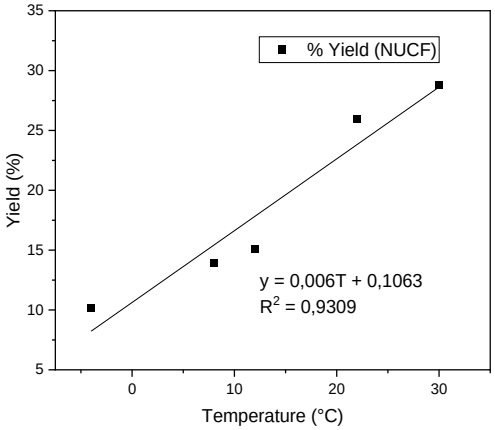


Figure 4 NUCF yield in various crystallization temperatures

MUFAs/PUFAS are known as omega fatty acids. They are essential fatty acids and very important for

human health. Omega fatty acids such as oleic, linoleic and linolenic acids must be in the right combination to consume, to reduce the risk of cardiovascular and degenerative disease efficiently.

The amount of MUFAs/PUFAs concentrate is also influenced by the crystallization temperature. Lower temperature leads to more SFA adsorbed into the urea molecule (UCF), so the MUFAs/PUFAs remaining in the concentrate fraction (NUCF) are higher. Yield is the amount of NUCF compared to the initial fatty acid (w/w), so the lower crystallization temperature provides less NUCF yield (Figure 4). Otherwise, the yield in the UCF got higher.

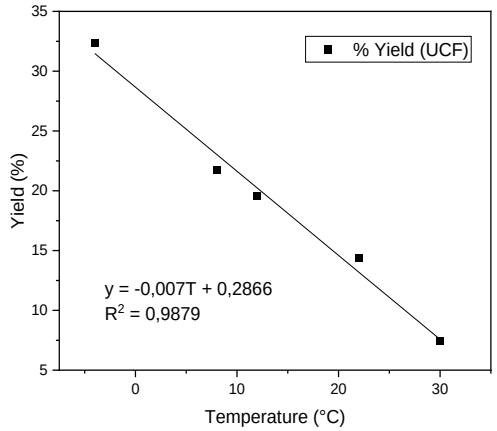


Figure 5 UCF yield in various crystallization temperatures

To estimate the UCF and NUCF yield of fatty acids concentrate which is influenced by crystallization temperature, predicted equations were developed using a linearization approach of the data, as shown in Figure 4 for NUCF and Figure 5 for UCF. This approach is quite accurate as indicated by the R^2 values were 0.93 and 0.98 for the NUCF and UCF, respectively. The amount of yields predicted (in %) as a function of crystallization temperature (in °C) are shown in Eq. (5) and Eq. (6):

$$\text{NUCF Yield} = 0.006\ T + 0.1063 \quad (5)$$

$$\text{UCF Yield} = -0.007\ T + 0.2866 \quad (6)$$

3.2 Solvent Composition Effect

Solvent compositions that were applied in this study ranged between 0% (pure water) to 100% (pure ethanol). As shown in Figure 6, higher ethanol concentration leads to higher MUFAs/PUFAs composition in the NUCF, which means that MUFAs and PUFAs successfully concentrated in liquid fraction, separated from the saturated ones (SFAs). Urea complexation is not only influenced by the solubility of fatty acids in the wetting agent (water, methanol, or ethanol) but also determined by the solubility of the urea crystals. The solubility of fatty acids in alcohol is higher than in water, due to their polarity [11]. Water is more polar than ethanol, while fatty acids are less polar, hence fatty acids dissolve more easily in ethanol than in water. Otherwise, urea is highly soluble in water and less soluble in ethanol. Thus, pure ethanol is better as a wetting agent than water for urea complexation due to the solubility between the solute and the solvent.

Fatty acid composition in NUCF is controlled by the complexation of SFA molecules into the urea crystals. SFA can be included in urea crystals because they have a smaller molecular diameter due to their straight chains. The more SFA molecules are bound to the urea crystal, the higher concentration of unsaturated fatty acids remaining in the liquid phase.

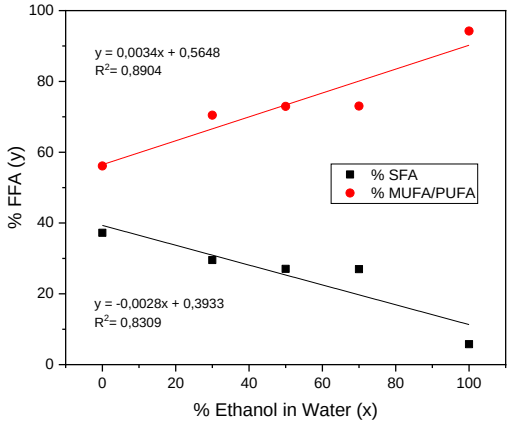


Figure 6 Predicted fatty acids composition in NUCF with various solvent ratio

In general, several previous research used 95% aqueous ethanol to be a wetting agent in the urea complexation process. However, different solvents were utilized for wetting agents in urea complexation such as methanol [18], [24], [30]. It is found difficult to compare these experiment results to previous similar research due to the lack of references.

For predicting the MUFAs/PUFAs content in the liquid fraction (NUCF), a linear approach was applied to develop a quantitative prediction. This is necessary for determining the concentration of MUFAs/PUFAs in unsaturated fatty acid concentrates. From Figure 6 it can be shown that the prediction equation for SFAs and MUFAs/PUFAs content in NUCF is as follows:

$$[\text{UFAs}]_{\text{NUCF}} = 0.0034\ X + 0.5648 \quad (7)$$

$$[\text{SFAs}]_{\text{NUCF}} = -0.0028\ X + 0.3933 \quad (8)$$

X is the % volume of the ethanol solution.

