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Research article

Effect of solvent pre-treatment on the physicochemical, thermal profiles and morphological behavior of *Mangifera pajang* seed fat

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ABSTRACT

In this work, the effect of solvent pre-treatment (hexane, petroleum ether and ethanol) on the physicochemical, thermal and morphology behavior of *Mangifera pajang* seed fat (MPSF) were investigated. Fat extraction was performed using Soxhlet method, and results showed that the yield, physicochemical, and crystalline structures of the MPSF were significantly ($p < 0.05$) influenced by the extraction solvents. Hexane gave the highest fat yield (7.67 %) with low unsaturation value (52.13 g iodine/g) compared with petroleum ether and ethanol. Hexane MPSF also had low oxidation rate (peroxide value of 1.1 mEq/g). Both non-stabilized and stabilized hexane MPSF showed a single melting endothermic peak at high temperature with onset, maximum peak and offset temperature of 16.23 ℃-18.21 °C, 28.22 ℃-31.25 °C and 34.85 ℃-39.58 °C, respectively. Hexane MPSF crystallized rapidly at high temperature with single maximum peak starting at 16.51 ℃-16.68 °C and ending at 0.23 ℃-1.13 °C. In comparison with ethanol extract, hexane MPSF demonstrated a compact crystalline structure with a large densely packed center. Therefore, MPSF obtained from hexane presented better overall quality than those obtained from other extraction solvents. MPSF exhibited similar melting and morphological behavior to mango kernel fat and commercial cocoa butter. These results suggested that hexane was the best solvent for the extraction of MPSF. This fat also has the potential to be applied as a cocoa butter alternative fat or functional fat.

1. Introduction

Mangifera pajang (MP) is an indigenous fruit that grows widely in Borneo Island and is locally known as mawang or bambangan (Ling et al., 2020). This fruit is one of the prominent underutilized fruits with great economic values because it is readily available (locally) but universally uncertain (Hassan et al., 2011). The cultivation of the fruit in Sabah, Malaysia, is reported with a constant increment from 115.3 metric tons to 121.6 metric tons in 2013–2015 (Jahurul et al., 2019a). In Malaysia, ripe MP fruit are preferred for consumption; they are also utilized for functional food (i.e. minimally processed fruits, juice powder, dehydrated fruit, and health drink) production and flavoring ingredient in food (Al-Sheraji et al., 2011, 2012; Jahurul et al., 2019a). The flesh are often used for consumption and food processing, whereas the peel and kernel are discarded as agricultural waste. The wastes, specifically the kernel and peel, reportedly have considerable antioxidant compounds that exhibited remarkable health benefits (Abu Bakar et al., 2009; Jahurul et al., 2019a; Tangah et al., 2017). The seed constitutes about 15%–27% of the total fruit weight and is composed of 8.6%–9.9 % of fat, which has potential application in the food industry (Abu Bakar et al., 2009; Jahurul et al., 2019b; Norazlina et al., 2020a).

The extraction of the MP seed fat (MPSF) can be performed by various methods, such as pressurized solvent extraction (Dunford and Zhang, 2003), supercritical fluid extraction (Jahurul et al., 2014a), ultrasonic-aided extraction (Tian et al., 2013), microwave-aided extraction (Balasubramanian et al., 2011; Taghvaei et al., 2014), adequous enzymatic oil extraction (Latif and Anwar, 2011), and Soxhlet extraction (Okeleye and Betiku, 2019). Among these methods, the Soxhlet method is most known because it is simple to use and economical (Oladipo and Betiku, 2019). Moreover, the solvent used for the extraction can be recovered after the extraction process, thus lowering the operational cost by producing re-useable solvent which can be used for another solvent

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extraction (Handa, 2008). The extraction process also can be carried out simultaneously, resulting in high oil recovery efficiency. The Soxhlet method is the standard extraction method at which the sample is in contact with the fresh solvent portions repeatedly throughout the process, leading to the equilibrium transfer (Kittiphom and Sutasinee, 2013). The extraction can be conducted using a non-polar solvent (i.e. hexane and petroleum ether), polar-aprotic solvent (such as chloroform) or polar-protic solvent (e.g., ethanol) (Jedidi et al., 2020). Several studies have investigated the yield and quality of oil extracted based on the polar and non-polar solvents used (Kittiphom and Sutasinee, 2013; Okeleye and Betiku, 2019; Jedidi et al., 2020). The choice of solvents for extraction plays an important role in the final outcome, which can affect the oil yield (Ajala and Betiku, 2015), fatty acid profiles (Abdolshahi et al., 2015), and physicochemical properties (Attah and Ibemesi, 1990). Hexane is one of the most common organic solvents used extensively for oil seeds extraction (Danlami et al., 2014). Hexane has low melting point, easy recovery, high availability, and high solubility in oil corresponding to its non-polar properties (Liu and Mamidipally, 2005; Mujdalipah et al., 2016).

