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Separation of Saturated and Unsaturated Fatty Acids of Palm Fatty Acid Distilled via Low-temperature Methanol Crystallization†

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This study aimed to optimize conditions for the separation process of unsaturated fatty acid (USFA) and saturated fatty acid (SFA) from palm fatty acid distilled by using low-temperature methanol solvent crystallization method. Several parameters were optimized to improve the solvent crystallization. The fatty acid composition analysis was carried out using a gas chromatography-flame ionization detector as fatty acid methyl esters. The results showed that a high percentage of SFA was found to be more than 95% with the percentage yield of 52%, and USFA was more than 93% with the percentage yield of 48%. This observation was found under the optimal conditions of which fatty acids-to-methanol ratio of 1: 15 (w/v), the crystallization temperature of -15 °C, and the crystallization time of 24 hours, respectively. The composition of separated SFA in the solid fraction contained 80% of palmitic acid (C16:0) as a dominant component, 12.5% of stearic acid (C18:0) and 2.5% of myristic acid (C14:0). The composition of separated USFA in the liquid fraction contained 70.4% of oleic acid (C18:1) as a principal component and 22.6% of linoleic acid (C18:2). The results showed that utilizing methanol as a crystallization solvent was recommended owing to its high efficiency, low cost, stability, availability, the comparative ease of recovery and its ability to form needle-like crystals which had good filtering and washing characteristics.

Key words: palm oil, fatty acid, separation, solvent crystallization

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Palm fatty acid distilled (PFAD) comprises 52% saturated fatty acid (SFA) and 48% unsaturated fatty acid (USFA). The USFA from PFAD can be utilized for a broad array of scientific and industrial applications including biolubricants, bioplastics, pharmaceuticals, surfactants, dispersants, and a variety of synthetics [1, 2]. In order to separate USFA and SFA from PFAD, research centres and industries have employed appropriate techniques such as chromatography, fractional or molecular distillation, enzymatic splitting, low temperature crystallization, solvent fraction, silver ion complexation, lipase concentration, lead salt-ether method, ion exchange resins, and urea complexation [3, 4]. Wanasundara and Peterson stated that separation of saturated fatty acids from unsaturated fatty acids could be achieved by complexing a blend of the fatty acids with urea [5]. Urea has been found to be a selectively complex saturated fatty acid relative to unsaturated fatty acid, creating a saturated fatty acid-enriched solid fraction and an unsaturated fatty acid-enriched liquid fraction. The solvent of choice for use in this step of the process is alcohol, with or without water, at a weight ratio of at least 2:1 (solvent to urea), or between 3:1 and 10:1, and most preferably between 4:1 and 5:1. A weight ratio of less than 2:1 tends to result in incomplete complexation of the unsaturated fatty acids. Meanwhile, a weight ratio greater than 10:1 would result in increased processing cost without a concomitant increase in yield or processability. In a similar study, Bist and Tao [6] developed a controlled separation method for the fatty acids mixture using urea complexation by controlling three process parameters: (i) urea-to-fatty acids ratio from 0 wt/wt to 1 wt/wt; (ii) alcohol-to-fatty acids ratio from 4 vol/wt to 8 vol/wt; and (iii) cooling temperature ranging from −2°C to −26°C. After heating the mixture containing fatty acids, urea, and alcohol to a temperature at which a homogenous mixture would be obtained, the homogenous mixture is then cooled to a temperature where the solid phase and a liquid phase will be formed. The solid phase was separated from the liquid phase via a controlled removal of the saturated fatty acid-rich fraction. The unsaturated fatty acid-rich fraction was obtained in the range of 65−98% of the

