Molekul, Vol. 18. No. 2, July 2023: 300 – 306

MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2023.18.2.8107

Fatty Acid Profile and Squalene Content in Cucumber Seed Oil (*Cucumis sativus* Linnaeus)

Hartati Soetjipto*, Teresa Febriyanti, Agustinus Ignatius Kristijanto

Jurusan Kimia Fakultas Sains dan Matematika, Universitas Kristen Satya Wacana, Salatiga 50711

*Corresponding author email: Hartati.sucipto@uksw.edu

Received March 06, 2023; **Accepted** May 16, 2023; **Available online** July 20, 2023

ABSTRACT. Cucurbitaceae is known as a source of vegetable oil that can be used in the fields of food, medicine, and cosmetics. Several studies showed that vegetable oil from the Cucurbitaceae contains squalene. Squalene is a high-economic value compound that was originally found in shark liver oil. This compound is proven to be very beneficial for health and cosmetics. The objectives of the study are to determine the fatty acid profile and squalene content of cucumber seed oil using the Gas Chromatography-Mass Spectrometry (GC-MS) method. Starting with the preparation of cucumber seeds by drying the clean seed in an oven at 60 ^{0}C for 1 hour, then the seeds were extracted using 2 methods namely maceration and a continuous extraction method with a soxhlet extractor. The yield of crude oil obtained by the soxhlet extractor is higher than maceration, which was 19.38 ± 0.94%, yellow colour, and distinct aroma, 2% water oil content; oil density of 0.96 g/mL; free fatty acid levels of 3.51%; an acid value of 4.97 mg NaOH/g oil; and peroxide value of 0.82 meq O_2/g oil. The results of the GC-MS analysis showed that cucumber oil was composed of 3 main components namely palmitic acid, linoleic acid, and squalene.

Keywords: Cucumber seed oil, *Cucumis sativus*, fatty acids, squalene.

INTRODUCTION

Vegetable oil is used globally for food production and industrial use. Globally, vegetable oil consumption is dominated by soybean, palm oil, rapeseed, and sunflower oil with each consumption of 31.6%; 30.5%; 15.5%, and 8.6% tonnes per year (Stevenson et al., 2007). To meet market supply and demand, the development of vegetable oil needs to be improved, including finding new sources.

The seeds from the *Cucurbitaceae* family are rich in vegetable oil and protein and are widely used as cooking oil in several countries. Essential oil and fixed oil, saponin, steroid, carotene, flavonoid, amino acid, resin, tannin, protein, and proteolytic enzymes are the main chemical constituents in the *Cucurbitaceae* family (Sharma et al., 2012). One of the *Cucurbitaceae* member plants to be studied whether it has the potential as a source of vegetable oil is cucumber (*Cucumis sativus* L.). The cucumber is one of the most widely grown vegetable crops in the world (Paris et al., 2012). The stem of the cucumber has been used in traditional Chinese medicine for its anti-inflammatory activity (Tang et al., 2010). Cucumber seeds contain α- and β-amyrin, sitosterol, oleic acid, and linoleic acid (Kumar et al., 2010; Soltani et al., 2017). Cucumber seed oil can reduce cholesterol so that it can relieve cardiovascular disease (Achu et al., 2008). Kumar et al, (2010) also reported that cucumber seed oil contains tocopherols and tocotrienols, which are organic fat-soluble compounds that are referred to as vitamin E. Cucumber seed oil is also reported to be one of the vegetable oils that has an important role in drug, food and cosmetics to prevent several diseases such as inflammation, constipation, fever , bacterial invection etc. This oil need to be harness and introduced to consumers for desired applications (Ifeoma et al., 2021).

Several studies reported that vegetable oil from the *Cucurbitaceae* contains squalene. Squalene is a high-economic value compound that was originally found in shark liver oil. This compound is proven to be very beneficial for health and cosmetics. The new information showed that squalene is used as a component of some vaccines because it is known to act as an adjuvant. An adjuvant is a compound that increases the magnitude of immune responses. Squalene also had been used as an additive to a few COVID-19 vaccines in development (Block, 2021).

Based on this background, the objectives of the study are to obtain cucumber seed oil by maceration and continuous extraction methods with hexane solvent, to determine the physicochemical properties of cucumber seed oil before and after the refining process, then to determine the composition of cucumber seed oil and squalene content by using the Gas Chromatography-Mass Spectrometry (GC-MS) method.