Petroleum ether (lipophilic organic solvent) has also been used widely for the extraction of lipophilic compounds of fats and oils from the seed of the fruit plant (Feng et al., 2019). Meanwhile, green polar solvent (ethanol) is used due to the low toxicity properties as established by the European Directive, high availability and bio-based properties (Perrier et al., 2017). Different studies have reported about how various solvents affect the physicochemical properties of oil (Kittiphom and Sutasinee, 2013; Jedidi et al., 2020). The usage of different solvent extraction methods also influences the quality of fats and oils, especially bioactive compounds and their yield (Oladipo and Betiku, 2019; Stevanato & da Silva, 2019). However, information on the characteristics of MPSF extracted using different solvents is lacking. Also, there has been no previous research on the effect of organic solvents on the characteristics and quality of fat extracted from MP. Thus, this study aimed to assess the influence of the organic solvents on the physicochemical properties, thermal profiles, morphological properties and the quality of the MPSF. The applicability of the extracted MPSF also evaluated.

2. Materials and methods

2.1. Materials

Ripe MP fruits were collected from Ranau, Sabah, Malaysia. The extraction solvents (i.e. hexane, petroleum ether and ethanol) and analytical chemicals (cyclohexane, acetic acid, chloroform, Wijs solution, potassium hydroxide, sodium thiosulphate, potassium iodide (KI) and phenolphthalein indicator) were purchased from Merck, Germany.

2.2. Extraction of MPSF using hexane, petroleum ether, and ethanol

MP seed powder were prepared prior to the extraction process. The detailed procedure is described in the previous research (Norazlina et al., 2020a). MPSF extraction was conducted using liquid extraction or conventionally known as Soxhlet method. Approximately 80.0 g of MP seed powder was extracted using 400 mL of hexane, petroleum ether and ethanol (1:5 ratio) as described by Jedidi et al. (2020) and Kittiphom and Sutasinee (2013) for 8 h, and the solvent was evaporated by using rotary evaporator (4001, HEIDOLPH LABORTA, Germany). The extracted MPSF was then dried in the oven for 2 h for complete solvent removal. Total yield for MPSF was determined and expressed as Eq. (1).

$$\text{Total Yield}(\%) = \frac{Mass\ of\ extracts\ (g)}{Mass\ of\ sample\ (g)}\ x\ 100 \quad (1)$$

2.3. Physicochemical analysis

The changes in the physicochemical properties for pre-treated MPSF was determined according to the AOCS (2003) official method of Cc 3b-92, Cd 8–53, Cd 1b-87, Cd 3d-63 and Cc7-25 for slip melting point (SMP), peroxide value (PV), iodine value (IV), acid value (AV) and refractive index (RI), respectively.

2.3.1. SMP

An open-end capillary glass tube was used for the determination of SMP of MPSF extracted from different solvents. The tube was dipped into the fat samples until at 10 mm length, and the fat was chilled and solidified on an ice bath prior to analysis. Fat samples (10mm; lower end of the capillary glass tube) were attached to the bottom of the thermometer by a using rubber band and then immersed into the glass test tube before being placed into water bath (10 °C) for the analysis. The temperature rate was controlled and increased at 1 °C until the fat column rose. The SMP of fat samples was determined when the fat column rose to 30mm high.

2.3.2. IV

Approximately 0.5 g of melted fat samples was mixed with 20 mL of cyclohexane and 25 mL Wijs solution and placed in the dark for 1 h. About 20 mL of 15% KI was immediately added into the mixture after 1 h, followed by the addition of 100 mL of distilled water. The mixture was titrated with 0.1 N sodium thiosulphate solution. About 1–2 mL of starch indicator was added after the yellow solution turned colourless, and the solution was titrated until the blue solution turned colourless. The IV for fat samples was determined as presented in Eq (2);

$$\text{IV}(\text{g iodine / g}) = \frac{(Vol\ of\ blank\ titrant - vol\ of\ sample\ titrant)x\ Normality\ of\ titrant\ x\ 12.69}{mass\ (g)} \quad (2)$$

2.3.3. PV

MPSF was melted at 10 °C above the melting point prior of the analysis. Approximately 5.0 g of MPSF was added with 30 mL of acetic acid: chloroform mixture (3:2), 0.5 mL saturated KI, 30 mL of distilled water and then titrated with 0.1 N sodium thiosulphate. Once the yellow solution turned colourless, 2 mL of 1% starch indicator was added,

$$\text{PV}(\text{mEq / g}) = \frac{(Vol\ of\ sample\ titrant - vol\ of\ blank\ titrant)x\ Normality\ of\ titrant\ x\ 1000}{mass\ (g)} \quad (3)$$

Table 1. Physicochemical properties of MPSF extracted from different solvent.