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starting material, respectively. Jiang [7] concentrated on extracting unsaturated fatty acid methyl esters (FAME) by urea complexation from soybean-derived FAME. The study investigated the effects of urea-to-FAME ratio, 95% ethanol-to-FAME ratio, and crystallization temperature and time on the purification of unsaturated FAME through single-factor experiments. The optimum condition for obtaining maximum FAME yield of NUCF with a purity of unsaturated FAME greater than 98% was established using a Box-Behnken design method and Response Surface Methodology. Under the optimal condition, the FAME yield was 58.08%, and the purity of unsaturated FAME was 98% at a urea-to-FAME ratio of 1.23, 95% ethanol-to-FAME ratio of 7, and crystallization temperature of 0°C. Bist [8] showed that individual unsaturated acids could be isolated in a high state of purity by crystallization from an appropriate solvent at temperatures ranging from 0°C to −70°C. The study claimed that for many purposes, the low-temperature crystallization technique is preferable to the chemical methods on account of its simplicity. However, in the case of animal fat, Strohmeier [9] effectively separated the unsaturated fatty acid mono-alkyl esters using methanol and acetone as solvents at low temperatures. A pure fraction of unsaturated fats (90%) was obtained with a solvent-to-FA ratio of 10:1 v/w and storing the mixture for 4 h at −22°C, which implies its optimum condition. Haraldsson proved that low-temperature solvent crystallization (LTSC) is the simplest and most efficient technique to separate USFA concentrates from fatty acids, stating that it has been extensively used since decades ago [10]. The results mark methanol as comparatively effective and superior to both acetone and ethanol regarding the separation of saturated and unsaturated components of the fatty acids mixture. Japir [11] studied the separation of SFA from a high free fatty acid crude palm oil fatty acid mixture using the methanol crystallization method; as a result, SFA was successfully separated via this highly efficient method with the final product consisting of palmitic acid as a major component. The fatty acid composition of cottonseed was assessed by Brown and Kolb using a polar solvent. The cottonseed fatty acids were dissolved in 90% methanol with the solution maintained at −15°C [12]. The crystallized fraction containing SFA was filtered using vacuum filtration. The residual content was a liquid non-crystallized fraction of concentrated USFA. Methanol was evaporated from the non-crystallized fraction via a heat exchanger. The yield of USFA fraction was 70%, and the iodine value was elevated from 103 g/100g to 144 g/100 g.

The LTSC is a well-recognized and economical, yet effective technique for separating USFA by eliminating SFA. The application of LTSC is based on the theory that higher SFA is considerably less soluble than their corresponding USFA. The solubility of any specified acid is closely related to its melting point and dependent on the nature of the solvent at a certain level [5]. LTSC application is based on the variances in fatty acid melting points to produce two fractions, a non-crystallized fraction (liquid) of concentrated USFA and a crystallized fraction, which contains the SFA. Afterward, the SFA are removed by filtration while the solvent is recovered from the non-crystallized fraction under reduced pressure using a rotary vacuum evaporator [8].

Generally, the separation of USFA from a mixture of fatty acids is carried out using numerous methods. Related papers and literature have selected LTSC as the preferred method because it is economical and has a high yield capability of unsaturated fatty acids.

MATERIAL AND METHOD

Sample

Malaysian palm fatty acid distilled (PFAD) was obtained from Sime Darby Plantation oil refinery, Selangor, Malaysia. Fatty acid methyl esters (standard) were purchased from Sigma-Aldrich Chemical. Methanol and other chemical reagents used in this research were also treated with analytical grade and used without further purification.

Preparation of Fatty Acid Methyl Esters (FAME) for GC-FID Analyses

About 0.008 mol (2 g) of fatty acids were added to a small (50 ml) two-neck round-bottom flask, equipped with a standard taper joint (19/38) and a short condenser. Then, 0.2 mol (8.7 ml) methanol was added to 0.01 mol (0.3 ml) HCl 37%, and this was followed by adding 1.4×10^{-2} mol (1.5 ml) of toluene. After that, the mixture was refluxed at 65°C for 1.5 hours. Then, it was transferred to a separating funnel, where 10 ml distilled water and 15 ml of hexane were added to the mixture. Later, the mixture was left to stand until two complete layers were formed. Then, the upper layer was separated and dried using anhydrous sodium sulphate Na_2SO_4 overnight. Thus, hexane was recovered under reduced pressure using a vacuum rotary evaporator at 35°C [13, 14].

Gas chromatography (GC) analyses were performed using gas chromatograph (Model 5890 SERIES II GC, USA) software equipped with a flame ionization detector (FID) and a BPX70 fused silica capillary column (30 m, 0.25 mm, 0.25-µm film thickness). The injector temperature was maintained at 280°C. Operating conditions were as follows: helium as the carrier gas with a flow rate of 1 ml/min; injection volume of 1 µl; and a split ratio of 60:1. The oven temperature was maintained at 120°C, increased to 245°C, and then held for 15 min at a rate of 3°C per

minute for 56.6 min of analysis. The FAME peaks were classified and quantified by comparing their peak areas and retention times with that of the pure standard FAME [15].

