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Effects of the candlenut seed oil supplementation on the fatty acids profile of Swiss Webster mice

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Abstract. *Wonggo D, Agustin YC, Wongso S, Putra SED. 2023. Effects of the candlenut seed oil supplementation on the fatty acids profile of Swiss Webster mice. Asian J Nat Prod Biochem 21: 26-33.* Candlenut (*Aleurites moluccana* (L.) Wild) is a widely distributed plant in Asia and Australia. Previous studies show the benefits of consuming foods containing Unsaturated Fatty Acids (UFA) that can reduce saturated fatty acids in the body. However, to date, there has been on the effect of candlenut seed oil on Fatty Acid (FA) profile, so this study intended to determine the highest candlenut seed oil supplemented to feed/food ingredients and its effect on unsaturated and Saturated Fatty Acids (SFA) profile in the blood and adipose tissue. Thirty-two Swiss Webster mice were divided into 2 groups, Group I (control group, n=16) was given normal (regular) feed, and Group II (test group, n=16) was assigned 16% candlenut seed oil-supplemented feed. The experiment was carried out for 13 weeks. At the end of the study (week 13th), the mice were dissected, and the fatty acids profile from the adipose was analyzed using a GC-FID instrument. Supplementation of 16% candlenut seed oil in feed increases total fat content to 180%, decreases Saturated Fatty Acids (SFA) to 17,71%, and increases unsaturated fatty acids (UFA) to 82,29%. Mice treated with 16% candlenut seed oil in the feed for 13 weeks showed a significant reduction in SFA level (38,975% ± 11,178 vs. 22,148% ± 2,853, $p<0,05$), accompanied by a significant increase in UFA (60,633% ± 10,924 vs. 77,693% ± 2,685, $p<0,05$). In addition, a significant increase in linolenic acid (1,1% ± 0,572 vs. 7,925% ± 1,305, $p<0,05$) and the UFA with more than one double bond (Linoleic acid and Linolenic acid) (26,65% ± 8,783 vs. 42,245% ± 2,322, $p<0,05$). Regular consumption of candlenut seed oil could increase UFA levels and decrease SFA levels. Therefore, candlenut seed oil might be the potential as a food additive to maintain health.

Keywords: Adipose, *Aleurites moluccana*, GC-FID, saturated fatty acid, unsaturated fatty acid

Abbreviations: ALA: Linolenic Acid; FA: Fatty Acid; GC-FID: Gas Chromatography-Flame Ionization Detector; LA: Linoleic Acid; MUFA: Mono Unsaturated Fatty Acid; PUFA: Poly Unsaturated Fatty Acid; SFA: Saturated Fatty Acid; UFA: Unsaturated Fatty Acid

INTRODUCTION

Candlenut (*Aleurites moluccana* (L.) Wild), a plant widely distributed in Asia and Australia, belongs to the Euphorbiaceae family. Candlenut is also known as kemiri (Indonesia and kukui (Hawaii). Several plant species in the *Aleurites* include *A. montana, A. trisperma, A. cordata, A. fordii,* and *A. moluccana*. Candlenut is used as a spice, biofuel, and traditional medicine. Candlenut seeds have the highest economic value compared to the leaves and stem. Candlenut seeds contain a high level of oil (60%), dominated by unsaturated fatty acids, such as Linolenic Acid (23,01%), Linoleic Acid (38,25%), and Oleic Acid (29,05%) (Tambun et al. 2020). Candlenut seed oil contains up to 66-91% of unsaturated fatty acids with as low as 7-10% saturated fatty acids (Shaah et al. 2021). Opportunities for exploring the potential of candlenut are widely open due to limited studies on this plant; therefore, it is necessary to study the use of candlenut seed oil as a feed supplement and its effect on fatty acid profiles and its toxicity in rats.

The high oil content in candlenut seed can be used as an unsaturated fatty acid source similar to peanut, sesame, and sunflower oil (Yanti et al. 2021). Candlenut seed oil has not been used as vegetable oil, known as "healthy oil." These essential unsaturated fatty acids cannot be synthesized in the human body (Watanabe and Tatsuno 2020), so these fatty acids could be obtained from candlenut seeds.

Fatty acids could be as saturated and unsaturated fatty acids based on the presence or absence of double bonds between carbon atoms. Unsaturated fatty acids are fatty acids that have one or more double bonds in their structure, and saturated fatty acid is a fatty acids without double bonds (Adeva-Andany et al. 2019). Moreover, unsaturated fatty acids, which only have one double bond, are known as Mono Unsaturated Fatty Acids (MUFA). Unsaturated fatty acids with more than one double bond are called Poly Unsaturated Fatty Acids (PUFA).

Unsaturated fatty acids, such as Omega-3 (Linolenic Acid), Omega-6 (Linoleic Acid), and Omega-9 (Oleic Acid), have health benefits, including cardiovascular disease, cancer prevention, lower risk of diabetes, and anti-inflammation agent (Medeiros-De-Moraes et al. 2018;

Moloudizargari et al. 2018; Priatni et al. 2018; Shahidi and Ambigaipalan 2018; Gammone et al. 2019). Contrarily, saturated fatty acids, such as palmitic acid, stearic acid, myristic acid, and lauric acid, were linked to high cholesterol levels and cardiovascular disease. The altered lifestyle, mainly dietary modification, could effectively prevent cardiovascular disease as the leading cause of death worldwide. The study aims to replace a diet with high saturated fat with an unsaturated one using candlenut seed oil.