	Hexane extract	Petroleum ether extract	Ethanol extract
Physicochemical properties			
Total fat content (%)	7.67 ± 0.06[a]	6.40 ± 0.10[b]	2.83 ± 0.06[c]
Iodine value (g iodine/g)	52.13 ± 0.6[b]	51.30 ± 0.5[b]	69.00 ± 1.0[a]
Acid value (mgKOH/g)	3.85 ± 0.07[b]	3.74 ± 0.06[b]	7.41 ± 0.23[a]
Peroxide value (mEq/g)	1.1 ± 0.1[b]	1.0 ± 0.1[b]	6.2 ± 0.2[a]
Slip melting point (°C)	31.7 ± 0.6[a]	31.3 ± 0.6[a]	28.3 ± 0.6[b]
Refractive index	1.4651 ± 0.0[c]	1.4658 ± 0.0[b]	1.4663 ± 0.0[a]

Values are the mean ± standard deviation of triplicate; means a different letter within a row are significantly different ($p < 0.05$) as measured by Tukey test.

followed by second titration. PV was obtained after the blue solution turned colourless, and the results are as expressed in Eq. (3).

$$\mathrm{AV(mgKOH/g)} = \frac{(Vol\ of\ sample\ titrant - vol\ of\ blank\ titrant)x\ Normality\ of\ titrant\ x\ 56.1}{mass\ (g)} \quad (4)$$

2.3.4. AV

Approximately 5.0 g of MPSF was diluted with 50 mL of ethanol followed by 50 mL of phenolphthalein indicator and then titrated with 0.1 N potassium hydroxide until pink solution was observed. The AV for MPSF was determined as stated in Eq. (4).

2.3.5. RI

RI for MPSF extracted using different solvents was determined by using a refractometer (Abbe Refractometer, Atago NAR37, Japan). Melted fat samples were filtered (Whatman, No.1, 125mm) to remove the impurities prior to analysis. One to two drops of samples were placed onto the lower prism of the refractometer (adjusted to 40 °C, allowed to stand for 1–2 min), and the light and instrument were adjusted until the reading was obtained.

2.4. Thermal properties by differential scanning calorimetry (DSC)

Thermal properties were analyzed by DSC (PYRIS Diamond, PERKIN ELMER, USA) for monitoring melting and crystallization of the extracted MPSF. The procedure was conducted according to AOCS (2003) Official Method Cj 1–94 with slight modification. The extracted fat was melted at 80 °C and then transferred into standard DSC aluminum pans for the analysis. Approximately 3–5 mg of the molten samples was transferred to standard DSC aluminum pans by using a syringe with needle (1 mL) sealed hermetically. The pans for non-stabilized samples were transferred to the DSC head for analysis. An empty hermetically sealed DSC aluminum pan was used as a reference. For the stabilization of the fat, hermetically sealed fat samples in the DSC aluminum pan were placed in vials, melted at 80 °C for 30 min and then incubated at 26 °C for 7 days. After 7 days, the stabilized fat samples were transferred to the DSC head for analysis. For thermal analysis, the following conditions were used; cooling to -40 °C and melting the samples to 80 °C. The samples were heated rapidly at 30 ºC/min to 80 °C to ensure a completely liquid state, held for 10 min, cooled at 10 ºC/min to -40 °C, maintained at -40 °C for 30 min, heated at 5 ºC/min to 80 °C and held for 2 min. During melting and cooling, the onset temperature, maximum temperature, offset temperature and enthalpy change of fat samples was measured.

2.5. Polymorphic behavior

The polymorphic form for MPSF extracted using different solvents was determined by using an X-Ray Diffractometer (SmartLab, Rigaku, USA), according to the establish method Cj 2–95 (AOCS, 2009). MPSF samples were melted at 80 °C, placed in glass sample holder, and stabilized for 24 h at 25 °C before X-ray diffractometer (XRD) analysis. A detailed procedure is described in our previous study (Norazlina et al., 2020b). The relative polymorphic form for MPSF was determined based on the reported cocoa butter (CB) polymorphic form reported by Marangoni and McGauley (2003) and Schenk and Peschar (2004).

2.6. Crystal microstructure observation

A polarized light microscope (Polarization Microscope Leica DM2500P, Leica, Germany) was used to observe the crystal microstructure of MPSF according to the method developed by Narine and Marangoni (1999) with slight modification. Approximately 15 μL of melted MPSF was placed on top of microscopic glass slide (pre-heated with MPSF at 80 °C) and then incubated at 4 °C (1 h). The sample was stored at 23 °C–25 °C for 48 h after the incubation process. Magnification of 40x was used for the identification of the crystal microstructure.

2.7. Statistical analysis

The total fat content determination and all analyses were performed in triplicate, and the data were expressed as mean ± standard deviation. The significant difference in the treatment means was determined by IBM SPSS software version 24 using one-way analysis of variance (ANOVA) and Tukey test. $P < 0.05$ was considered significant.

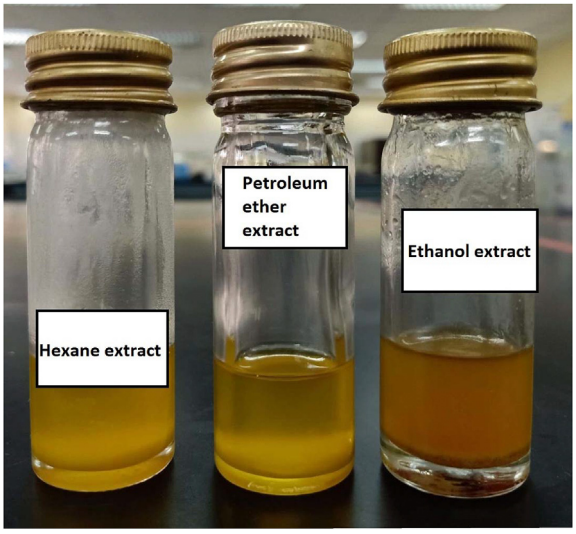

Figure 1. Physical appearance of the extracted MPSF using hexane, petroleum ether and ethanol.