Separation of Saturated and Unsaturated Fatty Acid Crystallization from PFAD

The separation of SFA and USFA from PFAD was performed in methanol using LTSC with supercooling in a refrigerator to enable temperature control. 10 g PFAD was subsequently mixed with 95% methanol and heated at 60°C. The mixture was continuously stirred until a homogeneous blend was obtained. The influence of factors controlling the separation of SFA and USFA constituents was evaluated within the minimum and maximum value of each parameter (Table 2). The ratio of methanol-to-FA was varied using different volumes of methanol (5–25 ml); the temperature of crystallization was changed from −15°C to 5°C, and the crystallization duration was varied from 6 h to 30 h. The USFA was concentrated into an amorphous fraction, while the SFA crystallized into a solid, which was removed by filtration. Afterward, the liquid fraction was re-filtered to eliminate any excess solids, while the methanol was evaporated from the non-crystallized fraction (liquid fractions) under low pressure using a vacuum rotary evaporator at 55°C.

RESULTS AND DISCUSSION

The Fatty Acid Composition of PFAD

Table 1 shows that $PFAD_{exp}$ consists of 52.8% saturated fatty acids and 47.2% unsaturated fatty acids. The saturated fatty acids comprise mainly myristic acid (1.18%), palmitic acid (48.91%), and stearic acid (2.7%), while oleic acid (37.4%) and linoleic acid (9.7%) are the main components of the unsaturated fatty acids. By comparison, this work proves that the fatty acid composition in $PFAD_{exp}$ is slightly different than that of the Malaysian palm oil distilled standard [16].

Separation of Saturated and Unsaturated Fatty Acids

The low-temperature solvent crystallization (LTSC) method with methanol was selected for this study due to the difference in melting points between saturated fatty acids and unsaturated fatty acids, which allows phase or fractional separation. The first is a non-crystallized fraction (liquid) of concentrated unsaturated fatty acids and the second is the crystallized fraction comprising the saturated fatty acids. The saturated fatty acids were subsequently removed by filtration while the solvent was recovered from the non-crystallized fraction under reduced pressure using a vacuum rotary evaporator.

The separation of PFAD by crystallization from methanol depends on the solubility differences between the various components of the mixture of fatty acids. Since higher saturated fatty acids are much less soluble than the corresponding unsaturated fatty acids, the mixtures are usually partially separated [18]. As a fatty acid, long chain saturated fatty acids, such as stearic acid and palmitic acid, are a relatively nonpolar compound. However, short chain saturated fatty acids, such as myristic acid, exhibit more polarity compared to stearic and palmitic acids. Monosaturated fatty acids and polyunsaturated fatty acids are characterized to be more polar than saturated fatty acids. The solubility of palm fatty acid distillate in methanol in the order of increased polarity is linoleic acid, oleic acid, myristic acid, palmitic acid, and stearic acid. Therefore, unsaturated fatty acids will be more soluble than saturated fatty acids in methanol. The solubility behaviour of PFAD in methanol at a specified temperature can also be explained based on

Table1. Fatty acid composition of PFAD.

Fatty acid composition	Relative composition (%)	
	$PFAD_{exp}$	$PFAD_{M*}$
Lauric acid C12:0	-	0.4
Myristic acid C14:0	1.1	1.2
Palmitic acid C16:0	48.9	47.1
Stearic acid C18:0	2.7	4.3
Oleic acid C18:1	37.1	36.7
Linoleic acid C18:2	9.1	9.0
Others	1.1	1.3
Σ Saturated Fatty acid	52.7	53
Σ Unsaturated Fatty Acid	46.2	45.7

$PFAD_{exp}$.: Palm fatty acid distillate of experimental; $PFAD_{M*}$: Malaysian palm fatty acid distillate [17]

the polarity and hydrogen bonding properties of the mixture components. As a result, the separation is due to the high solubility of oleic acid and linoleic acid, in methanol. However, SFA, particularly palmitic acid, is poorly soluble in methanol and is thus preferentially crystallized out. The slight increase in stearic acid is due to co-crystallization, given the solubility of other acids in PFAD. Myristic acids are saturated acids, but their concentrations are very low, and their solubility exceeds that of palmitic acid. Consequently, they are also soluble in methanol, thus leaving the sample enriched with palmitic acid [19]. The melting point of the FA changes with the number of carbons and the degree of unsaturation. Therefore it is probable to separate the mixture of fatty acids into saturated and unsaturated fatty acids [20]. The solubility of fatty acids increases with increase in temperature, and this is mainly reflected by their melting points: high melting point fatty acids are less soluble than low melting point fatty acids. Consequently, an increase of unsaturated fatty acid in the mixture usually leads to decreased melting point of the mixture and increased solubility of unsaturated fatty acid in methanol [21].

