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To cite this article: H Soetjipto *et al* 2018 *IOP Conf. Ser.: Mater. Sci. Eng.* **349** 012024

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Chemical characteristics and fatty acid profile of butterfly tree seed oil (*Bauhinia purpurea* L)

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Abstract. Butterfly tree (Kachnar) in Indonesia is only used as ornamental plants in garden, park, and roadsides. The seed of Butterfly tree was extracted with n-hexane and physicochemical properties were determined based on Standard Nasional Indonesia (SNI) 01-3555-1998 while the oil chemical composition was determined using GC-MS. The result showed that yield of the oil as 57.33 ± 1.14 % (w/w) and the chemical characteristic of seed oil include acid value (13.78 ± 0.23 mg KOH/g) saponification value (153.32 ± 1.85 mg KOH/g), peroxide value (43.51 ± 0.57 mg KOH/g). The butterfly tree seed oil showed that linoleic acid (28.11 %), palmitic acid (29.2%), oleic acid (19.82%) and stearic acid (10.7.4 %) were the main fatty acids in the crude seed oils. Minor amounts of neophytadiene and arachidic acid were also identified.

1 Introduction

To fill the need of world vegetable oil and fat which is continually increased. Moreover is predicted reach 236 million of ton at 2020 [1] while for this moment palm oil is still used as an important source of vegetable oil especially in Indonesia. **Indonesian Palm Oil Producers Association (Gapki) & Indonesian Ministry of Agriculture** said that around 81% of Indonesian palm oil products in 2015 was exported to other countries. As we know that palm oil plantation destroys the tropical forest in Indonesian national park and gives a lot of bad influence to the environmental. So that very important for Indonesia to look for new alternative plants for support as vegetable oil sources.

Butterfly tree (*Bauhinia purpurea* L.) is a small evergreen ornamental plant in Indonesia, but this plant has already cultivated in Pakistan [2] The mature seeds and young pods also can be eaten in Indian native countries [3]

Dewi et al (2014) analyzed the composition of butterfly tree seed oil, in the total lipids, free fatty acids were present [4]. The lipid composition including neutral lipids (94.91%), phospholipids (1.86%) and glycolipids (3.23%) . The major fatty acid composition of neutral lipids were linoleic acid (51.32%), palmitic acid (29.31%) [3-4].

Purification oil was needed to reduce unlikely aroma, taste, and color, especially for vegetable oil because of the presence of mucous, gum, ash, minerals, free fatty acids, sterol, hydrocarbon, mono and diglyceride and pigment. [5-6]

De-gumming, neutralization, bleaching, and deodorization were purification steps which were done to vegetable oil. Except for food industrial, usually only de-gumming and neutralization were done. Phosphoric acid (H_3PO_4) was used for washing vegetable oil in de-gumming step because phosphoric



acid can change nonhydratable fosfatide to be hydratable and follow by water washing. Neutralization is important to do and using sodium hydroxide which is easy to find and cheap. The alkali concentration was used influence to the amount of emulsion in saponification process [6]. The objective of the present study was to obtain knowledge about the chemical characteristic and fatty acid profile of butterfly seed oil before and after purification method.

2 Material and method

2.1. Sample preparation and Extraction process (modified from [4])

The dry Butterfly tree seeds (100 g) was extracted by Soxhlet method for 6 hours in n-hexane (300 ml). The butterfly tree seed oil was obtained after all the solvent removed by rotary-evaporation.

2.2. Physicochemical Property Assays for the crude Butterfly tree seed oil [7]

Important physicochemical properties of the crude oil comprises moisture, density, acid value saponification value, peroxide value was determined according to SNI 01-3555-1998. While the aroma and color of the oil were determined as descriptive.

2.3. Moisture and Density of Butterfly tree seed oil

Moisture of Butterfly tree seed oil (1.0 g) was measured by using Moisturizer balance (Ohaus, MB 150) while the density (1.0 ml) was measured by analytical balance (Mettler H80. Mettler Instrument Corp., USA).