EXPERIMENTAL SECTION

Materials and Methods

The equipment used in this study is a *soxhlet, rotary evaporator* (Buchi R0114, Switzerland), water bath (Memmert WNB 14, Memmert GmbH + KG, Germany), analytical scale with an accuracy of 0.0001 gram (Ohaus PA124, USA), analytical scale with an accuracy of 0.01 gram (Ohaus TAJ602, USA).

Sample Preparation

The seeds were separated from the flesh of the fruit, washed with clean water, rinse many times using running water to remove the mucus, and then dried at room temperature free of direct sunlight. In the next step, dry seeds were heated in a 60°C oven for 1 hour. The dried seeds were crushed with a grinder, stored in a dry container then ready for further analysis.

Cucumber Seed Oil Extraction

Maceration method

Cucumber seeds that have been grinded as much as 30 g were macerated with 150 mL of hexane solvent at room temperature for 24 hours, and stirred. After filtering, the filtrate is separated from the dregs, then the dregs were macerated again for 30 minutes with 50 mL of hexane solvent. Remaceration was carried out twice, then the filtrate was collected. The next step is the evaporation of the solvent from the filtrate using a rotary evaporator to obtain a solvent-free concentrated extract.

Continuous method

Cucumber seeds that have been grinded as much as 30 g were extracted with 150 mL of *n*-hexane solvent using a soxhlet extractor at a temperature of 80 °C (8 hours) until the solution became clear. The extraction yield was evaporated using a rotary evaporator with waterbath temperature of 50 °C until a solvent-free concentrated extract was obtained.

Characterization of the Physical-Chemical Properties of Cucumber Seed Oil

Determination of color and aroma was done descriptively, while quantitative determination is done to determine the yield (AOCS Aa 4-38, 1998a), density (AOCS Aa 4-38, 1998a), water content (AOCS Ca 2b-38, 1998b), acid value (AOCS Ca 5a-40, 1997a), and peroxide value (AOCS Cd 8-53, 1997b).

Refining process of Cucumber Seed Oil

The stages of refining cucumber seed oil include degumming and neutralization according to the AOCS method.

Degumming process (AOCS, 1989 modified)

The crude oil obtained was weighed as much as 5 grams and then 85% phosphoric acid was added to as much as 0.15% of the weight of the oil. The mixture was stirred at 75 °C using a magnetic stirrer at a speed of 400 rpm for 45 minutes. Then it was cooled and added 3-5% distilled water. In the next step, it was centrifuged at a rate of 2,600 rpm for 10 minutes to separate the gum. Then the oil was rinsed with distilled water until the rinse water is neutral.

Neutralization process (AOCS, 1989)

12.6 grams of NaOH 9.5% solution was added to the oil obtained from the degumming process, and the mixture was heated at 65 °C for 30 minutes. The oil was stirred for 30 minutes using a magnetic stirrer. After the stirring process was complete, the sample was cooled to room temperature. The oil and soap mixture formed was separated by using a centrifuge at 2,600 rpm for 10 minutes. Then followed by soap separation, the oil was rinsed several times until neutral rinsing water was obtained.

Analysis of Cucumber Seed Oil Profile

The chemical profiles of cucumber seed oil before and after the refining process were analyzed using Gas Chromatography-Mass Spectrometry (GCMS-QP2010 SE) at Semarang State University. The column type was AGILENT% W DB-1 with a length of 30 meters and a temperature of 65 °C. Injection temperature of 250°C at a pressure of 74.5 kPa with a total flow of 64.2 mL/minute with a linear velocity of 40 cm/sec. ID 0.25 mm with Helium carrier gas and ionizing EI +.

Data Analysis

The data obtained were statistically analyzed with 5 replications and as a treatment was an extraction method.

RESULTS AND DISCUSSION

The Yield of Cucumber Seed Oil

The yield of the cucumber seed oil using a continuous extraction method was higher than the yield of the maceration method (**Table 1**). The continuous extraction method used a soxhlet extractor, where the solvent will flow, evaporate and condense again so that the extraction occurs as if using a new solvent. The evaporated solvent will be condensed as a pure solvent and re-extract the existing sample. This process will continue to repeat until the oil is completely extracted. Different from the maceration method that occurs without solvent circulation, only ordinary stirring, then the oil is not fully extracted.