On the other hand, candlenut seeds contain phorbol ester and saponin, the two most plenteous substances found in candlenut seeds that are responsible for their toxicity (Corcoran et al. 2020; Lawani and Winter 2022). Phorbol ester is a toxic diterpene generally found in Euphorbiaceae and Thymelaceae. Candlenut seed is usually consumed as an herbal medicine for weight loss, could cause vomiting, diarrhea, gastrointestinal nuisance, cardiac arrest, and also death (González-Stuart and Rivera 2019; Corcoran et al. 2020; Rosa et al. 2022; Lawani and Winter 2022); due to its adverse effects. Maximum intake at ≥2 g/kg BW did not cause any mortality (de Castilho et al. 2021). Candlenut has also been banned for consumption as a weight-loss supplement in Brazil, Chile, and Argentine. In-vivo study indicates clinical signs of toxicity, including ataxia, anesthesia, and no response to audio and visual stimuli on rat models after oral administration of candlenut extract at a concentration of 2 g/kg body weight (de Castilho et al. 2021). The toxic effect after consuming other Euphorbiaceae plants, such as *Jatropha curcas* (Sawadogo et al. 2018), *Euphorbia bivonae* (Athmouni et al. 2019), and *Alchornea cordifolia* (Ansah et al. 2011) have also been reported.

Considering the potential use of candlenut seed oil for health and the high number of toxicity reports, it is crucial to determine the safe dose of candlenut seed oil supplemented in food. Furthermore, this study aimed to determine the highest content of candlenut seed oil in feed/food and its effect on saturated and unsaturated fatty acids profile in blood and adipose after consumption of candlenut seed oil.

MATERIALS AND METHODS

Animal model

Healthy Swiss Webster mice (*Mus musculus*) aged 3-4 months were distributed into polypropylene cages (20x30 cm). The animals were placed in a room at 27°C with free access to food and water (ad libitum). This research was approved by the Institutional Ethical Committee University of Surabaya, Indonesia, No. 192/KE/VIII/2021.

Proximate analysis

The proximate analysis comprises protein, water, ash, fat, carbohydrate, and crude fiber. Protein analysis: 0,5 g of each sample was digested with 1 mL H2SO4 and 1 g $CuSO_4$ at 300-400°C for 2 hours. The mixture was then cooled at room temperature and titrated with 0,1N NaOH. Analysis of moisture content: 2 g of sample was heated in the oven for 5 hours at 105°C. Analysis of ash content: 2 g of sample was heated in the furnace at 550°C for 4 hours. Fat content was performed by hydrolyzing the sample: 1,5 g of sample was added with 250 mL 3M HCL for 1 hour, then neutralized using aquadest. The dried sample was mixed with 60 mL hexane in the Soxhlet for 95 minutes, then dried in the oven at 105°C. Carbohydrate content was calculated using the formula: 100% - (water content + protein content + total fat content + ash content). Analysis of crude fiber content: 5 g of the sample was mixed with 200 mL of HCl 3% and refluxed for 3 hours. The mixture was cooled to room temperature, neutralized with NaOH 30%, then added with three drops of acetic acid 3% and 500 mL water. Ten mL of the solution was added with 25 mL Luffschroll solution and 15 mL water, then boiled for 3 minutes and cooled for 10 minutes. And then, the mixture was added with 15 mL KI 20% and 25 mL H2SO4 25% and titrated with 0,1N Na2S2O3.

Toxicity test of candlenut seed oil

Twelve mice were divided into two groups, Group I (Control Group, n=6) was given regular feed without candlenut seed oil addition. Group II (Test Group, n=6) was given candlenut seed oil with an increasing concentration of 2% per week in the feed, namely 0% of candlenut seed oil in the 1st week and 16% at the week 9th, At the end of the ninth week, 12 mice (6 from each group) were dissected, and blood samples were collected from the heart and inserted into the tubes containing EDTA. The fatty acids profile was then analyzed using Gas Chromatography-Flame Ionization Detector (GC-FID). The highest concentration of candlenut seed oil added, which did not cause toxicity in the animal model, was then used to determine the effect of candlenut seed oil addition on adipose tissue.

Fatty acids profile of candlenut seed oil effect on adipose tissue

Thirty-two Swiss Webster mice were divided into 2 groups, Group I (control group, n=16) was given normal (regular) feed, and Group II (test group, n=16) was assigned 16% candlenut seed oil-supplemented feed. The experiment was carried out for 13 weeks. At the end of the study (week 13th), the mice were dissected, and the fatty acids profile from the adipose was analyzed using a GC-FID instrument.

Each tube containing 0,1 g of blood/adipose sample was mixed with 1 mL KOH 1N in methanol and then put in the 85°C water bath for 15 minutes. The mixture was then cooled at room temperature before adding 1 mL of Boron Trifluoride (BF3), sprayed with N_2 gas, and put in the 90°C water bath for 30 min. It is followed by adding 1 mL n-hexane and 6 mL saturated NaCl. The mixture was centrifugated at 1,500 rpm for 10 min. The supernatant was collected and added with 1 g of anhydrous Na_2SO_4. The supernatant was taken out using a syringe, transferred to a new vial, and analyzed further using a GC-FID instrument. The standard solution for reference or blank in the GC-FID instrument containing 100 µL Tridecanoic Acid 10,000 ppm and 100 µL Tricosanoic Acid 1,000 ppm was treated the same as the sample.

Statistical analysis

Data were analyzed using the Statistical Product and Service Solution (SPSS program). All data were expressed as mean ± Standard Deviation (SD), and statistical differences between means were determined by independent T-test. P<0,05 was regarded as statistically significant for all statistical analyses.

RESULTS AND DISCUSSION

Proximate analysis for feed

The results of the proximate are shown in Table 1. Carbohydrates, protein, and water content are the top 3 components of the feed. The significant constituent change in feed supplemented with candlenut seed oil is the fat content. It increased 3x compared to the normal feed, while water, ash, protein, crude fiber, and carbohydrate content were insignificant changes.

Fatty acids profile of the feed

The saturated fatty acids content in the oil-supplemented feed decreased from 32.35% to 17.71%, while the unsaturated fatty acids increased from 14.64% to 82.29%. On the oil-supplemented feed, saturated fatty acids, i.e., lauric acid, myristic acid, and palmitic acid, were lower than normal. Linolenic acid was much higher in oil-supplemented feed (Table 2).