2.4. Acid value and acidity (SNI 01-3555-1998).

The amount of 2.0 g oil was added 50 ml ethanol 95% and 3 -5 drops phenolphthalein indicator, then was titrated by KOH 0.1 N until pale pink color.

$$\text{Acid value} = \frac{V \times T \times 56.1}{m} \quad \text{FFA (\%)} = \frac{M \times V \times T}{10 m}$$

V = Volume in ml standard KOH used

T = Normality of standard KOH

m = weight in gram of the sample

M = Molecular mass of dominant fatty acid (expressed as oleic acid)

2.5. Saponification value (SNI-3555-1998)

The amount of 2.0 g oil was added 25 ml KOH 0.5M, refluxed for 1 hour. After the sample have cooled the solution was added 0.5 ml phenolphthalein indicator and titrated by HCl 0.5M until the pink color disappeared

$$\text{Saponification value (mg KOH/g oil)} = \frac{56.1 \times T \times (V_0 - V_1)}{m}$$

V₀ = Volume in standard ml HCl 0.5 M used as a blank.

V₁ = Volume in standard ml HCl 0.5 M used as sample

T = Normality of standard HCl 0.5 M

m = weight in gram of the sample

2.6. Peroxide Value (SNI-3555-1998)

The amount of 0.3 g oil was added mixture of 55ml chloroform, 20 ml glacial acetic acid and 25 ml ethanol 95 %. Then 1 g KI was added to the solution and kept in the dark for 30 minutes. The next step 50 ml water-distillate free CO₂ was added to the mixture. The excess of Iod in the solution was titrated by Na₂S₂O₃ 0.02 N with a starch solution as indicator.

$$\text{Peroxyde value (mgrek/kg)} = \frac{(V_1 - V_0) \times T \times 1000}{m}$$

V_0 = Volume in ml standard sodium thiosulfate for blank

V_1 = Volume in ml standard sodium thiosulfate for sample

T = Normality of standard sodium thiosulfate solution

m = weight in gram of the sample

2.7. Fatty Acid Composition

The fatty acid composition of butterfly tree seed oil was analyzed according to IUPAC method 2.302 [8] And the analysis of methyl esters was performed on *Gas Chromatography-Mass Spectrometry* (GCMS-QP2010 SE Shimadzu) equipped with a flame ionization detector (FID) capillary column AGILENTTJ%W DB-(30 m x 0.25mm.). The column, injector, and detector temperatures were set at 80°C, 300 °C, and 300°C respectively. The flow rate of carrier gas Helium with split ratio 7.3.0 was set as 3.0 mL/min. The fatty acids were identified concerning the retention times of standard fatty acid methyl ester performed at the same condition.

2.8. Purification

2.8.1. Degumming process

Butterfly tree seed oil was heated at 7.0°C then added with 0.2% (v/w) solution of H₃PO₄ 20%, followed by stirring for 10 minutes at constant temperature. By using separating funnel, the impurities can be separated from the oil. Then the oil was washed with warm water (60°C) until pH of the washing water was neutral.

2.8.2. Neutralization Process

After the degumming process, oil is going to neutralization step. Oil was heated at 7.0-7.5°C, then add with 0.2% NaOH solution 0.3 N (0.87. ml), followed by stirring for 15 minutes then washed to neutral by warm water 60°C.

2.8.3. Statistical analysis

All determinations were carried out in triplicate and their mean value ($\pm$ standard deviation) is given.

3 Result and Discussion

3.1. The physicochemical properties of butterfly tree seed oil

Table 1 showed the physicochemical properties of butterfly tree seed oil before and after purification.

Table 1. Physicochemical properties of butterfly tree seed oil before and after purification		
Parameter	Crude oil	After Purification
Rendemen	14.18 %	8.12 %
Density (g/cm ³)	0.86 $\pm$ 0.02	0.82 $\pm$ 0.006
Color	Brownish yellow	Yellow
Aroma	specific	Specific
Acid value (mg KOH/g)	13.7.8 $\pm$ 0.23	3.87. $\pm$ 0.19
Peroxide value (mequiv O ₂ /kg)	43.51 $\pm$ 0.57.	15.62 $\pm$ 0.22
Saponification value (mg KOH/g)	153.32 $\pm$ 1.85	187.,39 $\pm$ 0.47.

