The effect of chemical refining process on the physicochemical and fatty acid composition of watermelon seed oil (*Citrulus lanatus* L.)

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**Abstract.** A lot of watermelon seed waste is wasted, generally its only used to make “kuaci”, a kind of salted saline a snack made from watermelon seeds. As a member of *Cucurbitaceae* family, watermelon seeds can be used as a source of vegetable oil that predicted contains squalene inside like other *Cucurbitaceae* members. This study aims to determine the effect of refining process on the physicochemical properties and composition watermelon seed oil. Watermelon seed oil which is obtained by using Soxhlet extractor in n-hexane solvent was refined in 3 steps, which was degumming, neutralization, and bleaching, respectively. Degumming process used 3 concentrations of H₃PO₄, which were 0.15%; 0.20%; and 0.25%, respectively; whereas 3 zeolit concentrations of 2%; 4%; and 8%, respectively were used in bleaching steps. Data were analyzed by using 3x3 Factorial design and it was laid out with Randomized Completely Block Design, 3 replications and as the block is time analysis. The optimum yield of the watermelon (*C. lanatus* L.) seed oil is obtained in the amount of 48.47±0.91% at 0.25% phosphoric acid concentration and 4% Zeolite concentration. The result of physicochemical analysis before and after the refining process have changed. The density, peroxide value and acid value show a significantly decrease, while only the saponification value shows an increase. Fatty acid composition (profile) of WSO before and after refining process does not change, comprises linoleic acid, palmitic acid, stearic acid and squalene (unsaturated hydrocarbon). Linoleic acid is the main compound of WSO (76.69%), followed by palmitic acid (14.60%), stearic acid (6.53%) and squalene (2.18%), respectively.

1. **Introduction**

Watermelon (*Citrulus lanatus* L.) is classified as a member of *Cucurbitaceae* family. Usually, people are only using the fruit flesh and the skin is preserved as a sweet snack while the seeds are removed. Watermelon seeds are only used to make “kuaci”, a kind of dry salty snacks made from watermelon seeds. These seeds contain high protein and unsaturated fatty acids as linoleic and oleic acid [1,2]. Watermelon seeds contains plant oil in amount of 16.79 %, also contains linoleic acid 75.71%, palmitic acid 18.10%, and stearic acid 6.19% [3].

Oil extracted generally contains phospholipid, free fatty acids, pigment and sap which affects the oil quality such as aroma, shelf life, and clarity of the oil [4]. The refining process will improve the shelf life of M.oleifera oil and the chemical refining process can increase the quality of kenaf (*Hibiscus cannabinus*) seed oil [5,6]. Therefore, improving the oil quality has to be done. Refining oil aims is to
avoid oil damage which usually occurs due to the hydrolysis and oxidation reactions. The presence of free fatty acid can trigger oil damage, which it affects the stability of vegetable oil [7].

The first step of the refining process is degumming, followed by neutralization, and then bleaching. In the degumming process, the main compound to be separated is the phosphatide. This compound will cause unwanted colors, flavors, and reduce the shelf life of the oil [8]. The sap or mucus, gum which contains phosphatide, protein, carbohydrate, residues, water, and resin will be separated to increase the quality of vegetable oil [9]. The neutralization step using sodium hydroxide (NaOH) is mostly done on an industrial scale because it is more efficient than other neutralizations. This step is also able to remove the color and dirt remains in the oil. And bleaching is the next step which will (want) to remove the unwanted colors remain inside the oil. Bleaching, as the last step, can be done using active carbon, zeolite, or bentonite [10].

Refining process using phosphoric acid in degumming step followed by a neutralization step affect the physicochemical properties of kachnar seed oil (Bauhinia purpurea L.) [11]. The refined oil obtained show the more brighter color, cleaner than the crude oil before refining and it also has a change in its fatty acid composition. In this study has focused on the removal of impurity especially of gummy materials and waxes to improve the physicochemical and composition of Watermelon Seed Oil.