Fatty acids profile of blood

The fatty acids profile of Swiss Webster mice fed with candlenut seed oil-supplemented feed, which increased gradually to 16% in week 9^{th,} showed no significant results on unsaturated fatty acids between the control (normal) group and oil-supplemented group (Figure 1).

At the same time, no toxicity or death was observed in this experiment. This finding showed that 16% candlenut seed oil showed no toxicity leading to death. The addition of the oil concentration was stopped at a concentration of 16% because this concentration was considered too high for the usual additional oil in a food ingredient.

Fatty acids profile of adipose tissue

Mice treated with 16% candlenut seed oil supplementation in feed for 13 weeks had higher weight gain than the control group (39.437g ± 3.054 vs. 35.062g ± 1.692, p<0.05) (Figure 2).

Furthermore, the total saturated fatty acids in the oil-supplemented group significantly decreased (38.975% ± 11.178 vs. 22.148% ± 2.853, p<0.05), while the total unsaturated fatty acids increased significantly (60.633% ± 10.924 vs. 77.693% ± 2.685, p<0.05) (Figure 3).

Based on the number of double bonds of unsaturated fatty acids showed that the unsaturated fatty acids with more than one double bond (Linoleic acid and Linolenic acid) had a significant increase compared to the control (26.65% ± 8.783 vs. 42.245% ± 2.322, p<0.05), and the unsaturated fatty acids with only one double bond (Palmitoleic acid and Oleic acid) also increased, but insignificant (33.985% ± 6.965 vs. 35.448% ± 0,6, p<0.05) (Figure 4).

The results showed that the saturated fatty acids, i.e., myristic acid, palmitic acid, and stearic acid in the oil-supplemented group were lower than the control (2.643% ± 2.412 vs. 1.095% ± 0.265, p<0.05), (26.925% ± 4.241 vs. 17.3% ± 1.995, p<0.05), and (9.443% ± 4.734 vs. 3.778% ± 0.747, p<0.05), respectively, and a combination of myristic acid and palmitic acid that known as harmful fatty acids, decreased significantly (29.535% ± 6.585 vs. 18.37% ± 2.18, p<0.05) (Figure 5).

Furthermore, it showed that only the level of linolenic acid increased significantly (1.1% ± 0.572 vs. 7.925% ± 1.305, p<0.05). In contrast, oleic acid and linoleic acid increased, but insignificant (31.575% ± 6.186 vs. 33.35% ± 0.889, p<0,05) dan (25.55% ± 8.281 vs. 34.3% ± 1.715, p<0.05), respectively. Palmitoleic acid decreased (2.4% ± 1.023 vs. 2.098% ± 0.873, p<0.05) (Figure 6).

Table 1. The results of proximate analysis of normal feed and oil-supplemented feed

Matrix	Proximate analysis results		% Change from normal feed
	Normal feed	16% Oil-supplemented feed	
Water	11.5	9.02	-21.2
Ash	5.82	5.52	-5.1
Fat	5.63	15.8	180.4
Protein	16	14.9	6.9
Crude Fiber	6.52	5.55	14.8
Carbohydrate	54.6	49.2	9.8

Table 2. Fatty acids content of normal feed and supplemented feed

Fatty acids		Concentration (%)	
		Normal feed	16% Oil-supplemented Feed
Saturated	Lauric acid	3.29	0.44
	Myristic acid	1.41	0.34
	Palmitic acid	24.2	13.8
	Stearic acid	2.73	2.87
	Others	0.7	0.25
	Total	32.35	17.71
Unsaturated	Palmitoleic acid	0.19	0.08
	Oleic acid	29	26.4
	Linoleic acid	35.5	36.9
	Linolenic acid	2.1	18.4
	Others	0.75	0.46
	Total	67.65	82.29

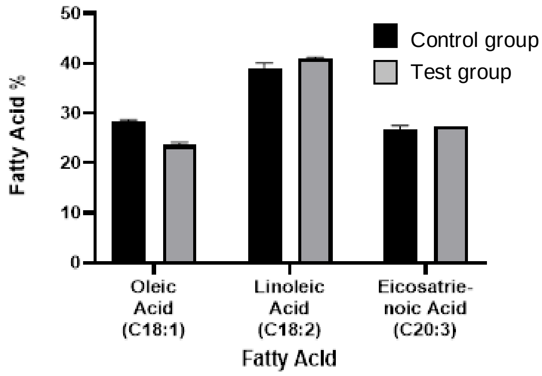

Figure 1. Profile of unsaturated fatty acids in the blood of mice after 9 weeks consuming feed supplemented with increasing gradually of candlenut seed oil to 16%

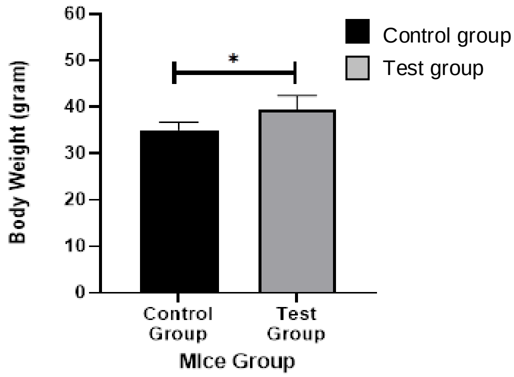

Figure 2. Body weight of mice body treated with normal feed and oil-supplemented feed. *Indicate significant differences between the control and the test groups with $p<0.05$

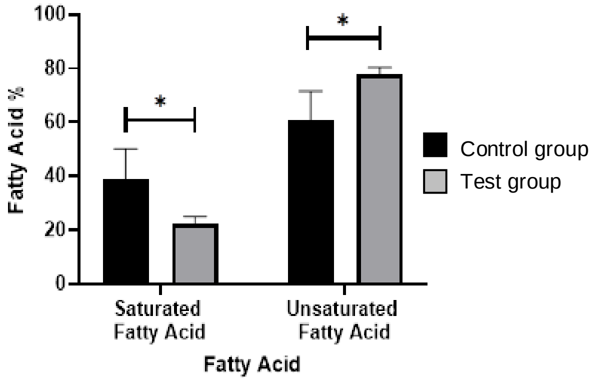

Figure 3. Saturated and unsaturated fatty acids in adipose tissue in mice treated with normal and oil-supplemented feed. *Indicate significance between the control and the test groups with $p<0.05$