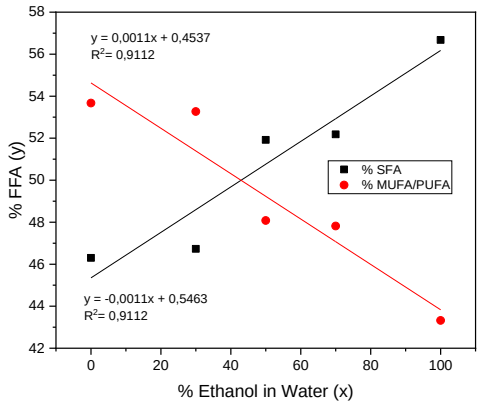


Figure 7 Predicted fatty acids composition in UCF with various solvent ratio

Figure 7 shows the linear approach of the MUFAs/PUFAs and SFAs concentration in the UCF (solid fraction), which are contrary to NUCF data. A high concentration of SFAs is preferred due to the effective inclusion of the saturated fatty acids in the urea crystal, remaining high concentration of MUFAs/PUFAs in the NUCF.

The solvent composition also affected the MUFAs/PUFAs yield in UCF and NUCF. More SFAs adsorbed in urea crystal reduced the UCF and NUCF yield of unsaturated fatty acids. Higher ethanol concentration caused lower yield but higher PUFAs concentration. This is shown in Figure 8.

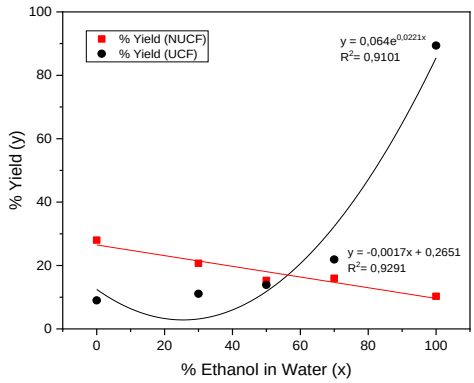


Figure 8 Predicted UCF and NUCF yield in various solvent ratios

To estimate the yield of MUFAs/PUFAs concentrate which is influenced by solvent composition, an exponential approach was developed for UCF yield, and a linear equation for the NUCF yield. These approaches were quite accurate as indicated by the R^2 value of higher than 0.9. Then, the amount of yield (%) can be predicted as a function of solvent composition (X, % volume) as shown in Eq. (9) and Eq. (10):

$$\text{NUCF Yield} = -0.0017\,X + 0.2651 \quad (9)$$

$$\text{UCF Yield} = 0.064\,e^{0.0221\,X} \quad (10)$$

3.3 Fatty Acids Composition

Table 2 summarizes fatty acids (SFAs, MUFAs and PUFAs) concentrations in various crystallization temperatures and ethanol concentrations. The NUCF obtained by urea-complexation was successfully improved %MUFAs/PUFAs from refined palm oil. Compared with the original material, MUFA and PUFA were all significantly purified in the NUCF, indicating that crystallization at −4 °C to 22 °C were appropriate for the enrichment of MUFAs/PUFAs. However, complexation was not well conducted at room temperature due to the slow crystallization under higher temperatures.

Pure ethanol contributed to be an adequate wetting agent due to the high improvement of 50.49% MUFAs/PUFAs to 94.22% after complexation. However, pure water only increased MUFAs/PUFAs up to 56.11%. Fatty acids are insoluble in water but highly soluble in ethanol, while urea is highly soluble in water but slightly soluble in pure ethanol. Urea complexation needs appropriate solvent composition for both urea and fatty acids.

Table 2 Comparison of Fatty Acid Composition in Palm Oil

Pre-Complexation (Refined palm oil [31]**)**		**Post-Complexation**		
% SFA	%MUFAs/PUFAs	NUCF (T,°C)	% SFA	% MUFAs/PUFAs
49.45	50.49	-4	11	89.01
		8	28.53	71.45
		12	39.05	60.95
		22	49.49	50.51
		30	83.49	16.51
		NUCF (% X)	% SFA	% MUFAs/PUFAs
		0	37.25	56.11
		30	29.54	70.46
		50	27.06	72.94
		70	26.98	73.02
		100	5.77	94.22

4.0 CONCLUSION

The separation of fatty acids using urea complexation was greatly influenced by the crystallization temperature and solvent composition. Reducing the temperature resulted in a decrease in the yield of non-urea complexed fraction (NUCF), but an increase in the concentration of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Whereas higher ethanol concentration reduced the NUCF yield but higher MUFAs/PUFAs concentration. The yield of NUCF achieved 28.79% with only 16.51% MUFAs/PUFAs content at 30°C. At lowest temperature (-4°C), NUCF reached the highest MUFAs/PUFAs concentration (89.1%). Higher ethanol concentration reduced the NUCF yield but higher MUFAs/PUFAs concentration. Pure ethanol obtained 10.3% yield with 94.2% MUFAs/PUFAs, otherwise, pure water generated 28% yield but only 56.1% MUFAs/PUFAs. Predictive

equations to estimate MUFAs/PUFAs concentration and yield after urea-complexation under certain crystallization temperature (T) and ethanol concentration (X) are:

$$[UFAs]_{NUCF} = 2.0041\ T + 15.055$$
$$NUCF\ Yield = 0.006\ T + 0.1063$$
$$[UFAs]_{NUCF} = 0.0034\ X + 0.5648$$
$$NUCF\ Yield = -\ 0.0017\ X + 0.2651$$

These quantitative data expressed in predictive equations are recommended for separation process design in chemical industries.

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Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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