Optimization of Separating Parameters

The main aim of this current study is to determine the optimal conditions for efficiently separating the total SFA as well as total USFA from PFAD as show in Table 2.

Effect of FA-to-solvent Ratio (w/v)

As a result, the experiments were done with solvent crystallization under different ratios of fatty acids-to-solvent (w/v), as shown in Figure 1.

Table 2. The operative conditions of experiments.

Parameter	Value
FAs-to-solvent ratio (w/v)	1:5, 1:10, 1:15, 1:20, 1:25
Crystallization temperature	−20, −15, −10, −5, 0, 5
Crystallization time (min)	6, 12, 18, 24, 30

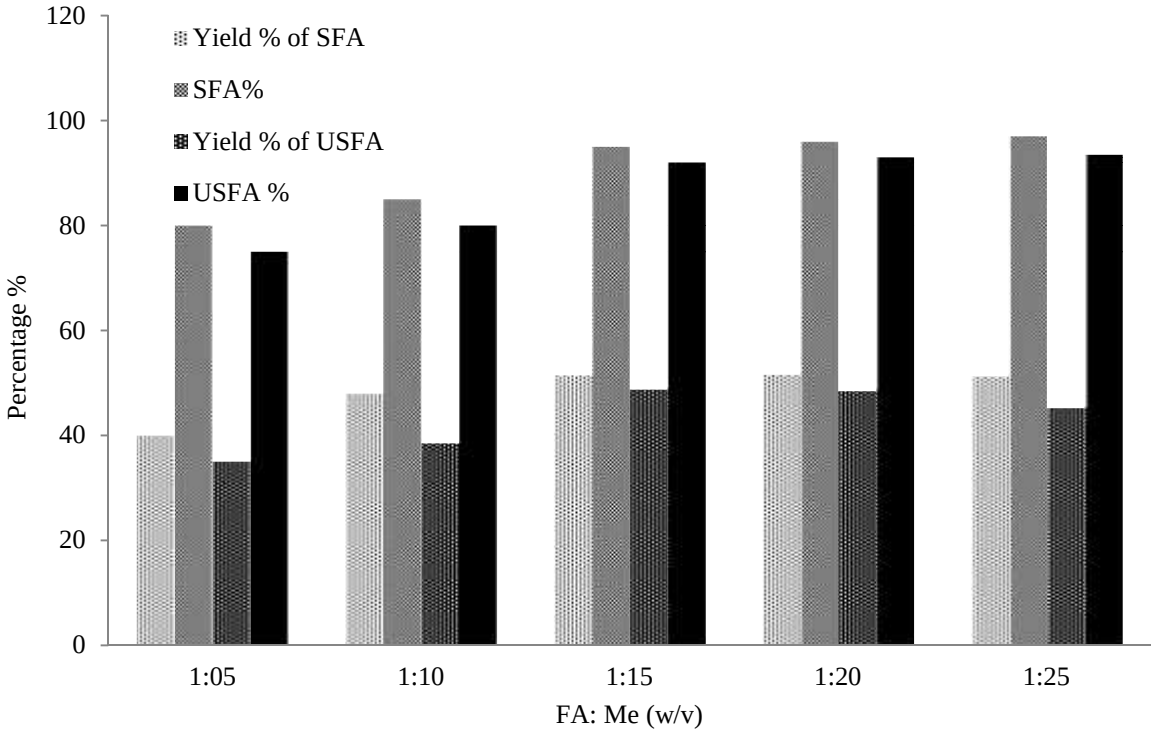

Figure 1. Effect of FA-to-solvent ratio (w/v) of fractionation of PFAD at −15°C and 24 h.