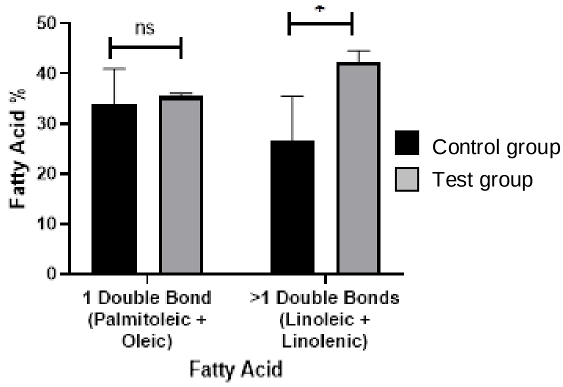

Figure 4. Percentage of 1 and >1 double bond groups on unsaturated fatty acid of mice of the control group and test group. Ns indicates no significant differences between the control group and the test group. *Indicate significant differences between the control and the test groups with $p<0.05$

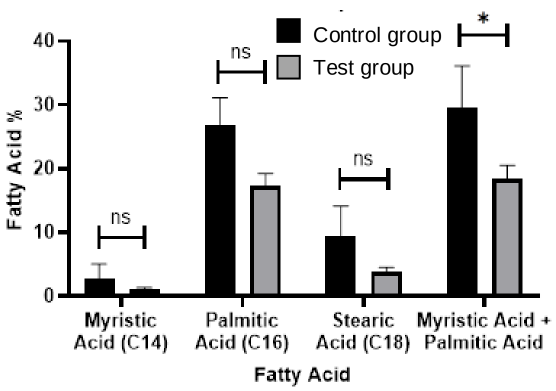

Figure 5. Percentage of Saturated Fatty Acids in adipose tissue of mice of the control and test groups. Ns indicates no significant differences between the control group and the test group. *Indicate significant differences between the control and the test groups with $p<0.05$

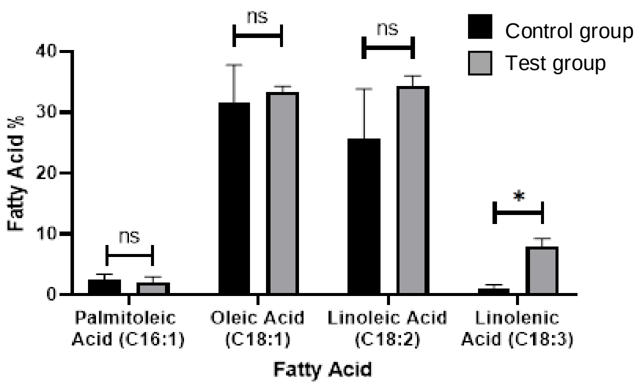

Figure 6. Percentage of unsaturated fatty acid in adipose tissue of mice of the control and test groups. Ns indicates no significant differences between the control group and the test group. *Indicate significant differences between the control and the test groups with $p<0.05$

Discussion

Supplementation of 16% candlenut oil in feed reduces saturated fatty acids and increases unsaturated fatty acids significantly. Furthermore, an increase was observed in linolenic acid and unsaturated fatty acids with more than one double bond. Decreased saturated fatty acid content (38,975% to 22,148%) and increased unsaturated fatty acids (60,633% to 77,693%) indicate the potential of candlenut seed oil as a food ingredient with beneficial health effects.

Increased levels of total fatty acids in feed supplemented with candlenut seed oil are due to the high oil content in candlenut seed (Cabral et al. 2016; da Silva et al. 2020). In this study, total fatty acids increased by 180% in the oil-supplemented feed compared to normal (regular) feed. The mice fed the high-fat diet (oil-supplemented feed) showed a significant increase in body weight compared to the control group (Figure 2). A study by Baumgardner et al. (2008) showed that adding corn oil increased weight gain in mice because of its high calories. Most of the fatty acid content in candlenut seed oil is unsaturated fatty acids with various health benefits. However, it is still necessary to determine the maximum limit of candlenut seed oil consumption so there is no adverse effect.

The unsaturated fatty acids profile in the blood was used to determine the highest level of candlenut seed oil supplementation in the feed that does not cause any adverse effect. (Figure 1). The fatty acids profile in adipose tissue was also determined because adipose tissue is the primary storage that/accumulates fatty acids. Blood fatty acids represent transient levels due to the breakdown of fatty acid stores within adipose tissue (Hodson et al. 2008). Fatty acids consumed are transported into the bloodstream as parts of more complicated lipid structures like triacylglycerols and phospholipids (Calder 2015). The fatty acids are then taken to adipose tissue to be re-esterified and stored as an excessive energy reservoir (Kuriyama et al. 2005; Suganami et al. 2012). The absorbed fatty acids accumulate and multiply in the body, especially in adipose tissue (Figueiredo et al. 2017).

Supplementation of 16% candlenut seed oil in the feed increased unsaturated fatty acids in blood and adipose tissue. In this study, mice fed with test feed (oil-supplemented feed) for 13 weeks showed an increase of UFA up to 17% compared to the control group. Huber et al. (2007) showed that mice fed with a high unsaturated fatty acid diet for six weeks with an average daily feed consumption of 7,6 g/day were able to increase levels of unsaturated fatty acids in the adipose tissue by 5 to 14% compared to mice with normal feed (Huber et al. 2007). Another study by Shin and Ajuwon (2018) showed that mice fed with a high unsaturated fatty acid diet for 12 weeks showed an increase in the average daily consumption of feed accompanied by increasing unsaturated fatty acids by 2-7% than mice with normal feed (Shin and Ajuwon 2018). The fatty acids in the feed are deposited in the body, especially in adipose tissue, for energy storage (Kuriyama et al. 2005; Suganami et al. 2012).