Refining Process of Cucumber Seed Oil by Degumming and Neutralization Methods

Selection is carried out based on the results of the oil obtained so that it is decided which oil from the continuous extract will be purified. The crude oil yielded from the continuous extraction method was refined using degumming and neutralization methods.

The addition of 85% H3PO4 as much as 0.15% to the oil was carried out in the degumming process and the addition of 9.5% NaOH was carried out in the neutralization process. The results of cucumber seed oil yield before and after the refining process were presented in **Table 2**.

After the refining process, the yield of cucumber seed oil decreased from 19.38 ± 0.94% to 11.62 ± 0.93%. The addition of H_3PO_4 in the degumming process made the phospholipid compound contained in cucumber seed oil which was insoluble in water become soluble in water (Mardani et al., 2016). Phosphatides, proteins, resins, or metals were gums and impurities which could damage the oil. The leaching of gums and various impurities at the end of the oil refining process, using distilled water, which is added many times until the oil wash water is neutral, will cause a decrease in the oil yield obtained (Soetjipto et al., 2018).

The Physico-Chemical Properties of Cucumber Seed Oil (*C. sativus*)

The physico-chemical properties of cucumber seed oil were shown in **Table 3**. The oil colour after the refining process in **Figure 2** (b) turned pale yellow, this was related to the addition of NaOH during the neutralization process. Oil color is one of the important physicochemical properties in determining oil quality. Color in vegetable oils is generally caused by the presence of color pigments such as chlorophyll and carotene which causes the color of the oil to become greenish or reddish. Mariod et al., 2012 reported that oxidation of the pigment molecule or reaction of oxidized triglycerides with carotenoids will cause the color of the oil to darken.

The water content of cucumber seed oil before the refining process (2 ± 0.06%) was greater than after the refining process (1 ± 0%). However, when compared with the allowable water content of vegetable oil in SNI, the oil obtained in this study still

Table 1. The average of the cucumber seed oil yield using maceration and continuous extraction method.

Yield	Hexane Maceration	Continuous Hexane
$\bar{x}$ ± SE (% b/b)	13.26 ± 0.99	19.38 ± 0.94

Table 2. The results of cucumber seed oil yield before and after the refining process

Yield	Before	After
$\bar{x}$ ± SE (% b/b)	19.38 ± 0.94	11.62 ± 0.93

Table 3. The comparison of physico-chemical properties of cucumber seed oil before and after the refining process with SNI (Indonesian National Standard) of cooking oil

Characteristics	Before Refining process ($\bar{x} \pm$ SE)	After Refining process ($\bar{x} \pm$ SE)	SNI 3741: 2013 Cooking Oil
Aroma	Distinct aroma	Distinct aroma	Normal
Colour	Yellow	Pale Yellow	Pale Yellowish
Water Content (%)	2 ± 0.06	1 ± 0	Max 0.15
Density (g/mL)	0.96 ± 0.02	0.91 ± 0.01	(-)
Acid Value (mg KOH/g oil)	4.97 ± 0.71	2.132 ± 0.01	Max 0.6
FFA (%oleic acid)	3.51 ± 0.50	1.50 ± 0.00	(-)
Peroxide Value (meqiuv O_2/g oil)	0.82 ± 0.18	0.54 ± 0	Max 1

(a) (b)

Figure 2. Cucumber seed oil Before the refining process; (**b**) After the refining process (Source: Personal documentation, 2019)

does not meet the requirements because it is still higher than the allowable value. The water content of the oil should be kept as little as possible because it will cause a hydrolysis reaction in the oil and cause a rancid odor (Budiman et al., 2012).