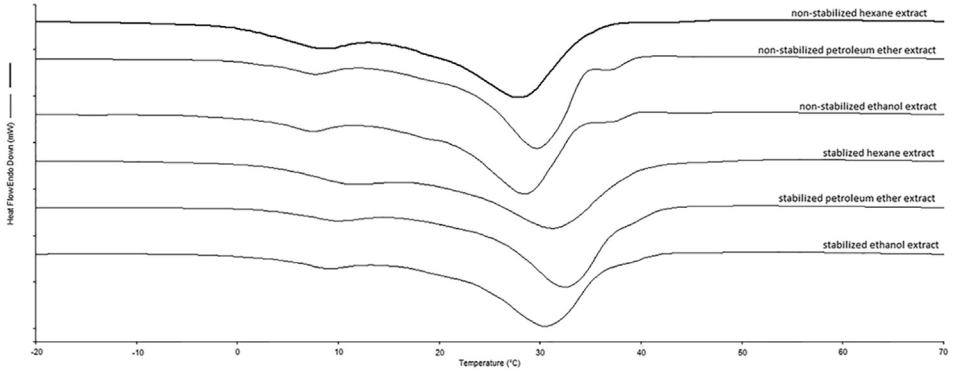

Figure 2. Differential Scanning Calorimetry (DSC) melting curve for non-stabilized and stabilized MPSF extracted from different solvent.

3. Results and discussion

3.1. Total fat content

Table 1 shows the total percentage of MPSF extracted from hexane, petroleum ether and ethanol. The physical appearances of the extracted MPSF are presented in Figure 1. The melted hexane and petroleum ether MPSF were yellow liquid solution and turned to yellow solid fat at 25 °C. By contrast, the melted ethanol extracts were a cloudy golden-yellow liquid solution and changed to golden-yellow solid fat at 25 °C. Similar results also reported by Okeleye and Betiku (2019) in which golden-yellow liquid oil was obtained from the extraction of kariya seed oil (KSO) using the polar solvent (ethyl acetate and acetone). The hexane extracts showed the highest total fat content, followed by petroleum ether and the ethanol extracts. The trends in the given values were in agreement with the mango seed fat extracted using hexane, petroleum ether and ethanol reported by Kittiphom and Sutasinee (2013). The efficacy of the extraction may be due to the interaction of the solutes with the solvent, solvent polarity, boiling temperature and composition of the MPSF. These findings were supported by Ferreira-Dias et al. (2003), who reported the efficacy of hexane as the best extraction solvent of *Quercus suber* seed oil. Thus, hexane gave the best extraction yield compared to the two other solvents.

3.2. Physicochemical characteristics of MPSF extracted from different solvents (IV, SMP, AV, PV and RI)

The physicochemical properties for the extracted MPSF were significantly ($p < 0.05$) affected by the usage of different extraction solvents. Major parameters for the characterization of oil quality such as the IV, AV, and PV is presented in Table 1. The IVs for the hexane and petroleum ether extracts were found to be lower than that of the ethanol extract. By contrast, ethanol MPSF showed high unsaturation values, indicating that this fat had more unsaturated fat than the hexane and petroleum ether extracts. This result was similar to the mango kernel fat (MKF) extracted by Kittiphom and Sutasinee (2013). The IVs for hexane and petroleum MPSF were comparable with the IV of MPSF extracted using hexane as reported by Jahurul et al. (2018) and Norazlina et al. (2020a) with IVs of 53.3 and 50.3 g iodine/g, respectively. MPSF has four main fatty acids, namely, palmitic (8.35%–8.40%), stearic (36.35%–36.40%), oleic (44.40%–44.50%), and linoleic acid (5.35%–5.40%) with significant presence of arachidic and behenic acids. The high content of unsaturated fatty acids such as the oleic and linoleic acids explains the high unsaturation value in the MPSF.

Similar to the IV, the AV and PV of the MPSF were higher after exposure to the ethanol. AV is the measure of the lipid total acidity corresponding to the fatty acid constituents that makes up the glyceride component (Ekpa and Ekpa, 1995). By contrast, the PV is the measure of the peroxide and hydroperoxide concentrations that form from the first stage of lipid oxidation. High PV values indicate high oxidation and degradation of the oil quality (Matthäus, 2010). The regulation for the PV of edible lipid by FAO/WHO (1993) should not exceed 10 ± 20 mEq/g lipids, and the results complied with the regulation. The MPSF for the hexane and petroleum ether extracts showed comparable AV and PV with the MPSF and MKF extracted by Azrina et al. (2015), Jahurul et al. (2014c) and Sonwai and Ponprachanuvut (2014) with values of 2.81–5.11 mgKOH/g and 0.4–2.0 mEq/g respectively. By contrast, the ethanol extract showed high AV and PV of 7.41 mgKOH/g and 6.2

Table 2. Melting and crystallization properties for non-stabilized and stabilized MPSF extracted from different solvent.