2. Materials and methods

Watermelon seed was obtained from a field in Trengguli village, Demak, Central Java Indonesia. All the solvents and chemicals used were purchased of analytical grade (E-Merck Germany, and SMART lab Indonesia). The instruments used in this work were analytical balance to the accuracy 0.0001 g (Ohaus PA124, USA), soxhlet apparatus, waterbath (Memmert WNB 14), rotary evaporator (Buchi R-114, Swiss), Gas-chromatography-Mass Spectrophotometer (GC-MS) perkin-Elmer.

2.1. Sample preparation method [2]
The seeds are washed and dried in oven at 60°C for 24 h and then it is crushed with a grinder.

2.2. Refining process of Watermelon Seed Oil (WSO)

2.2.1. Degumming process [12] modified
Degumming process using three concentrations of phosphoric acid (85% conc.) whichater are 0.15%, 0.20%, and 0.25%, respectively.

2.2.2. Neutralization process [13] modified
In this process, a concentration of 9.5% sodium hydroxide was used.

2.2.3. Bleaching process [14] modified
The neutralized oil added using three zeolite concentrations which are 2%, 4%, and 8%, respectively. The natural zeolite was used in this experiment, before use zeolite was activated by heating in a furnace.

2.3. Physicochemical characteristics of WSO
All of the items were determined according to the AOCS method. The yield of oil extraction obtained was determined by gravimetric method using 4 digits balance [15], density [16], water content [17], acid value (AV) [18], peroxide value (PV) [19], and saponification value (SV) [20], respectively.

2.4. Statistical analysis
Data were analyzed using a 3x3 Factorial design, and it was laid out with a Randomized Completely Block Design (RCBD), 3 replications and as a group is the time of analysis. The first factor is H₃PO₄ concentration consisted of 3 concentrations which are 0.15%; 0.20%; and 0.25%, respectively. The second factor is the zeolite concentration also consisted of 3 concentrations are: 2%, 4%, 8%,
respectively. The comparison between treatment means was carried out with honestly significant difference test with 5% of level significance [21].

3. Result and discussion

Table 1 summarizes the yield of oil obtained after refining process using phosphoric acid 0.15%, 0.20% and 0.25%. The optimal yield of WSO was obtained with the use of 0.15% as much as 33.43 ± 4.35 % because the use of phosphoric acid 0.15% and 0.25% statistically give the same results, while on the use of 0.20% phosphoric acid give lower yield.

Table 1. Yield of WSO in various concentration of 85% phosphoric acid.

| Yield (%) | 85% H₃PO₄ |
|-----------|-----------|
|           | 0.15%     | 0.20%     | 0.25%     |
| Average ±SE⁹ | 33.43±4.35 | 26.77±11.06 | 35.45±9.63 |
| W⁹ = 5.54 | (b)⁵⁺     | (a)⁵⁺     | (b)⁵⁺     |

⁹ SE = Standard Error of Estimate; W = Honestly Significant Difference (HSD) at 5% level of significance.

⁵ Numbers followed by the same letter at the same row or column indicate that the treatment means are not significantly different, whereas the numbers followed by different letter at the same row or column indicate that the different treatment means are significant different. This note is also used for table 3.

According to the previous studies, during oil refining process showed that the oil requires phosphoric acid in unequal amounts depending on the oil type, and generally its ranged between 0.15–0.25%. The addition of phosphoric acid in degumming step will change unhydratable gum to be hydratable and then remove both of them [22,23]. Gum must be removed in the degumming step, because it will inhibit the oil separation due to the soap formation in the saponification process. The gum is also able to act as an emulsifier that’s why it will emulsify the oil causing the yield to be decreasing [9].

![Degumming reaction](image)

Figure 1. Degumming reaction [24].

After degumming WSO was neutralized by using NaOH until the rinse water neutral, then followed by bleaching step. The yield obtained after bleaching process is shown in table 2.