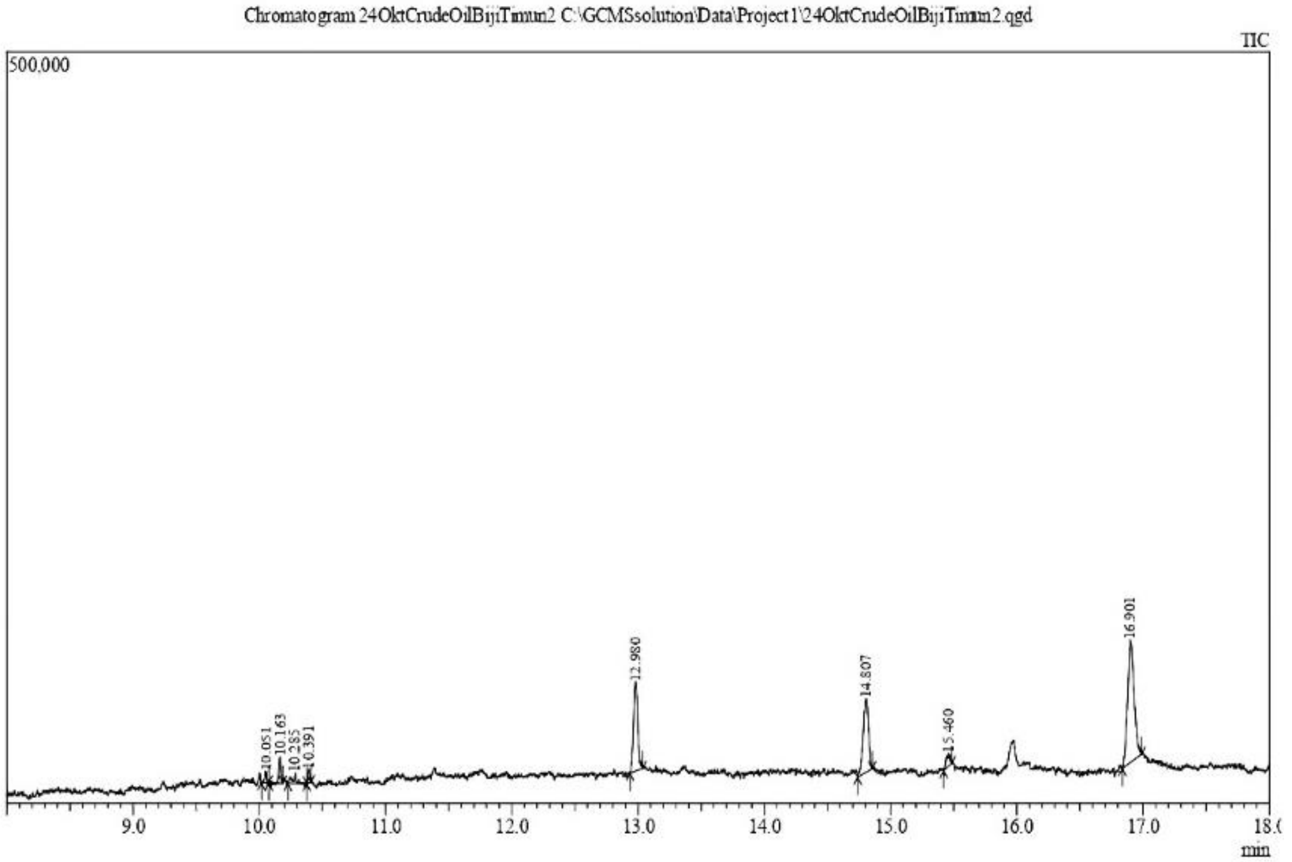
The density of cucumber seed oil after the refining process has decreased from 0.96 ± 0.02 g/mL to 0.91 ± 0.01 g/mL. The decrease in oil density after refining was due to the addition of NaOH in the purification process (neutralization) which caused a saponification reaction. The saponification process will remove free fatty acids as sodium soap so that the free fatty acid content decreases drastically. The loss of free fatty acids and impurities in the oil causes a decrease in oil mass, so it will affect the density of the oil. The same reasons as before caused a decrease in Acid value (AV) 4.97± 0.71 to 2.132 ± 0.01 (mg KOH/g oil) and free fatty acid (FFA) also 3.51± 0.50 to 1.50 ± 0.00 (% oleic acid) (Meilano et al., 2017). When compared with SNI for vegetable oil, the acid value after the refining process did not meet SNI quality standards. The high acid value was possible because there was a heating or oxidation process during the evaporation of hexane solvents, so it is possible also that there are many small molecules resulting from the oxidation reaction.