	Hexane extract	Petroleum ether extract	Ethanol extract
Melting and crystallization properties for non-stabilized sample			
Melting behavior			
Onset Temperature (°C)	16.23 ± 0.07^{a}	21.41 ± 0.15^{c}	19.22 ± 0.12^{b}
Max Temperature (°C)	28.22 ± 0.00^{a}	29.71 ± 0.00^{c}	28.23 ± 0.00^{b}
Offset Temperature (°C)	34.85 ± 0.15^{c}	34.71 ± 0.00^{b}	34.13 ± 0.04^{a}
Enthalpy (J/g)	60.42 ± 0.08^{b}	57.87 ± 0.02^{a}	62.29 ± 0.58^{c}
Crystallization behavior			
Onset Temperature (°C)	16.68 ± 0.15^{a}	18.31 ± 0.00^{b}	18.28 ± 0.00^{b}
Max Temperature (°C)	12.30 ± 0.00	16.92 ± 0.00	16.92 ± 0.00
Offset Temperature (°C)	1.13 ± 0.05^{a}	7.83 ± 0.15^{b}	8.00 ± 0.00^{c}
Enthalpy (J/g)	-117.88 ± 2.90^{b}	-125.50 ± 2.02^{a}	-107.11 ± 0.58^{c}
Melting and crystallization properties for stabilized sample			
Melting behavior			
Onset Temperature (°C)	18.21 ± 0.0.7^{a}	23.15 ± 0.01^{c}	20.39 ± 0.08^{b}
Max Temperature (°C)	31.25 ± 0.00^{b}	32.24 ± 0.00^{c}	30.73 ± 0.00^{a}
Offset Temperature (°C)	39.58 ± 0.01^{c}	38.32 ± 0.00^{b}	37.17 ± 0.61^{a}
Enthalpy (J/g)	65.63 ± 0.08^{c}	60.22 ± 0.04^{a}	62.00 ± 0.00^{b}
Crystallization behavior			
Onset Temperature (°C)	16.51 ± 0.02^{a}	18.41 ± 0.01^{c}	18.15 ± 0.00^{b}
Max Temperature (°C)	11.30 ± 0.00	14.43 ± 0.00	13.40 ± 0.00
Offset Temperature (°C)	0.23 ± 0.04^{a}	6.75 ± 0.04^{c}	4.11 ± 0.00^{b}
Enthalpy (J/g)	-101.13 ± 0.52^{b}	-105.28 ± 0.52^{b}	-121.58 ± 0.52^{a}

Values are the mean ± standard deviation of triplicate; means a different letter within a row are significantly different ($p < 0.05$) as measured by Tukey test.

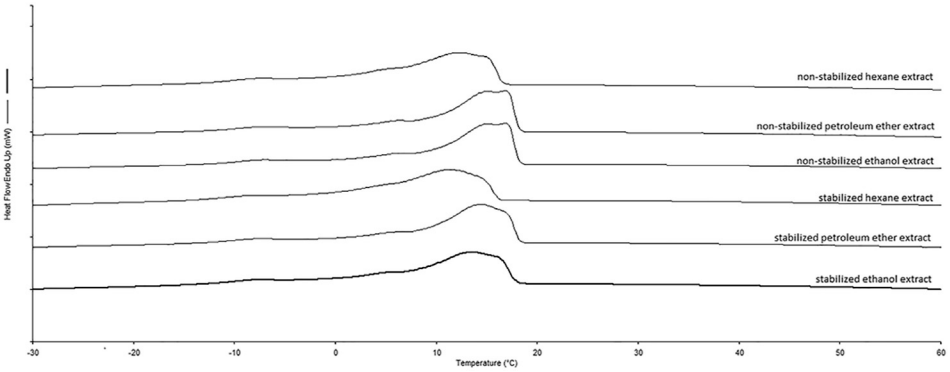

Figure 3. Differential Scanning Calorimetry (DSC) crystallization curve for non-stabilized and stabilized MPSF extracted from different solvent.

mEq/g, respectively. These values showed that the ethanol extract had less stable fats with high rancidity compared with hexane and petroleum ether solvents. Although PV for the three extracts did not exceed 10 mEq/g, the ethanol extract was more unstable and prone to oxidation compared with hexane and petroleum ether. Thus, hexane and petroleum ether MPSF can be easily stored with minimal deterioration than the ethanol MPSF (Kittiphom and Sutasinee, 2013).

