Composition of Fatty Acids and Squalene Content of Wild Spinach (Amaranthus dubius Mart) Seed Oil

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Composition of Fatty Acids and Squalene Content of Wild Spinach 
(*Amaranthus dubius* Mart) Seed Oil

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Abstract

This study aims to determine the yield of wild spinach seed oil obtained by continuous extraction methods using Soxhlet apparatus for 10.5 hours in *n*-hexane solvent, the physio-chemical properties, and the composition of wild spinach seed oil using the Gas Chromatography-Mass Spectrometry (GC-MS) method. Refinement of wild spinach seed oil was done by degumming and neutralization. The yield of wild spinach seed oil extraction before refinement was 4.36 ± 0.29% (w/w) while after refinement was 0.68 ± 0.033% (w/w). Physio-chemical properties of wild spinach seed oil after refinement was as follows: water content 0.37%; peroxide value 9.5 meq/kg; acid value of 0.53 mg KOH/g oil; saponification value 208.9 mg KOH/g oil. The results of GC-MS analysis showed that the wild spinach seeds were composed of 5 main components: dodecanoic acid (0.28%); tetradecanoic acid (0.08%); palmitic acid (1.31%); squalene (94.9%); and linoleic acid (3.34%).

Keywords: spinach seed oil, physio-chemical properties, GC-MS, squalene

Introduction

Squalene is a type of lipid that acts as a biosynthetic precursor for all steroids in animals and plants. Squalene was first discovered in the shark liver oil by a Japanese researcher Tsujimoto Mitsumaru in 1916 [1] The name of squalene is assigned from the genus of Shark, which was isolated, *Squalus* spp. [2] His compound is widely used in the field of beauty in the form of ingredients for cosmetics and also plays an important role in the health field because it can reduce the risk of cancer and reduce cholesterol levels in the blood [3]. This causes squalene to have high economic value, and demand for squalene continues to increase. The various benefit of squalene mentioned above is one of the reasons for increased hunting and killing of sharks for their fins and hearts [4]. To maintain the sustainability and preservation of marine mammals, research on vegetable squalene has been started to be developed several years ago. The results showed several plants contain squalene such as olive (564 mg/100 g oil) [5] pumpkin seed 8600mg/100 g oil [6], and palm (0.1-1300mg/100 g oil) [7]. The *Amaranthus* genus in the Amaranthaceae family type has been reported to contain squalene in relatively high amounts on its seed oil (4.20% to 6.14%) compared to other vegetable oils despite having a little yield [8]. The genus of Amaranthus, belonging to the family of Amaranthaceae have more than 60 species. One of them was known as a wild amaranth (*Amaranthus dubius*), it is a species of Amaranth that has not been widely used.

Spinach is a plant that originated in America, but now spinach has spread throughout the world. There are many examples of spinach types both used in the food sector and those that have not been used as in the type of wild spinach. Wild spinach is a plant of the Amaranthaceae family which is commonly known as “chowlai” which is a wild vegetable and is also often considered a weed. Wild spinach often grows in the yard of the house, school yard, bushes, and on the edge of the river. As the name implies, wild spinach, this type of spinach is easily found and available in large quantities and has not been utilized [9].

Types of spinach that are often used in the extraction of spinach seed oil are *A. cruentus* L., *A. hypochondriacus* L., and *A. hybridus* [8]. There are several types of wild spinach in Indonesia, one of which is *Amaranthus dubius* Mart spinach which is quite abundant in nature and can be easily obtained because it grows wildly but has not been widely used. The appearance of wild spinach (*A. dubius* Mart) is very similar to or resembles thorn spinach (*A. spinosus*). The presence of thorns in thorn spinach is an easy feature to distinguish it with *A. dubi*
us Mart. In this study, we want to determine the yield of wild spinach (A. dubius Mart) seed oil, the physiochemical properties, the fatty acid and the squalene content.

Materials and Methods

This research was carried out in the chemistry laboratory, Faculty of Science and Mathematics, Universitas Kristen Satya Wacana from 19 August 2019 to 27 November 2019.

The raw material used was wild spinach seeds (A. dubius Mart) obtained from Salatiga and surrounding areas. All reagents and solvents used from Smart Lab Indonesia, with pro analysis degree including n-hexane, ethanol 96%, chloroform, glacial acetic acid, hydrochloric acid, potassium iodide, phenolphthalein, methyl orange, potassium hydroxide, sodium thiosulfate, anhydrous sodium sulfate, phosphoric acid, and sodium hydroxide. The equipment used in this study was Soxhlet apparatus, rotary evaporator (Buchi R0114, Switzerland), water-bath (Memmert WNB 14, Memmert GmbH+KG, Germany), analytical scale with accuracy of 0.0001 gram (Ohaus PA124, USA), analytical balance with 0.01-gram accuracy (Ohaus TAJ602, USA). The Gas Chromatography-Mass Spectrometer (GCMS-QP2010 SE Shimadzu, Japan) analysis was conducted at Semarang State University.