The peroxide value indicates the formation of hydroperoxides caused by primary oxidation. The peroxide value reduced from 0.82 ± 0.18 to 0.54 ± 0 (meq O_2/g oil) after refining which was less than SNI (max 1 meq O_2/g oil). A high peroxide value means poor quality of the oil, but a low peroxide value does not always show good quality (Saleh et al., 2013), because it is possible to decompose the peroxide in the oxidation reaction of the oil. To avoid any mistakes, it is better to measure Acid value and Conjugated Dienes as secondary oxidation products (Hamilton et al., 1998). In this study, the oil obtained showed a low peroxide value but a relatively high acid value.

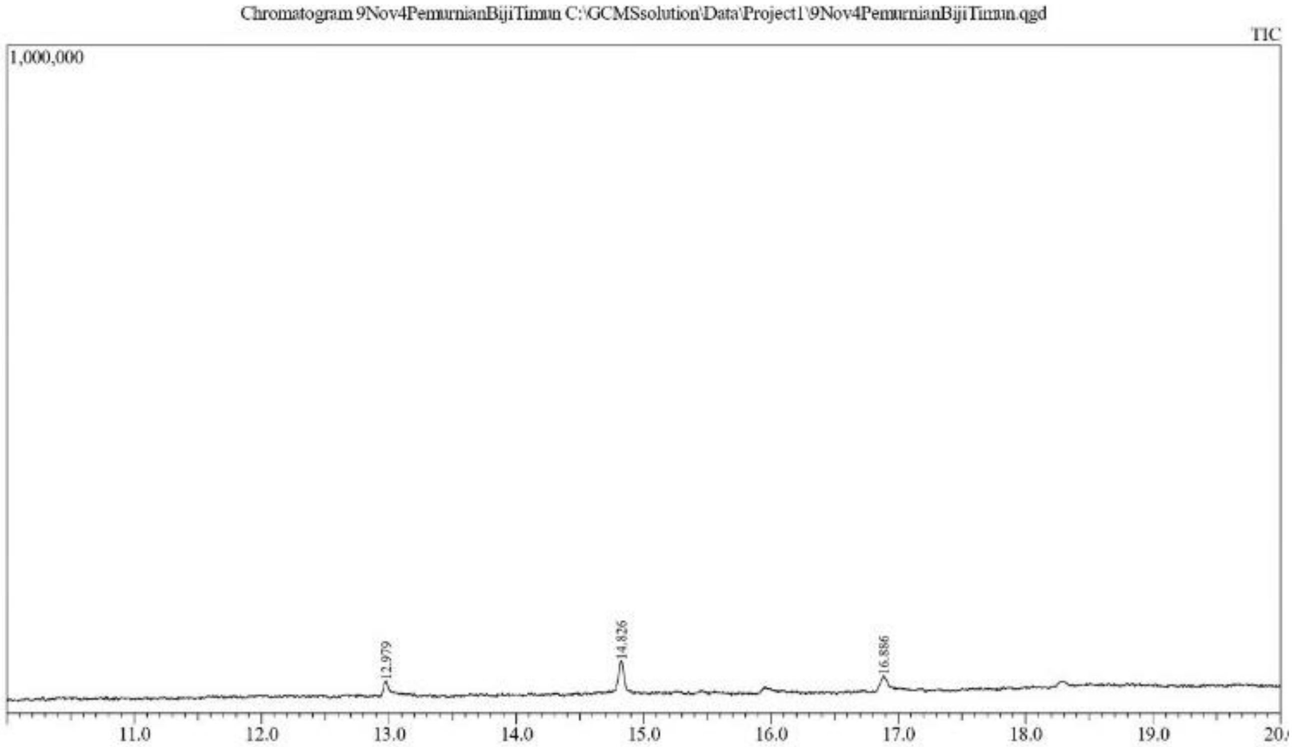
Composition of Cucumber Seed Oil (*C. sativus*) Using Gas Chromatography-Mass Spectrometry (GC-MS) Before and After the Refining process

The composition of fatty acids in cucumber seed oil before and after the refining process was presented in **Figure 3** and **Table 4**. **Figure 3** (**a**) showed that the cucumber seed oil before the refining process was identified to have 8 peaks, while **Figure 3** (**b**) showed that the cucumber seed oil after the refining process was identified to have 3 peaks.

Then each peak that appeared on the chromatogram was analyzed by MS, and the spectra of the fractionation results were compared with the Wiley database to determine the compound, and the results were demonstrated in **Table 4**.