Corresponding to the IV, the SMPs of MPSF for the hexane and petroleum extracts were significantly ($p < 0.05$) higher than that of ethanol MPSF. The SMP for ethanol MPSF decreased with the increased in the IV. This finding was due to the increment of the unsaturation values due to the presence of the low melting point unsaturated fatty acid such as the linoleic acid. However, the values were in line with the desirable melting properties of the commercial CB (25.3 ℃–35.0 °C) and palm oil-mid fraction (28.1 ℃-39.25 °C) reported by Saadah and Nazaruddin (2010), Nazaruddin et al. (2014), Sonwai et al. (2014), Jin et al. (2016), Jin et al. (2017), and Jin et al. (2018). In addition, the changes in the RI of MPSF were observed; ethanol MPSF had the highest RI values, followed by petroleum ether and then hexane MPSF. This finding was associated with the changes in the unsaturation values of the fat. According to Jahurul et al. (2018), high RIs were due to the increment in the composition of stearic, oleic and linoleic acids. However, the changes in the RI for the three solvent extracts were not significantly different, and these results were similar to changes in the RI values of KSO extracted using hexane, ethyl acetate and acetone (Okeleye and Betiku, 2019). The RI values for MPSF were found to be higher than those for the reported MPSF (Jahurul et al., 2018), and MKF (Munchiri et al., 2012; Jahurul et al., 2014a) but similar to those for KSO (Okeleye and Betiku, 2019) with RI of 1.4594, 1.4562–1.4597, and 1.464–1.465, respectively.

The physicochemical properties for the petroleum ether MPSF were not significantly ($p < 0.05$) different from those of hexane MPSF, but the yield obtained was lower than that of hexane MPSF. The physicochemical properties showed that hexane was the best solvent for the extraction of MPSF given that it had the highest total fat yield with good stability and desirable melting profiles compared with ethanol MPSF.

3.3. Effect of solvent pre-treatment on thermal properties of MPSF

3.3.1. Melting behaviour

The melting curves for non-stabilized and stabilized MPSF extracted using hexane, petroleum ether, and ethanol are shown in Figure 2. The onset temperature, maximum temperature, offset temperature, and enthalpy of the fat samples were measured (Table 2). The melting properties for the extracted MPSF were influenced by solvents exposure, resulting in high melting temperature of petroleum ether extract, followed by hexane and ethanol extract with the onset and offset temperature of 21.41–23.15 °C and 34.71–38.32 °C, 16.23–18.21 °C and 34.85–39.58 °C, 19.22–20.39 °C and 34.13–37.17 °C respectively. Although, most of the fat samples showed a single endothermic peak.

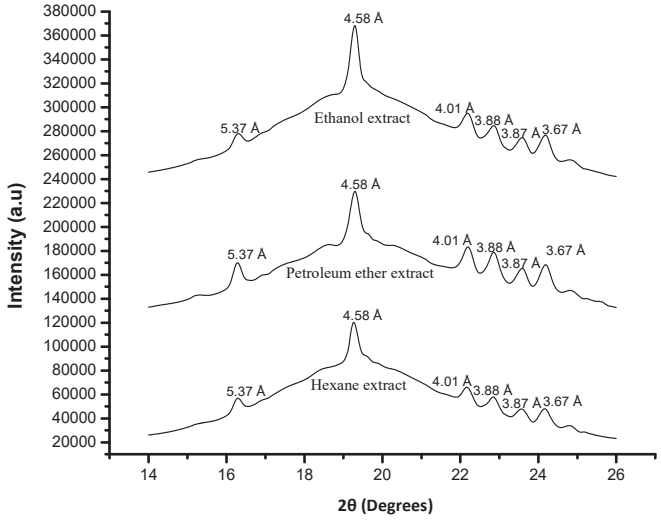

Figure 4. Diffraction pattern and short spacing of MPSF extracted from different solvent at 25 °C.

Petroleum ether showed narrow melting peak similar to the hard chocolate fat reported by Jin et al. (2018), whereas hexane and ethanol MPSF exhibited broad peaks comparable with the melting curves of hard palm oil-mid fraction. Both melting profiles for non-stabilized and stabilized MPSF showed significant ($p < 0.05$) changes, and petroleum ether MPSF had the highest onset temperature, followed by ethanol and the hexane MPSF. By contrast, different trends were observed in the offset temperature; hexane MPSF had the highest offset temperatures, followed by petroleum ether and ethanol MPSF. The melting profiles for the stabilized fat greatly improved as the offset temperature shifted to 37.17 °C–39.58 °C, indicating the presence of the high-melting symmetrical triacylglycerols (TAGs). MPSF is mainly composed of SOS (28.7%–40.71%), POS (11.6%–11.93%) and SOO (11.2%–26.88%), corresponding to the high melting properties of the fat (Jahurul et al., 2018; Norazlina et al., 2020b). Moreover, the offset temperature for MPSF was similar to the melting profiles of commercial CB (26.8 °C–40.7 °C), which is desirable for the production of chocolate (Jin et al. 2016, 2018; Kadivar et al., 2016; Huang et al., 2021; Zhang et al., 2020). Ethanol MPSF showed low offset temperature, which corresponded to the high unsaturation value that was due to the presence of the unsaturated fatty acid and low-melting TAGs compared with hexane and petroleum ether MPSF.