Table 2. Yield of WSO in various concentration of zeolite.

| Yield (%) | Zeolite concentrations |
|-----------|------------------------|
|           | 2%         | 4%         | 8%         |
| Average ±SE | 34.75±8.79 | 31.68±6.0  | 29.22±10.02 |
| W = 5.54   | (a)        | (a)        | (a)        |

The optimum yield in the amount of 34.75%±8.79 was obtained in the use of 2% zeolite concentration. The more the use of zeolites up to 8% neither increases nor decreases yield. Zeolite has
3 layers structure that allows triglyceride molecules to be absorbed due to interactions with H⁺ ions in the envelope contained in the zeolite structure [25]. Zeolite also has an open pores structure with a large surface area so as to allow the uptake of dye molecules. Zeolite can be used to remove oil colorant because it has a high adsorptive. The addition of 4% zeolites’ concentration combined with 0.25% of phosphoric acid (85% conc.) gave the maximum WSO yield in the amount of 48.47±0.91% [26]. The maximum WSO yield obtained is suspected due to the oil is relatively clean that the soap result in the purification process does not absorb many impurities and does not reduce the yield [27].

Table 3. The average yield of WSO resulting from interaction after the refining process.

| H₃PO₄ Concentration (%) | Zeolite concentration (%) |
|-------------------------|---------------------------|
|                         | 2            | 4          | 8          |
| 0.15% W= 9.59           | 34.79± 4.42 (a) | 21.06± 11.67 (a) | 44.43±0.73 (b) |
| 0.20% W= 9.59           | 33.51±3.33 (a) | 25.50±0.46 (a) | 21.30±8.28 (a) |
| 0.25% W= 9.59           | 35.96±3.86 (a) | 48.47±0.91 (b) | 21.30±0.99 (a) |
|                         | (b)          | (a)        | (ab)       |
|                         | (b)          | (c)        | (a)        |

3.1. Physicochemical properties of WSO (C.lanatus) pre- and post- refining.
Physicochemical properties of WSO are shown in table 4. Crude oil of WSO was obtained as much 23.22%, this result is lower than Baboli (50%) [1], but is higher than the Ariani result of 16.79% [3]. This difference is possible due to several reasons, for example: a watermelon varieties, soil conditions, and the environmental conditions in which watermelons grow [28].

Table 4. Physicochemical analysis of WSO.

| Parameter                        | WSO Before | WSO After | Ariani [3] | Baboli [1] |
|----------------------------------|------------|-----------|------------|------------|
| Yield (%)                        | 23.22      | 48.47     | 16.79±0.82 | 50±0.2     |
| H₂O concentration (%)            | 9.14       | 0.49      |            |            |
| Density (g/ml)                   | 0.82       | 0.80      |            |            |
| Peroxide value (Mequiv O₂/Kg Oil)| 4.50       | 0.90      | 73.50±1.59 | 3.24±0.1   |
| Saponification value (mg KOH/g Oil) | 26.92     | 88.86     | 77.87±2.26 | 200±0.1    |
| Acid Value (mg NaOH/g Oil)       | 4.13       | 1.55      | 5.05±0.00  | 2.4±0.1    |

After the refining process changes occur in the physicochemical properties of the oil, for example, a decrease in the water content of oil from 9.14% to 0.49%, peroxide value from 4.5 to 0.9. Decrease of water content occur because in the degumming process using phosphoric acid, the water contained in oil will be washed into the acid solution, so that the water content of the oil will drop. The water content in the oil is an important parameter due to the hydrolysis reaction that can reduce the quality of the oil. The decrease of peroxide value cause of the present of sodium hydroxide in neutralization process. This
compound will react with free fatty acid and peroxide polymers [29]. Oil oxidation is strongly influenced by fatty acid constituent, heating time and storage condition [30]. The higher the peroxide value the worse the oil condition [31].

The density of the oil before and after refining does not differ greatly from 0.82 to 0.80 because the refining process removed the oil impurities so that it affects its density [32]. The saponification value of WSO increased after the refining process. The low saponification value indicate that the oil has large fatty acid molecules and vice versa [33].