(a)



(b)

Figure 3. Chromatogram of cucumber seed oil. (**a**) Before the refining process; (**b**) After the refining process

Table 4. Components of cucumber seed oil before and after the refining process

Component Name	BM	Molecular Formula	Before Refining process		After Refining process	
			Retention Time (minutes)	% area	Retention Time (minutes)	% area
Octadecanoic acid, methyl ester (CAS)	298	$C_{19}H_{38}O_2$	10.051	1.20	-	-
9-Octadecenoic acid(Z)-, methyl ester (CAS)	296	$C_{19}H_{36}O_2$	10.163	2.84	-	-
Dodecanoic acid (CAS) Lauric acid	287	$C_{16}H_{33}O_3$	10.285	1.89	-	-
Ethyl linoleate	294	$C_{19}H_{34}O_2$	10.391	1.63	-	-
Hexadecanoic acid (CAS) (Palmitic Acid)	270	$C_{17}H_{34}O_2$	12.980	21.28	12.979	21.37
2,6,10,14,18,22-Tetracosahexaene (squalene)	410	$C_{30}H_{50}$	14.807	22.72	14.826	53.28
Octadecanoic acid (CAS) (Stearic Acid)	284	$C_{18}H_{36}O_2$	15.460	2.95	-	-
9,12-Octadecadienoic acid (Z,Z) (Linoleic Acid)	294	$C_{19}H_{34}O_2$	16.901	45.50	16.886	25.36
TOTAL				**100**		**100**

The result of GC-MS analysis of cucumber seed oil before the refining process was dominated by linoleic acid (45.50%) then followed by squalene (22.72%), palmitic acid (21.28%), stearic acid (2.95%) and 9-Octadecanoic acid methyl ester (2.84%) and other components with <2% content. After the refining process, squalene was the dominant compound (53.28%) followed by linoleic acid (25.36%) and palmitic acid (21.37%).

Based on the results of GC-MS analysis before and after the refining process, there was a slight difference in the percent content of each compound in oil. Some compounds are lost after the refining process Octadecanoic acid, methyl ester (CAS), 9-Octadecenoic acid (Z)-, methyl ester (CAS), 9-Octadecenoic acid(Z)-, methyl ester (CAS), Dodecanoic acid (CAS) Lauric acid, Ethyl linoleate, and Stearic acid. The loss of some compounds has occurred in the refining process, especially in the presence of NaOH in the neutralization step causing the formation of soap which will also be washed away when washing the oil with distilled water (Ratih & Wuriyanti, 2016).

In this study, there was a squalene component, the level of squalene before the refining process was 22.72%, retention time was 14.807 minutes, molecular weight 410, and molecular formula $C_{30}H_{50}$. After the refining process, squalene content increased to 53.28% with a retention time of 14.826 minutes. Squalene is an unsaponifiable compound so it is not lost in washing. With the refining process, the impurities were lost and pure oil is obtained so that the squalene content increased.

CONCLUSIONS

The yield of cucumber seed oil obtained by continuous extraction method at 19.38 ± 0.94% (w/w) is higher than oil obtained by maceration at 13.26 ± 0.99 % (w/w).

The physicochemical properties of cucumber seed oil after purification showed changes such as the color of the oil becoming pale yellow, and there is a decrease in the value of water content, density, acid number, free fatty acid, and peroxide number.

The composition of cucumber seed oil constituents before the refining process was dominated by palmitate acid (21.28%), linoleic acid (45.50%), and squalene (22.72%). While the composition of cucumber seed oil constituents after the refining process was dominated by palmitic acid (21.37%), linoleic acid (25.36%), and squalene (53.28%).

ACKNOWLEDGEMENTS

The Acknowledgment is addressed to the Ministry of Research, Technology, and Higher Education for funding this research through the PDUPT research program grants in 2019-2020.

REFERENCES

Achu, M. B., Fokou, E., Tchiégang, C., Fotso, M., & Mbiapo, F. (2008). Atherogenicity of Cucumeropsis mannii and Cucumis sativus oils from Cameroon. *African Journal of Food Science*, 2, 21–25. Retrieved from https://www.researchgate.net/publication/242213303_Atherogenicity_of_Cucumeropsis_mannii_and_Cucumis_sativus_oils_from_Cameroon

American Oil Chemist Society Champaign. (1997a). *Official methods and recommended practices of the american oil chemists' society*. Ca 5a-40.