The refining process for vegetable oil is necessary to do to eliminate the impurities from crude oils especially the phosphatides or so-called gums (degumming process). It was started with degumming then follow by neutralization, bleaching, and deodorization. For nonfood industrial consumption, the third and fourth steps (bleaching and deodorization) is not done. This process aims to neutralize water degummed or partially purified oils by using NaOH (alkali neutralization).

Seeds of *B. purpurea* (100 g) contained 14.18 % (w/w) of oil. This seed oil content is not quite different with previously reported [4]. The density of this oil $0.86 \pm 0.02 \text{ g/cm}^3$ similar to other edible oil [9] The acid value is an important variable for the oil quality because it showed the content of free fatty acid inside the oil. The lower the free fatty acid, the better the quality of oil [10] Peroxide value is indicated by the oxidative state of oils. The peroxide value of butterfly tree seed oil is relatively high 43.51 ± 0.57 . (mequiv O₂/kg). Oils easy become rancid when the peroxide value ranges from 20 to 40 mequiv O₂/kg of oil. Saponification value is expressed as the number of mg KOH required to saponify 1 g of sample [11]. Saponification value of Butterfly tree seed oil indicates that the oil contains high molecular weight fatty acids on average 153.32 ± 1.85 (mg KOH/g) or very high proportion of low molecular weight triacylglycerols.

The result showed that purification step increases the quality of the oil. The colour of the oil to be clean, bright yellow and reduced acid value (13.78 ± 0.23 to 3.87 ± 0.19 mg KOH/g) and peroxide value (43.51 ± 0.57 . to 15.62 ± 0.22 mequiv O₂/kg) whereas increased saponification value (153.32 ± 1.85 to 187.39 ± 0.47 . mg KOH/g). As a comparison with another species of Bauhinia, *B. variegata* seed oil showed the level of Free Fatty Acid (FFA) *B. variegata* 0.6 mgKOH/g while 0.9 mgKOH/g sample for *B. linnaei* [3]. The lower acid value, the more stable of oil. A low acidity and peroxide value indicate a good quality and stability. The oils which have high peroxide values are not so stable and easily become rancid with an undesirable odor.

3.2 Fatty acid profile of butterfly tree seed oil

The fatty acid composition is presented in Table 2. The 4 major fatty acids of crude butterfly tree seed oil were linoleic acid (28.11%), palmitic acid (25.42%), oleic acid (19.82%), stearic acid (10.74%) and palmitic acid (3.7.8%). Another compound (6 compounds) which are each compounds less than 3%.

After purification, the formation of fatty acid profile of butterfly tree seed oil changed. Linoleic acid, palmitic acid, and stearic acid were reduced to be 16.00%, 22.60%, and 7.23% respectively, whereas oleic acid increased to 25.53%. As a comparison, the major fatty acids in butterfly tree seed oil obtained is similar to Arain et al., [3] A very low acidity in oil indicate is good quality and stability [12]. The difference between species, or the same species but come from different places which have a different climate, soil or environment will influence the quality of the plants. The phytochemical variations and content of the compounds of species is dependent on geographical location. Purification process also influenced the fatty acid profile but only in content not in various kind of fatty acid.

Table 2. Fatty acid composition of crude and purified butterfly tree seed oil

No	Fatty acids	Content (%)		
		This study		Arain et al. [3]
		Crude oil <i>B. purpurea</i>	Purified oil <i>B. purpurea</i>	<i>B. variegata</i>
1	Linoleic acid (C18:2)	28.11	16.00	42.1 ± 1.8
2	Palmitic acid (C16:0)	29.20	22.60	22.1 ± 1.5
3	Oleic acid (C18:1)	19.82	25.53	0.5 ± 0.1
4	Stearic acid (C18:0)	10.7.4	7.32	17.5 ± 1.7 .
5	Arachidic acid	1.17.	-	

4 Conclusions

The physicochemical of butterfly seed oil and its fatty acid profile were influenced by degumming and neutralization process (purification). The moisture, density, acid value, saponification value and peroxide value of bauhinia tree seed oil were changed after purification. This oil could be a good source of natural oil rich in linoleic acid and oleic acid. Purification method reduced the content of linoleic acid but increase the oleic acid

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