Animal models supplemented with 16% candlenut seed oil in their diet for 13 weeks also significantly decreased saturated fatty acids and increased unsaturated fatty acids (Figure 3). A study by Pedrosa et al. (2002) that used candlenut leaf extract showed only a decrease in LDL levels without interfering with their HDL content. This difference in results may be due to differences in lipoprotein cholesterol distribution (Krause and Hartman 1984). These results indicate that candlenut oil can potentially prevent cardiovascular disease caused by the blood accumulation of Low-Density Lipoprotein (LDL) cholesterol. The increase in LDL levels will lead to the formation of plaque or atherosclerosis and subsequent stroke and abrupt heart attack (Luo et al. 2022).

Extraction of candlenut seed produces 42-62% oil, with total unsaturated fatty acids of as much as 66-91% and a low level of saturated fatty acids (7-10%) (Shaah et al. 2021). Consuming a high level of unsaturated fatty acids was closely related to cardiovascular risk prevention, cancer, diabetes, obesity, and autoimmune deterrence (Medeiros-De-Moraes et al. 2018; Moloudizargari et al. 2018; Shahidi and Ambigaipalan 2018; Gammone et al. 2019). Unsaturated fat influences the genes that control fat metabolism and inflammation, so the risk of cardiovascular disease decreases (Larsen et al. 2021). One example is the downregulation of FGF18 expression, a Fibroblast Growth Factor (FGF) family involved in apoptosis and cell survival. Furthermore, the upregulation of SEPTIN 14, a protein implicated in cell proliferation, was also observed. The expression change in FGF18 and SEPTIN14 may lead to the prevention of atherosclerosis and reduced Vascular Smooth Muscle Cell (VSMC) proliferation level (Larsen et al. 2021).

Unsaturated fatty acids are fatty acids that have double bonds in their structure. There are two types of unsaturated fatty acids, unsaturated fatty acids with only one double bond (Mono Unsaturated Fatty Acid/MUFA) and unsaturated fatty acids with more than one double bond (Poly Unsaturated Fatty Acid/PUFA). Both have an equal effect on preventing cardiovascular disease (Miller et al. 2016). However, Lada and Rudel (2003) showed that MUFA was better at lowering LDL than PUFA because MUFA could lower HDL levels without lowering LDL levels, while PUFA can reduce LDL and HDL levels. Figure 4 shows that only PUFA levels increased significantly (26.65% ± 8.783 vs. 42.245% ± 2.322, $p<0,05$) in the oil-supplemented group. Previous studies showed that PUFA could reduce triglyceride levels and improve endothelial function, better at preventing heart disease than MUFA (Miller et al. 2016). However, Sheashea et al. (2021) showed that MUFA was more consistent in preventing heart disease. In addition, PUFA and MUFA did not have a different effect on the rate of fat oxidation (Jones et al. 2008; Casas-Agustench et al. 2009).

This study revealed that all detected saturated fatty acids, i.e., myristic acid, palmitic acid, and stearic acid, were decreased (Figure 5). It is consistent with the study by Mensink (2016) that three unsaturated fatty acids are the most abundant in humans and play a significant role in the body. Saturated Fatty Acid (SFA) is a type of fatty acid with no double bonds in its chain (Adeva-Andany et al. 2019) and is also called "bad fat." Interestingly, whereas stearic acid is classified as a saturated fatty acid, it hasn't played a significant role in lipid metabolism; thus, it showed no effect on cardiovascular risk and had a neuroprotective effect against cerebral ischemia (Chen et al. 2020). The study showed that saturated fatty acids, namely myristic acid and palmitic acid, were decreased in the mice treated with oil-supplemented feed. These saturated fatty acids are associated with significant health problems, such as cholesterol and heart disease (Fatima et al. 2019; Saraswathi et al. 2022). Palmitic acid is known for its role as an intracellular signaling molecule and is closely related to metabolic syndrome, cancer, a neurodegenerative disorder, and cardiovascular disease (Fatima et al. 2019). Myristic acid, although accumulated in small amounts, has a significant role in cholesterol-upregulating action (hypercholesterolemia) and insulin resistance causing diabetes (Saraswathi et al. 2022).

Four unsaturated fatty acids were detected in this study, i.e., Palmitoleic Acid, Oleic Acid, Linoleic Acid, and Linolenic Acid (Figure 6). The four unsaturated fatty acids are also the most abundant unsaturated fatty acids found in adipose tissue (Ratcliffe et al. 2020). The unsaturated fatty acids belonging to MUFA are Palmitoleic Acid and Oleic Acid, while those belonging to PUFA are Linoleic Acid and Linolenic Acid. Palmitoleic acid, or (9Z)-hexadec-9-enoic acid, is Omega-7 monounsaturated fatty acid that contributes to anti-obesity, diabetes, and cardiovascular risk (Hu et al. 2019). The other unsaturated fatty acid in high levels was oleic acid or Omega-9. It is used as an anti-inflammation, anti-cancer, and emulsifier or solubilizing agent in the industry (Farag and Gad 2022). Omega 9 is commonly used for lowering LDL (bad cholesterol) and increasing HDL (good cholesterol) (Alagawany et al. 2022). Omega-7 and Omega-9 in food can reduce cholesterol levels due to an immunosuppressive effect, lowering triglycerides and reducing lymphocyte proliferation by Concanavalin A (ConA) (Yaqoob et al. 1994).

Omega-6 (Linoleic Acid) (LA) was the highest unsaturated fatty acid found in this study (Figure 6). LA is abundant in the body and in our food (Marangoni et al. 2020). LA is metabolized into other PUFAs with more carbon chains and is associated with immune and inflammatory responses (Patterson et al. 2012). Linolenic acid or Omega-3 Alpha-Linolenic Acid (ALA) is commonly found in flaxseed, chia seed, walnut, and fish oil (Shahidi and Ambigaipalan 2018). ALA act as Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) precursor. Increasing ALA levels in the body are linked to cardiovascular health benefits (Watanabe and Tatsuno 2020; Weinberg et al. 2021), lowering cholesterol level (Rouhanipour et al. 2022), cancer prevention (Wei et al. 2022), and anti-inflammation (Ishihara et al. 2019). LA and ALA combination also served as a proven anti-inflammation agent, even though the complex mechanism is not fully understood yet (Innes and Calder 2018).