3.4. Crystallization behaviour

Contrasting trends were observed in the crystallization properties of the studied fat. As shown in Table 2, the hexane extracts crystallized

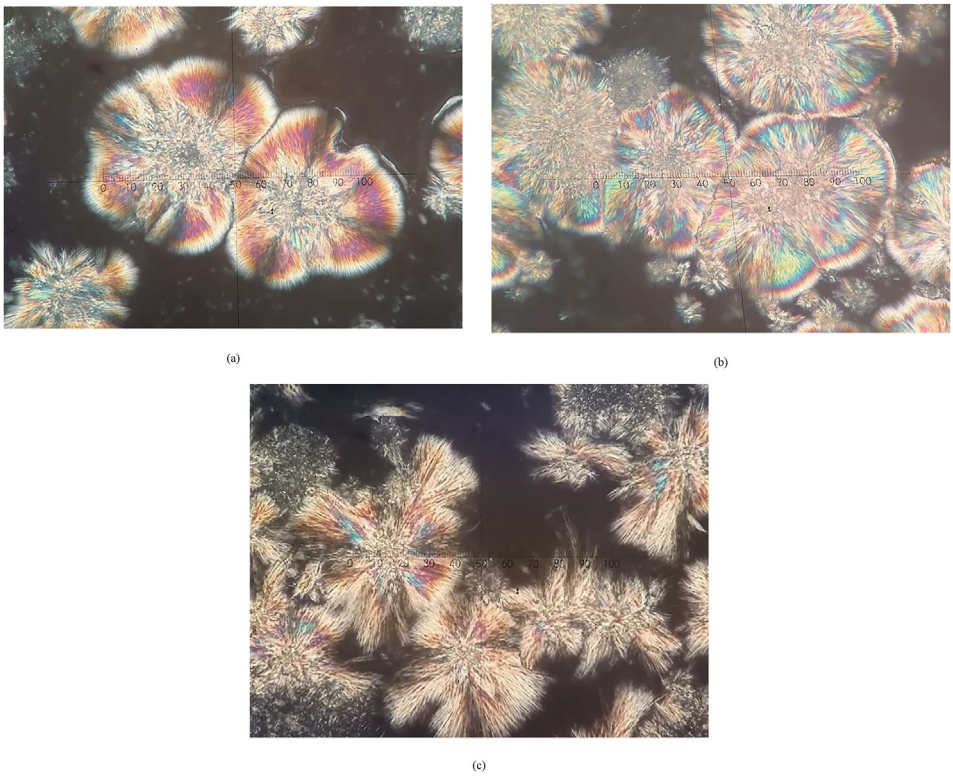

Figure 5. Crystal microstructure of MPSF extracted from hexane (a), petroleum ether (b) and ethanol (c).

slowly as the temperature decreased compared with the petroleum ether and the ethanol extracts for both non-stabilized and stabilized samples. The petroleum ether and ethanol extracts had high onset (18.15 °C–18.41 °C) and off set (4.11 °C–8.00 °C) temperatures compared with the hexane extracts. These values were higher than the reported stabilized (started at 14.95 °C and ended at -13.33 °C) and non-stabilized (start at 14.32 °C and ended at 13.24 °C) MPSF (Jahurul et al., 2019b) and CB (started at 15.5 °C and ended at 3.50 °C), indicating high content of high-melting TAGs such as the 1,3-disteroyl-2-oleoyl-glycerol (Jin et al., 2018). However, the hexane (started at 16.51 °C–16.68 °C and ended at 0.23 °C–1.13 °C) extracts for both non-stabilized and stabilized samples showed comparable values with the abovementioned MPSF and CB. The onset and offset values for all fat samples significantly ($p < 0.05$) decreased after stabilization for 7 days at 26 °C. The crystallization range for the stabilized fat samples widened as the exothermic peak shifted to the left. The crystallisation curve for the non-stabilized petroleum ether and ethanol extracts (Figure 3.) shows that the fat had two unseparated peaks, similar to the hard palm oil-mid fraction as reported by Jin et al. (2018). These results suggested that such properties were not suitable for chocolate manufacturing. However, hexane MPSF showed a broad single exothermic peak, which could be improved by some modification to narrow the curve. A narrow melting range of fat is desirable for easy tempering process (Beckett, 2008).

3.5. Changes on the morphology behaviour of MPSF extracted from different solvents

3.5.1. Polymorphic form

The polymorphic form is closely related to the crystallization profiles of a fat, and it is important for industrial application especially in confectionery industry (for chocolate formulation) (Norazlina et al., 2020b). This is because poor crystallization results in chocolate blooming or the formation of a grayish white film (Schenk and Peschar, 2004). The individual polymorphic form/type has a specific melting point, unique molecular organization and stability (Solís-Fuentes et al., 2005); thus, the polymorphic form of the MPSF was observed to determine the potential application of the fat. The changes in the fingerprint pattern and the evaluated short spacings for MPSF from the three extraction solvents are presented in Figure 4. The polymorphic form of MPSF was not affected by the extraction solvents, and similar fingerprint patterns were observed for the three MPSFs. The β-form was found to be the dominant polymorphic form in the MPSF with short spacing of 4.58 Å (β_2). These results showed similar dominant peak (β2, 4.58 Å) and small peak of β_1 (5.37 Å) form with the CB reported by Norazlina et al. (2020b). Small peak of β_1 (4.01 Å), β_1' (3.88 Å), β_1' (3.75 Å), and β_1 (3.67 Å) were also present in the diffraction pattern for the MPSF short spacings. This pattern indicated the presence of mixture β' form in MPSF (Mohammad Ali, 2013).