Optimal crystallization temperature and crystallization time were achieved at −15°C, and 24 h, respectively, with a fatty acids-to-methanol ratio of 1:15 (w/v). It can be observed from Figure 1 that there is a relationship between solvent volume, yield of SFA, percentage of SFA, yield of USFA, and percentage of USF wherein the yield of SFA and USFA decreased, and the percentage of SFA and USFA increased, with an increase in the volume of solvent from 1:5 to 1:25 (w/v). In all cases, increasing the amount of solvent from 1:5 to 1:25 (w/v) led to lower yields and higher purity of USFA fraction and SFA fraction [22]. If the concentration of FA in a solvent is too high, then the precipitate tends to form a slimy mass, which cannot be handled or washed except perhaps in small quantities under laboratory conditions [23]. In this study, the concentration of fatty acid-to-methanol was varied from 1:5 to 1:25.

Effect of Crystallization Temperature

One of the crucial factors that affect LTSC efficiency is temperature. The saturated fatty acids start to crystallize with a decrease in mixture temperature [24]. More crystals were observed when the temperature reached −15°C. For this reason, we selected −20°C to be the higher range for the crystallization temperature. The higher temperature of −15°C in the case of the 1:15 (gml^{-1}) ratio and 24 h led to a slightly higher yield of precipitate mass [25], as shown in Figure 2.

Effect of Crystallization Time

The optimization of the crystallization time required for fractionation is shown in Figure 3, where the results of fractionation experiments using 95% methanol at a ratio 1:15 (g: ml) and −15°C with crystallization times of 6 h, 12 h, 18 h, 24 h, and 30 h are outlined. The yields of the precipitates showed a slight difference, although a more significant difference is observed for unsaturated fatty acids. The separation of PFAD by crystallization from methanol depends mainly on the solubility differences between the various components of the mixture of fatty acids [26]. While it is known that higher SFA is much less soluble than the corresponding USFA, this fact has been used to separate the mixture of fatty acid partially [27]. After 6 h of crystallization, the fraction of unsaturated fatty acids in the precipitates remained low because the temperature only reached −15°C at 4 h. After 12 h, the amount of USFA in the precipitate remained relatively constant with no significant improvement. However, the precipitate at a crystallization time of 24 h exhibited the highest content of SFA. Therefore, a crystallization time of 24 h was selected for further experiments. Moreover, prolong the crystallization time (30 h) did not improve the results, as shown in Figure 3. The solution temperature has to be slowly decreased to a specific crystallization temperature (1–6 h) and needs to be maintained at this point for 6–24 h [28].

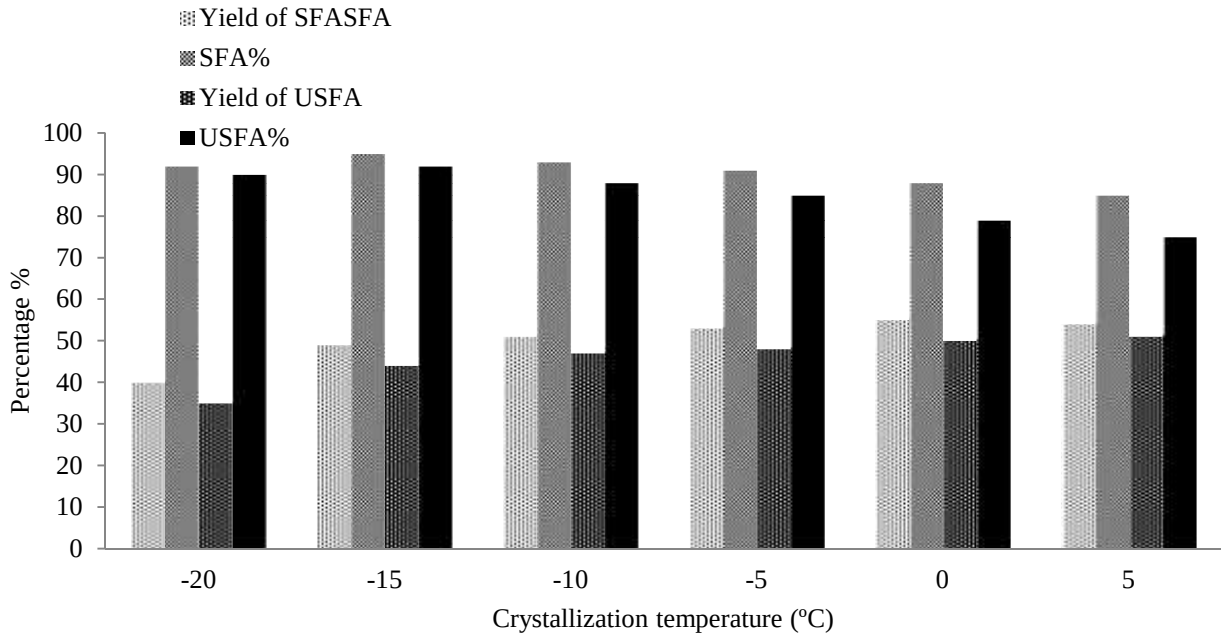

Figure 2. Effect of crystallization temperature of fractionation of PFAD using methanol with a ratio of 1:15 (w/v) at 24 h.