Candlenut has been known to contain phorbol ester and saponin, found in abundant amounts in candlenut seed, and is believed to be responsible for its toxicity (Rosa et al. 2022; Lawani and Winter 2022). However, adding 16% candlenut seed oil to the mice feed does not cause death in the animal model, and this shows that candlenut is not detrimental up to 16%. Further research was needed to determine the long-term effects, metabolism, and mechanisms of its toxicity to ensure the safety of candlenut seed oil consumption. Vomiting, diarrhea, gastrointestinal nuisance, cardiac arrest, and death are reported after consuming candlenuts as an herbal weight-loss supplement (Lawani and Winter 2022; Rosa et al. 2022). Due to the adverse effects, the candlenut supplement has been banned for consumption in Brazil, Chile, and Argentine. In addition, an in-vivo study indicates clinical signs of toxicity, including ataxia, anesthesia, and no response to audio and visual stimuli on rat models after oral administration of candlenut extract at a concentration of 2 mg/kg body weight (de Castilho et al. 2021). Other Euphorbiaceae plants, such as *J. curcas* (Sawadogo et al. 2018), *E. bivonae* (Athmouni et al. 2019), and *A. cordifolia* (Ansah et al. 2011), also reported having toxic effects.

Supplementation of 16% candlenut seed oil in the feed reduces saturated fatty acids and increases unsaturated fatty acids significantly (38.975% to 22.148% and 60.633% to 77.693%, respectively). Furthermore, an increase was observed in linolenic acid and unsaturated fatty acids with more than one double bond. However, no significant changes were observed in the other unsaturated fatty acids. Decreased levels of saturated fatty acids and increased levels of unsaturated fatty acids indicate the potential of candlenut to be used as a food ingredient with health benefits.

REFERENCES

Adeva-Andany MM, Carneiro-Freire N, Seco-Filgueira M, Fernández-Fernández C, Mouriño-Bayolo D. 2019. Mitochondrial β-oxidation of saturated fatty acids in humans. Mitochondrion 46: 73-90. DOI: 10.1016/j.mito.2018.02.009.

Alagawany M, Elnesr SS, Farag MR, El-Sabrout K, Alqaisi O, Dawood MAO, Soomro H, Abdelnour SA. 2022. Nutritional significance and health benefits of omega-3, -6 and -9 fatty acids in animals. Anim Biotechnol 33 (7): 1678-1690. DOI: 10.1080/10495398.2020.1869562.

Ansah C, Oppong E, Woode E. 2011. Subacute oral toxicity assessment of *Alchornea cordifolia* (Schumach and Thonn) Müll Arg (Euphorbiaceae) extract in rats. Trop J Pharm Res 10 (5): 587-594. DOI: 10.4314/tjpr.v10i5.7.

Athmouni K, Feki EA, Ayadi H. 2019. Hepatotoxic effects of Euphol-rich fractions from *Euphorbia bivonae*—Relevance to cytotoxic and anti-tumor activities. Pathophysiology 26 (1): 69-76. DOI: 10.1016/j.pathophys.2018.10.003.

Baumgardner JN, Shankar K, Hennings L, Albano E, Badger TM, Ronis MJJ. 2008. *N*-Acetylcysteine attenuates progression of liver pathology in a rat model of nonalcoholic steatohepatitis. J Nutr 138 (10): 1872-1879. DOI: 10.1093/jn/138.10.1872.

Cabral MRP, Santos SAL, Stropa JM, Silva RCL, Cardoso CAL, Oliveira LCS, Scharf DR, Simionatto EL, Santiago EF, Simionatto E. 2016. Chemical composition and thermal properties of methyl and ethyl esters prepared from *Aleurites moluccanus* (L.) Willd (Euphorbiaceae) nut oil. Ind Crops Prod 85: 109-116. DOI: 10.1016/j.indcrop.2016.02.058.

Calder PC. 2015. Functional roles of fatty acids and their effects on human health. JPEN J Parenter Enteral Nutr 39: 18S-32S. DOI: 10.1177/0148607115595980.

Casas-Agustench P, López-Uriarte P, Bulló M, Ros E, Gómez-Flores A, Salas-Salvadó J. 2009. Acute effects of three high-fat meals with different fat saturations on energy expenditure, substrate oxidation and satiety. Clin Nutr 28 (1): 39-45. DOI: 10.1016/j.clnu.2008.10.008.

Chen P, Wu CY, Clemons GA, Citadin CT, Silva CA, Possoit HE, Azizbayeva R, Forren NE, Liu CH, Rao KNS et al. 2020. Stearic acid methyl ester affords neuroprotection and improves functional outcomes after cardiac arrest. Prostaglandins Leukot Essent Fatty Acids 159: 102138. DOI: 10.1016/j.plefa.2020.102138.

Corcoran J, Gray T, Bangh SA, Singh V, Cole JB. 2020. Fatal yellow oleander poisoning masquerading as benign candlenut ingestion taken for weight loss. J Emerg Med 59 (6): e209-e212. DOI: 10.1016/j.jemermed.2020.07.026.

da Silva JCM, Nicolau CL, Cabral MRP, Costa ER, Stropa JM, Silva CAA, Scharf DR, Simionatto EL, Fiorucci AR, de Oliveira LCS et al. 2020. Thermal and oxidative stabilities of binary blends of esters from soybean oil and non-edible oils (*Aleurites moluccanus*, *Terminalia catappa*, and *Scheelea phalerata*). Fuel 262: 116644. DOI: 10.1016/j.fuel.2019.116644.

de Castilho PF, Dantas FGS, Araújo RP, Castro LHA, Araújo FHS, Negri M, Santos AC, Souza RIC, Cardoso CAL, Oesterreich SA et al. 2021. General and genetic toxicology studies of *Aleurites moluccana* (L.) Willd. seeds in vitro and in vivo assays. J Ethnopharmacol 280: 114478. DOI: 10.1016/j.jep.2021.114478.