Sample preparation. Wild spinach seeds were separated from the wrapper petals. After being grinded and sifted with a 60-mesh sieve the sample stored in a dry container ready for the next step.

Extraction by continuous extraction method (modified AOCS Aa 4-38-1998). Fifty grams of wild spinach seeds that had been mashed were extracted with 250 mL of n-hexane solvent using continuous extraction method using Soxhlet extractor apparatus at 80 °C for 10.5 hours or until the solvent turned colorless. The extract was concentrated using a rotary evaporator under vacuum pressure and temperature around 60 °C until the solvent was used up then steamed for ± 1.5 hours to remove the n-hexane which still remained after the evaporation process from the oil.

Characterization of the physico-chemical properties of wild spinach seed oil. In this study, the physicochemical parameters which were tested included descriptive determination of the aroma and color. Yield and density determination using methods described in AOCS Aa 4-38-1998 and AOCS Cc 10A-25-2005, respectively. To determine water content, acid value, peroxide value, and saponification value using methods described in; SNI 01-3555-1998.

Oil refinement. The refining wild spinach seed oil include degumming (AOCS, 1989 with modification) and neutralization (AOCS, 1989 with modification).

Chemical composition analysis of wild spinach seed oil. Analysis of the chemical composition of wild spinach seed oil (A. dubius Mart) was done using Gas Chromatography-Mass Spectrometry. The test was conducted in the laboratory of the Faculty of Mathematics and Natural Sciences, Semarang State University. The type of column used was AGILENT% W DB-I (30 m x 0.25 mm) at 65 °C. Injection temperature of 250 °C at 74.5 kPa with a total flow of 64.2 mL/min and a linear velocity of 40 cm/sec. The carrier is Helium and ionizing method E1+.

Results and Discussion

The yield of wild spinach seed oil has been presented in Table 1. The average yield of wild spinach seed oil before refinement was 4.36 ± 0.29% (w/w). The yield was lower compared to the yield of spinach oil from A. spinosus L and A. viridis L that are also found in Indonesia. The oil yield of A. spinosus L and A. viridis L were 7.21% and 5.23%, respectively, with Soxtec HT 6 (Tecator, Sweden) as extractor apparatus [8]. The oil was extracted by organic solvents, so it requires a refining process to remove unwanted compounds such as pigments, free fatty acid, phospholipid etc to give a better-quality oil. After refinement, the yield of wild spinach seed oil decreased from 4.36 ± 0.002% to 0.68 ± 0.032%.

Less oil yield was due to the addition of H3PO4 in the degumming process which made phospholipid compounds in wild spinach crude oil that was insoluble in water became soluble [12]. Hydratable phosphatides were easily separated by adding water at low temperature of ± 40 °C. Also, gum and impurities that caused damage to oil such as phosphatides, proteins, resins or metals, was settled and rinsed away when distilled water added which contribute to reduction in oil yield [6]. The degumming process was carried out before the neutralization process.

The soap formed from the reaction between fatty acids and NaOH in the neutralization process would absorb gum resulting from the degumming process and would also be wasted when rinsing with distilled water [12].

Table 1. Calculation Result for Average Yield (% ± SE) of Wild Spinach Seed Oil

| Yield of oil/gram seeds | Before refinement | After refinement |
|-------------------------|-------------------|-----------------|
| % ± SE (w/w)            | 4.36±0.29         | 0.68±0.032      |

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The results of the physio-chemical measurements of wild spinach seed oil presented in Table 2. It showed that the refined oil yield almost met the SNI 3741 for cooking oil used as a reference for vegetable oil, except for the water content. Wild spinach seed oil before refinement and after refinement are presented in Figure 1.

The SNI standard for cooking oil for the water content value is ≤ 0.15%. The refined wild spinach seed oil results had a water content of 0.37 ± 0.07%. This was due to the repeated rinse using distilled water, causing the water content in the produced oil exceeded the SNI standard. The density value of oil after refinement was 0.84 ± 0.01 g/mL. This value approximates the pure squalene density value which is 0.85 g/mL. This was because the oil has lost free fatty acids and other impurities during the refinement process that make the oil contain dominated by squalene (Table 3) [13].

The peroxide value is an important parameter in determining oil quality because it is used in determining damage to oil. Unsaturated fatty acids can bind oxygen to their double bonds to form peroxide. If the peroxide value increases, it can be said that the oil will soon be rancid or damaged [14]. In Table 2 the peroxide value in the refined oil has met SNI cooking oil standards with a value of less than 10 meq/kg (9.5 ± 4.75 meq/kg).