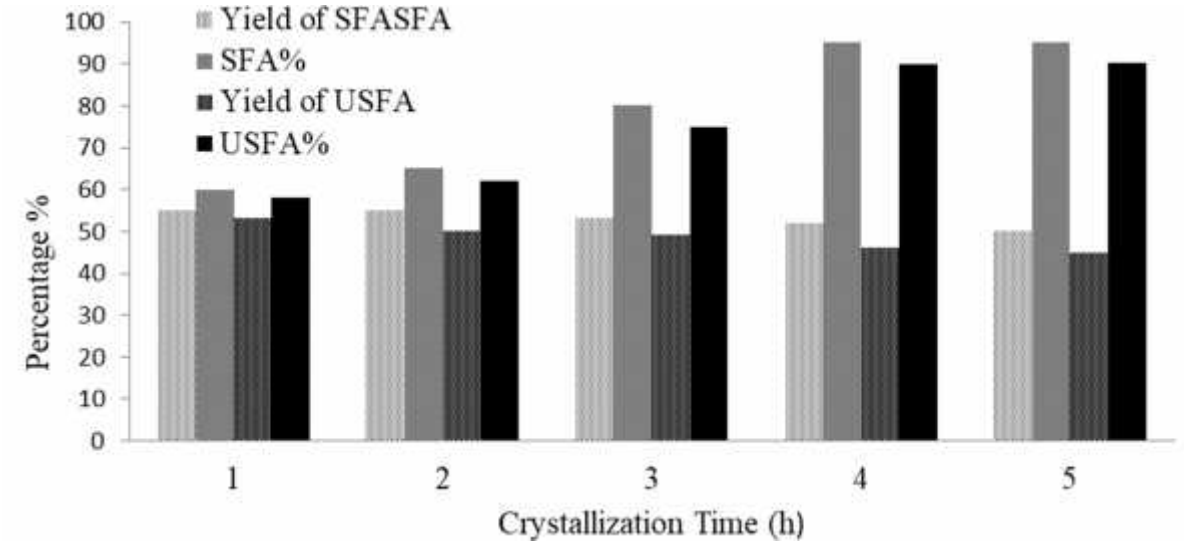

Figure 3. Effect of crystallization time of fractionation of PFAD using methanol with a ratio of 1:15 (w/v) at −15°C.

Composition of Saturated and Unsaturated Fatty Acids

The fatty acids composition was obtained after esterification using GC-FID analysis. The composition of FA is outlined in Table 1. It was observed that there were highly significant differences between the composition of FA in PFAD, and SFA in the solid fraction after methanol crystallization. The composition of FA in PFAD comprised 1.1% myristic acid (C14:0), 48.9% palmitic acid (C16:0), 2.7% stearic acid (C18:0), 37.4% oleic acid (C18:1), and 9.7% linoleic acid (C18:2), respectively. However, the composition of SFA after recrystallization at optimum conditions consists of 0.6% myristic acid (C14:0), 88.5% palmitic acid (C16:0), 8.4% stearic acid (C18:0), 3.6% oleic acid (C18:1), and 0.5% linoleic acid (C18:2), as shown in Figure 4. A maximum SFA purity of 95% was achieved. It is shown that palmitic acid (C16:0) increased from 48.9% to 88.5% and stearic acid (C18:0) increased from 2.7% to 8.4%. The monounsaturated fatty acids (MUSFA), oleic acid (C18:1), was decreased from 37.4% to 3.6% and for the polyunsaturated fatty acids (PUSFA), linoleic acid