Farag MA, Gad MZ. 2022. Omega-9 fatty acids: potential roles in inflammation and cancer management. J Genet Eng Biotechnol 20 (1). DOI: 10.1186/s43141-022-00329-0.

Fatima S, Hu X, Gong RH, Huang C, Chen M, Wong HLX, Bian Z, Kwan HY. 2019. Palmitic acid is an intracellular signaling molecule involved in disease development. Cell Mol Life Sci 76 (13): 2547-2557. DOI: 10.1007/s00018-019-03092-7.

Figueiredo PS, Inada AC, Marcelino G, Cardozo CML, Freitas KDC, Guimarães RDCA, Castro AP, Nascimento VA, Hiane PA. 2017. Fatty acids consumption: The role metabolic aspects involved in obesity and its associated disorders. Nutrients 9 (10): 1158. DOI: 10.3390/nu9101158.

Gammone MA, Riccioni G, Parrinello G, D'orazio N. 2019. Omega-3 polyunsaturated fatty acids: Benefits and endpoints in sport. Nutr 11 (1). DOI: 10.3390/nu11010046.

González-Stuart AE, Rivera JO. 2019. Herbal weight loss supplements: from dubious efficacy to direct toxicity. In: Watson R, Preedy VR (eds). Dietary Interventions in Liver Disease: Foods, Nutrients, and Dietary Supplements. Elsevier, Amsterdam. DOI: 10.1016/B978-0-12-814466-4.00014-8.

Hodson L, Skeaff CM, Fielding BA. 2008. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res 47 (5): 348-380. DOI: 10.1016/j.plipres.2008.03.003.

Hu W, Fitzgerald M, Topp B, Alam M, O'Hare TJ. 2019. A review of biological functions, health benefits, and possible de novo biosynthetic pathway of palmitoleic acid in macadamia nuts. J Funct Foods 62: 103520. DOI: 10.1016/j.jff.2019.103520.

Huber J, Löffler M, Bilban M, Reimers M, Kadl A, Todoric J, Zeyda M, Geyeregger R, Schreiner M, Weichhart T et al. 2007. Prevention of high-fat diet-induced adipose tissue remodeling in obese diabetic mice by n-3 polyunsaturated fatty acids. Intl J Obes 31 (6): 1004-1013. DOI: 10.1038/sj.ijo.0803511.

Innes JK, Calder PC. 2018. Omega-6 fatty acids and inflammation. Prostaglandins Leukot Essent Fatty Acids 132: 41-48. DOI: 10.1016/j.plefa.2018.03.004.

Ishihara T, Yoshida M, Arita M. 2019. Omega-3 fatty acid-derived mediators that control inflammation and tissue homeostasis. Intl Immunol 31 (9): 559-567. DOI: 10.1093/intimm/dxz001.

Jones PJH, Jew S, AbuMweis S. 2008. The effect of dietary oleic, linoleic, and linolenic acids on fat oxidation and energy expenditure in healthy men. Metabolism 57 (9): 1198-1203. DOI: 10.1016/j.metabol.2008.04.012.

Krause BR, Hartman AD. 1984. Adipose tissue and cholesterol metabolism. J Lipid Res 25 (2): 97-110. DOI: 10.1016/s0022-2275(20)37830-5.

Kuriyama H, Liang G, Engelking LJ, Horton JD, Goldstein JL, Brown MS. 2005. Compensatory increase in fatty acid synthesis in adipose tissue of mice with conditional deficiency of SCAP in liver. Cell Metab 1 (1): 41-51. DOI: 10.1016/j.cmet.2004.11.004.

Lada AT, Rudel LL. 2003. Dietary monounsaturated versus polyunsaturated fatty acids: Which is really better for protection from coronary heart disease? Curr Opin Lipidol 14: 41-46. DOI: 10.1097/00041433-200302000-00008.

Larsen SV, Holven KB, Christensen JJ, Flatberg A, Rundblad A, Leder L, Blomhoff R, Telle-Hansen V, Kolehmainen M, Carlberg C et al. 2021. Replacing saturated fat with polyunsaturated fat modulates peripheral blood mononuclear cell gene expression and pathways related to cardiovascular disease risk using a whole transcriptome approach. Mol Nutr Food Res 65 (24): e2100633. DOI: 10.1002/mnfr.202100633.

Lawani O, Winter M. 2022. Heart block initiated by candlenut ingestion. Case Rep Cardiol 2022: 3679968. DOI: 10.1155/2022/3679968.

Luo J, Wang J, Song B. 2022. Lowering low-density lipoprotein cholesterol: from mechanisms to therapies. Life Metabolism 1: 25-38. DOI: 10.1093/lifemeta/loac004.

Marangoni F, Agostoni C, Borghi C, Catapano AL, Cena H, Ghiselli A, la Vecchia C, Lercker G, Manzato E, Pirillo A et al. 2020. Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects. Atherosclerosis 292: 90-98. DOI: 10.1016/j.atherosclerosis.2019.11.018.

Medeiros-De-Moraes IM, Gonçalves-De-Albuquerque CF, Kurz ARM, de Jesus Oliveira FM, Pereira de Abreu VH, Torres RC, Carvalho VF, Estato V, Bozza PT, Sperandio M et al. 2018. Omega-9 oleic acid, the main compound of olive oil, mitigates inflammation during experimental sepsis. Oxid Med Cell Longev 2018: 6053492. DOI: 10.1155/2018/6053492.

Mensink RP. 2016. Effects of saturated fatty acids on serum lipids and lipoproteins: A systematic review and regression analysis. World Health Organization.