Acid value is the amount of KOH (mg) that is used to neutralize one gram free fatty acids. The higher of free fatty acids content in oil, the higher damage to the oil will happen [14]. The acid value of wild spinach seed oil after refinement complied to the SNI value of cooking oil’s acid value because the oil acid value was less than 0.6 mg KOH/g. This is because the amount of free fatty acids decreased due to the neutralization process [15]. The acid value of wild spinach seed oil showed that the oil quality was good.

![Figure 1. Wild Spinach Seed Oil before Refinement (a) and After Refinement (b)](image)

| Characteristics            | Wild Spinach Seed Oil | SNI 3741 : 2013* Cooking Oil |
|----------------------------|-----------------------|-----------------------------|
| Aroma and colour           | Distinct aroma of spinach seed and yellow colour. | Normal |
| Water Content (%)          | 0.37±0.07             | Max 0.15                    |
| Density (g/mL)             | 0.84±0.01             | (-)                         |
| Acid Value (mg KOH/g)      | 0.53±0.23             | Max 0.6                     |
| Peroxide Value (meq/kg)    | 9.5±4.75              | Max 10                      |
| Saponification Value (mg KOH/g) | 208.9±1.57       | (-)                         |

| Component Name                               | BM | Molecular Formula | Retention Time (minutes) | %area |
|----------------------------------------------|----|-------------------|--------------------------|-------|
| Dodecanoic acid, methyl ester (CAS)/Methyl Laurate | 214| C13H26O2          | 7.505                    | 0.28  |
| Tetradecanoic acid, methyl ester (CAS)/Methyl tetradecanoate | 242| C15H30O3          | 8.414                    | 0.08  |
| Hexadecanoic acid (CAS)/Palmitic acid        | 270| C17H34O2          | 12.970                   | 1.31  |
| 2,6,10,14,18,22-Tetracosahexaene/Squalene (Z,Z) 9,12-Octadecadienoic acid–(CAS)/Linoleic acid | 410| C30H50            | 14.859                   | 94.94 |
| TOTAL                                        | 280| C18H32O2          | 16.886                   | 3.39  |

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The saponification value is the amount of KOH needed to convert 1 gram of oil into soap [16]. The saponification value is affected by the molecular weight of its constituent components [16]. The value of the saponification of each oil must be different and never be the same. The higher the saponification number, the better the oil quality will be. The saponification value of the wild spinach seed oil after refinement was 208.9 ± 1.57 mg KOH/g.

The GC-MS spectra of Wild Spinach Seed Oil is presented in Figure 2. It showed that the wild spinach seed oil contained 5 compounds which indicated by the presence of 5 peaks. The results of MS analysis for each peak were presented in Table 3. A study conducted by [8] used 11 types of spinach seeds of which the dominant fatty acids were palmitic acid and linoleic acid. Palmitic acid value from 11 species of spinach ranged from 19.1% to 23.4%. Whereas for linoleic acid ranged from 36.7% to 49.9%. When compared, the values of linoleic acid and palmitic acid from spinach seed oil in this study had quite a difference. This was because in the 11 types of spinach studied by [8] the refinement step did not conducted. Refinement reduced the number of fatty acids in the oil. This was because the fatty acids in the oil were saponified by NaOH in the neutralization process and were rinsed away after the saponification process [15]. The decrease in fatty acid content was quite a lot due to the addition of excessive NaOH affected the decrease in fatty acid content which was saponified [16].

![Figure 2. GC Chromatogram of Wild Spinach Oil](image)

![Figure 3. The Fragmentation of Squalene Found in Sample (Up); Compared to WILEY7.LIB Database of Squalene (Down)](image)
Based on the previous result, Amaranth seed oil contains 6000-8000 mg of squalene [8]. In this study, refining oil of A. dubius (wild amaranth) showed squalene is the main compound. Figure 2 shows the dominant compound with area 94.94% was found at Rt = 14.859. Comparing the fragmentation pattern of the compound and WILEY7 LIB database (Figure 3), the result showed the dominant compound was squalene (C30H50) with molecular weight of 410. This result exceeded the content of other vegetable squalene which was generally below 50%, for example in refined pumpkin seed oil (18.77%) [4] and refined luffa seed oil (46.32%) [8]. This was consistent with the results of research by [8] which said that spinach seed oil had a higher squalene content than other vegetable oils.

**Conclusion**

The yield of wild spinach seed oil before and after refinement was 4.36 ± 0.22% (w/w) and 0.68 ± 0.032% (w/w), respectively. Chemical properties of wild spinach seed oil after refinement were as follow: water content 0.37%; peroxide value 9.5 meq/kg; acid value 0.53 mg KOH/g of oil; saponification value 208.9 mg KOH/g of oil. GC-MS analysis results showed that wild spinach seeds were composed of 5 components which were dominated by squalene (94.9%), then followed by linoleic acid (3.34%), palmitic acid (1.31%), dodecanoic acid (0.28%), and tetradecanoic acid (0.08%).

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