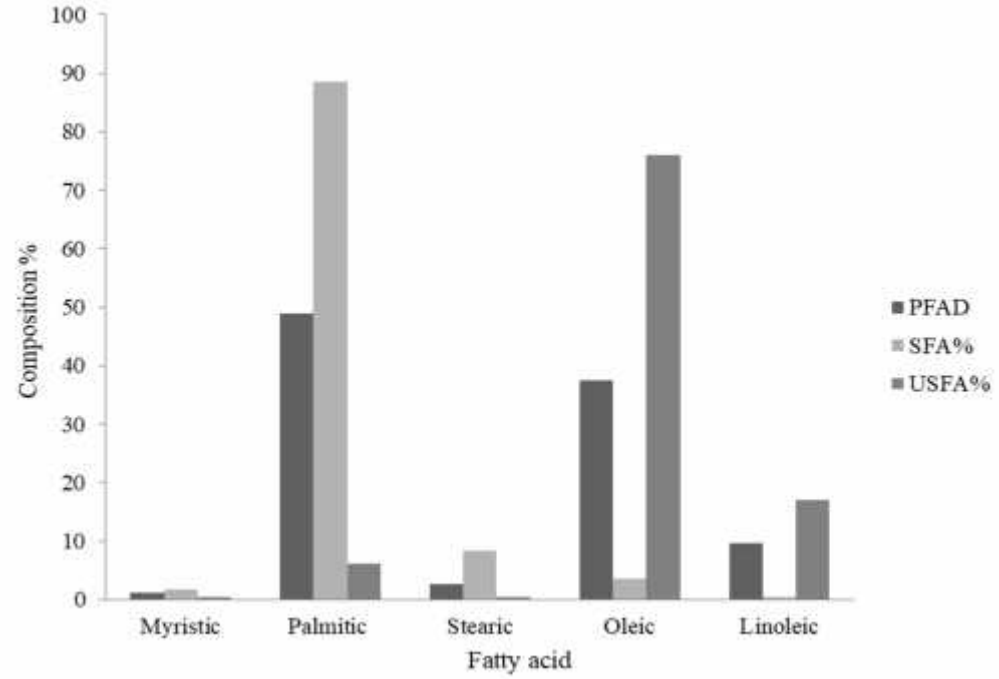

Figure 4. Fatty acids profile of PFAD and SFA and USFA after methanol crystallization.

(C18:2) was decreased from 9.7% to 0.5%. This indicates that methanol crystallization is an efficient technique for separating SFA concentrates from PFAD because of differences in the PFAD solubility [29].

A significant difference was discernible between fatty acids composition prior to and after low-temperature crystallization under optimum extraction conditions, with fatty acids-to-methanol ratio of 1: 15 (w/v), the crystallization temperature of -15 °C, and the crystallization time of 24 hours. A remarkable difference could be observed in the composition of palmitic acid (C16:0) and oleic acid (C18:1). Palmitic acid as the main constituent of saturated fatty acids in PFAD declined fast to 6.2%, while oleic acid surged to 76%. The maximum percentage of USFA was determined to be 93%. The entire volume of saturated fatty acids (SFA), comprising myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) decreased from 1.1% to 0.5%, 48.9% to 6.2% and 2.7% to 0.5%, respectively, as per Figure 4. Therefore, it is plausible to hypothesize that low-temperature solvent crystallization is an effective method for the separation of USFA from PFAD [30].

CONCLUSION

Fatty acid composition and physicochemical parameters of PFAD obtained from Sime Darby were checked and compared with reported studies. Most of the quality parameters of PFAD showed comparable values with data from earlier surveys. Saturated fatty acids and unsaturated fatty acids were successfully separated from PFAD using a highly efficient method for which the final products were SFA and USFA. The fatty acids-to-methanol ratio (w/v) had a highly significant influence on the yield and percentage of SFA and USFA. Meanwhile, crystallization temperature and crystallization time had a moderately significant impact on the yield and percentage of SFA and USFA. The maximum percentage of SFA was over 95% when including over 88.5% palmitic acid as a major component. Also, the maximum percentage of USFA was over 93% when comprising over 76% oleic acid as a major component. The ratio of the yield was up to 52% for SFA and 48% for USFA under the optimal conditions, where the ratio of FAs-to-MeOH was 1:15 (w/v), and the crystallization temperature was −15°C, with a crystallization time of 24 h. In conclusion, methanol crystallization was a promising method for obtaining high palmitic acid concentrates as a major component in SFA in a solid fraction and high oleic acid concentrates as a major component in USFA in a liquid fraction from PFAD. It could also be potentially used for the large-scale production of SFA and USFA. Also, the methanol (95%) used in this study could be recycled to support the principle of green chemistry. This method could be one of the most efficient, cheapest, and simplest methods that could be applied for the separation of fatty acids mixture.

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