Miller M, Sorkin JD, Mastella L, Sutherland A, Rhyne J, Donnelly P, Simpson K, Goldberg AP. 2016. Poly is more effective than monounsaturated fat for dietary management in the metabolic syndrome: The muffin study. J Clin Lipidol 10 (4): 996-1003. DOI: 10.1016/j.jacl.2016.04.011.

Moloudizargari M, Mortaz E, Asghari MH, Adcock IM, Redegeld FA, Garssen J. 2018. Effects of the polyunsaturated fatty acids, EPA and DHA, on hematological malignancies: A systematic review. 9 (14): 11858-11875. DOI: 10.18632/oncotarget.24405.

Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. 2012. Health implications of high dietary omega-6 polyunsaturated fatty acids. J Nutr Metab 2012: 539426. DOI: 10.1155/2012/539426.

Pedrosa RC, Meyre-Silva C, Cechinel-Filho V, Benassi JC, Oliveira LFS, Zancanaro V, Dal Magro J, Yunes RA. 2002. Hypolipidaemic activity of methanol extract of *Aleurites moluccana*. Phytother Res 16 (8): 765-768. DOI: 10.1002/ptr.1046.

Priatni S, Ratnaningrum D, Kosasih W, Sriendah E, Srikandace Y, Rosmalina T, Pudjiraharti S. 2018. Protein and fatty acid profile of marine fishes from Java Sea, Indonesia. Biodiversitas 19: 1737-1742. DOI: 10.13057/biodiv/d190520.

Ratcliffe N, Wieczorek T, Drabińska N, Drabińska N, Gould O, Osborne A, de Lacy Costello B. 2020. A mechanistic study and review of volatile products from peroxidation of unsaturated fatty acids: An aid to understanding the origins of volatile organic compounds from the human body. J Breath Res 14 (3): 034001. DOI: 10.1088/1752-7163/ab7f9d.

Rosa MCdB, Ribeiro PR, Silva VO, Selvati-Rezende DAC, Silva TP, Souza FR, Cardoso MG, Seixas JN, Andrade EF, Pardi V et al. 2022. Fatty acids composition and in vivo biochemical effects of *Aleurites moluccana* seed (Candlenut) in obese Wistar rats. Diabetol Metab Syndr 14 (1): 80. DOI: 10.1186/s13098-022-00847-4.

Rouhanipour H, Sharifi SD, Irajian G-H, Jalal MP. 2022. The effect of adding L-carnitine to omega-3 fatty acid diets on productive performance, oxidative stability, cholesterol content, and yolk fatty acid profiles in laying hens. Poult Sci 101 (11): 102106. DOI: 10.1016/j.psj.2022.102106.

Saraswathi V, Kumar N, Ai W, Gopal T, Bhatt S, Harris EN, Talmon GA, Desouza C. 2022. Myristic acid supplementation aggravates high fat diet-induced adipose inflammation and systemic insulin resistance in mice. Biomolecules 12 (6): 739. DOI: 10.3390/biom12060739.

Sawadogo S, Sanou SD, Dabiré AP, Belemtougri GR, Sawadogo L, Leiris J de, Tanguy S, Boucher F. 2018. In vivo evaluation of *Jatropha curcas* L (Euphorbiaceae) leaves acute and subacute toxicity in mice. J Sci Res 10 (2): 187-193. DOI: 10.3329/jsr.v10i2.35267.

Shaah MA, Allafi F, Hossain MS, Alsaedi A, Ismail N, Kadir MOA, Ahmad MI. 2021. Candlenut oil: Review on oil properties and future

liquid biofuel prospects. Int J Energy Res 45 (12): 17057-17079. DOI: 10.1002/er.6446.
Shahidi F, Ambigaipalan P. 2018. Omega-3 polyunsaturated fatty acids and their health benefits. Ann Rev Food Sci Technol 9: 345-381. DOI: 10.1146/annurev-food-111317-095850.
Sheashea M, Xiao J, Farag MA. 2021. MUFA in metabolic syndrome and associated risk factors: is MUFA the opposite side of the PUFA coin? Food Funct 12 (24): 12221-12234. DOI: 10.1039/D1FO00979F.
Shin S, Ajuwon KM. 2018. Effects of diets differing in composition of 18-C fatty acids on adipose tissue thermogenic gene expression in mice fed high-fat diets. Nutrients 10 (2): 256. DOI: 10.3390/nu10020256.
Suganami T, Tanaka M, Ogawa Y. 2012. Adipose tissue inflammation and ectopic lipid accumulation. Endocr J 59 (10): 849-857. DOI: 10.1507/endocrj.EJ12-0271.
Tambun R, Tambun JOA, Tarigan IAA, Sidabutar DH. 2020. Activating lipase enzyme in the candlenut seed to produce fatty acid directly from candlenut seed. J Phys: Conf Ser 1542 (1): 012006. DOI: 10.1088/1742-6596/1542/1/012006.
Watanabe Y, Tatsuno I. 2020. Prevention of cardiovascular events with omega-3 polyunsaturated fatty acids and the mechanism involved. J Atheroscler Thromb 27 (3): 183-198. DOI: 10.5551/jat.50658.
Wei L, Wu Z, Chen YQ. 2022. Multi-targeted therapy of cancer by omega-3 fatty acids-an update. Cancer Lett 526: 193-204. DOI: 10.1016/j.canlet.2021.11.023.
Weinberg RL, Brook RD, Rubenfire M, Eagle KA. 2021. Cardiovascular impact of nutritional supplementation with omega-3 fatty acids: JACC focus seminar. J Am Coll Cardiol 77 (5): 593-608. DOI: 10.1016/j.jacc.2020.11.060.
Yanti S, Saputri DS, Lin HY, Chou YC, Agrawal DC, Chien WJ. 2021. Fatty acid evaluation of seeds and nuts by spectroscopy and chromatography. Food Sci Technol (US) 9 (3): 58-68. DOI: 10.13189/fst.2021.090302.
Yaqoob P, Newsholme EA, Calder PC. 1994. The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. Immunology 82 (4): 603-610.