The presence of these peaks was weak in the hexane extracts. Thus, hexane was preferable for the extraction of fat for producing specialty fat, because β-phases, β (V) or β (VI) are desirable in chocolate manufacturing, and these phases are based on the saturated TAG composition (Schenk and Peschar, 2004). CB is mainly composed of 15.6% of POP, 45.4% of POS and 27.6% of SOS, thereby contributing to this unique β-form (Jin et al., 2018). Meanwhile, MPSF exhibited similar TAG types to CB but had less POP (5.9%) and POS (11.6%) contents and comparable SOS (28.7%) content with CB (Jahurul et al., 2018). Thus, MPSF and CB had similar diffraction patterns. The diffraction pattern for the extracted MPSF was also similar to the fingerprint region of β (V)-form for CB after the isothermal crystallization at 22 °C (Schenk and Peschar, 2004) and CB reported by Wang and Maleky (2018). These results suggested that the extraction of MPSF using hexane was preferable for producing specialty fat such as fat with resemblance to CB for industrial application.

3.5.2. Crystal microstructure

The polarized light microscopy was used to visualize the crystal network of the extracted fat to clarify differences in the textures of the fat mixture and to identify the type of crystallinity/morphological changes that occurred during crystal growth (Khairy & Tajul, 2016). This morphology is closely related to the polymorphic behavior of a fat (Mahisanunt et al., 2017). Futhermore, the concentration, morphological behavior (such as

crystal microstructure and polymorphic form), and interactions of the fat crystals were used to determine the texture of a product for use in confectionery (Rios et al., 2014). The crystal microstructure of MPSF obtained from different organic solvents after static crystallization for 2 days at 23 °C–25 °C is presented in Figure 5. All three fat crystal structures exhibited needle-like crystals that branched outward. The crystals had granular centers surrounded by feather needle-like crystal. Such crystal form were associated with the β polymorphic form (Ramel et al., 2017). Similar observation were reported by Bahari and Akoh (2018), Jahurul et al. (2018), Jahurul et al. (2014a,b) and Sonwai et al. (2014) who studied the MKF crystal structure and the influence of the addition of hard fat (palm stearin and palm oil-mid fraction) into MKF and illipe butter as specialty fat. These aforementioned fats were investigated as CB alternative fat, suggesting that MPSF could also be applied as CB alternative. Remarkable changes were observed on the crystalline structure of MPSF obtained from ethanol. For hexane (Figure 5a.) and petroleum ether MPSF (Figure 5b.), spherulitic crystals were observed with diameter of 40–80 μm. Both had a compact fat crystal structure, in which the crystals accumulated together. These crystalline structures also had a large densely packed center. Meanwhile, ethanol MPSF shows a less compact crystalline microstructure compared with hexane and the petroleum ether MPSF. The changes in the microstructure were due to the saturation degree of MPSF (Veronica et al., 2018). Corresponding to the IV, the ethanol extract had a high unsaturation value compared with the other two solvents. Unsaturated ethanol MPSF showed a loose, scattered microstructure (Figure 5c.), with needle-shaped crystals having small densely packed center. This structure was similar to the crystal structure of Kaew-Morakot (Thai cultivar) mango reported by Sonwai and Ponprachanuvut (2014). In addition, the fatty acid compositions, TAGs, and polymorphic behavior of fat could influence the crystal microstructure.

4. Conclusion

This work is the first report on the changes in the physicochemical, thermal and morphological properties of MPSF extracted using different types of organic solvents (hexane, petroleum ether and ethanol). The physicochemical and thermal behaviors of MPSF were significantly ($p < 0.05$) influenced by the extraction solvents, and results suggested that the MPSF extracted with hexane had better quality than the MPSF extracted with petroleum ether and ethanol. Hexane solvent had the highest fat yield with good rancidity and stability compared with the other two solvents. Hexane MPSF showed intermediate high melting properties with onset and offset temperature of 16.23–18.21 °C and 34.85–39.58 °C respectively. This behavior is desirable for CB fat, which is suitable for chocolate manufacturing. The polymorphic behavior of the MPSF was not affected by the extraction solvents, but obvious changes were observed in the crystal microstructure of the fat. The essential morphological features also indicate that hexane MPSF has the similar structure as CB which makes it applicable as CB alternatives provided by the similar properties that will give similar texture to the final product. Thus hexane is suggested as the suitable solvent of extraction. In addition, hexane is the most common organic solvent that has been used widely by the industry due to its high availability, great solubility with oil (non-polarity) and easy recovery. This findings are beneficial in providing information such as the choice of solvents for extraction. More studies on the composition of fat such as the fatty acid and TAGs compositions will be beneficial for further information on the influence of the extraction solvents on the properties of fat. The physicochemical properties are also influenced by the composition of fat.

Declarations

Author contribution statement

Norazlina, M. R.: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Tan, Y. S.: Performed the experiments; Analyzed and interpreted the data.

Hasmadi, M., Jahurul, M. H. A.: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

The authors are unable or have chosen not to specify which data has been used.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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