The refining process also decreased acid value because when saponification process occurred, free fatty acid will react with sodium hydroxide and separated to become soapstock. The higher the acid value of the oil, the worse the quality of the oil because a lot of triglyceride molecule hydrolyzed [34]. The refining process can increase oil quality [33].

3.2. Fatty acid composition of the WSO

The fatty acid composition of the crude and refined WSO is shown in table 5, while the results of the gas chromatogram are shown in figure 2 and figure 3. Four fatty acids are detected from WSO before refining process, each of the peaks shows time retention is 24.523, 16.069, 15.799, and 13.473 minutes respectively, while after refining process WSO also demonstrated four fatty acid with retention time 24.537, 16.149, 15.914, and 13.523 minutes respectively. Every peak was analyzed using MS to determine the compound then compare with Wiley database figure 2 and figure 3.

Figure 2 is a spectrum of one compound from WSO shows a similar fragmentation with figure 3 spectrum of 9,12-Octadecadienoic acid from Wiley database, so it is believed that spectrum in figure 2 is 9,12-Octadecadienoic acid. By using the similar way all the peaks that appear in WSO spectra have been identified. The result of identification from all the peaks before and after refined are shown in table 5.

After the refining process the composition of fatty acid is similar, a little change in fatty acid composition was observed especially in the concentration of the compounds. The reason for this change is the present of alkali solution in neutralization which causes the saponification reaction.

The linoleic acid content in this study which are 77.80% is higher in comparison with Baboli finding 68.3% [1]; and a little bit higher than linoleic acid of Ariani report is in the amount of 75.71% [3]. Table 5 also shown the presence of a squalene compound in relatively small amount (2.18%) after purification. Squalene is a powerful antioxidant compound so it is widely used in cosmetics and treatment for skin health. This compound is also able to reduce the toxicity of drugs consumed and has antitumor activity [35].

![Figure 2. Chromatogram of WSO before refining process.](image-url)
Figure 3. Chromatogram of WSO after refining process.

Figure 4. Spectrum of 9,12-octadecadienoic acid from WSO.

Figure 5. Spectrum of 9,12-octadecadienoic acid from WSO (Wiley database).

Table 5. Fatty acid profiles from all the peaks of WSO before and after refined.

| Name of compounds                     | MW | Molecule structure | Before Retent. Time (min) | Area (%) | After Retent. Time (min) | Area (%) |
|--------------------------------------|----|--------------------|---------------------------|----------|--------------------------|----------|
| Hexadecanoic acid (Palmitic acid)    | 256| C_{16}H_{32}O_{2}   | 13.473                    |          | 13.04                     |          |
| 9,12-Octadecadienoic acid (Z,Z)-     | 280| C_{18}H_{32}O_{2}   | 15.799                    | 77.80    |                          |          |
| (Linoleic acid)                      |    |                    |                           |          |                          |          |
| Octadecanoic acid (Stearic acid)     | 284| C_{18}H_{36}O_{2}   | 16.069                    | 5.43     |                          |          |
| Squalene                             | 410| C_{30}H_{50}        | 24.523                    | 3.73     |                          |          |

^a MW = molecule weight.
4. Conclusion
The best results of WSO were obtained with the addition of phosphoric acid 0.25% with 4% zeolite is 48.47±0.91%. Refining process influence physicochemical properties and concentration of fatty acid but it is not influence the profile of WSO. The density, peroxide value and acid value show a decrease, while the saponification value shows an increase. Fatty acid composition (profile) of WSO before and after refining process does not change, comprises linoleic acid, palmitic acid, stearic acid, and squalene (unsaturated hydrocarbon). Linoleic acid is the main compound of WSO (76.69%), follow by palmitic acid (14.60%), stearic acid (6.53%), and squalene (2.18%).

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Conflict of interest
The authors declare they have no conflict of interest.

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