American Oil Chemist Society Champaign. (1997b). *Official methods and recommended practices of the american oil chemists' society*. Cd 8-53.

American Oil Chemist Society Champaign. (1998a). *Official methods and recommended practices of the american oil chemists' society*. Aa 4-38.

American Oil Chemist Society Champaign. (1998b). *Official methods and recommended practices of the american oil chemists' society*. Ca 2b-38.

American Oil Chemists' Society Champaign. (1989). *Official methods and recommended practices of the american oil chemists' society*.

Block, E. (2021). *VERIFY: Are sharks being used in COVID-19 vaccines?* Retrieved from https://www.wusa9.com/article/news/verify/what-is-squalene-do-some-covid-19-vaccines-have-shark-in-them-squalene-what-is-squalene-what-are-the-ingredients-in-some-of-the-covid-19-vaccines-fact/65-5afbbdc7-a462-4b27-b4db-fe5358a501d0

Budiman, F., Ambari, O., & Surest, A. H. (2012). Pengaruh waktu fermentasi dan perbandingan volume santan dan sari nanas pada pembuatan virgin coconut oil (VCO) (Effect of fermentation time and volume ratio of coconut milk and pineapple juice on the production of virgin coconut oil (VCO). *Jurnal Teknik Kimia*, *18*(2). Retrieved from http://jtk.unsri.ac.id/index.php/jtk/article/view/16

Hamilton, R. J., Kalu, C., Mcneill, G. P., Padley, F. B., & Pierce, J. H. (1998). *Effect of Tocopherols, Ascorbyl Palmitate and Lecithin on Autoxidation of Fish Oil*. *75*(7).

Ifeoma P., O., Azubike, J., O., Ifeanyi V., E., Precious O., O., Osuagwu, C., C., … James O., O. (2021). Phytochemical and proximate composition of cucumber (*Cucumis sativus*) seed oil. *International Journal of Research and Scientific Innovation*, *08*(02), 244–250. https://doi.org/10.51244/ijrsi.2021.8207

Kumar, D., Kumar, S., Singh, J., Narender, Rashmi, Vashistha, B. D., & Singh, N. (2010). Free radical scavenging and analgesic activities of *Cucumis sativus* L. fruit extract. *Journal of Young Pharmacists*, 2(4), 365–368. https://doi.org/10.4103/0975-1483.71627

Mardani, S., Ghavami, M., Heidary-Nasab, A., & Gharachorloo, M. (2016). The effects of degumming and neutralization on the quality of crude sunflower and soyabean oils. *Journal of Food Biosciences and Technology*, *6*(2), 47–52.

Mariod, A., Matthäus, B., Eichner, K., & Hussein, I. H. (2012). Effects of deodorization on the quality and stability of three unconventional sudanese oils. *Gida*, *37*(4), 189–196.

Meilano, A. R., Soetjipto, H., & Cahyanti, M. N. (2017). Pengaruh proses degumming dan netralisasi terhadap sifat fisiko kimia dan profil asam lemak penyusun minyak biji gambas (Luffa acutangula Linn.). *Chimica et Natura Acta*, *5*(2), 50. https://doi.org/10.24198/cna.v5.n2.14604

Paris, H. S., Daunay, M. C., & Janick, J. (2012). Occidental diffusion of cucumber (Cucumis sativus) 500-1300 CE: Two routes to Europe. *Annals of Botany*, *109*(1), 117–126.

https://doi.org/10.1093/aob/mcr281
Ratih, R., & Wuriyanti, H. (2016). Karakterisasi dan penentuan komposisi asam lemak dari hasil pemurnian limbah pengalengan ikan dengan variasi (Characterization and determination of fatty acid composition from the process). *Berkala Sainstek*, *IV (1)*. Retrieved from https://jurnal.unej.ac.id/index.php/BST/article/download/4461/3493/
Saleh, B., Ezzatpanah, H., Aminafshar, M., & Safafar, H. (2013). *The Effect of Refining Process on the Conjugated Dienes in Soybean Oil*. (November).
Sharma, S., Dwivedi, J., & Paliwal, S. (2012). Evaluation of antacid and carminative properties of Cucumis sativus under simulated conditions. *Der Pharmacia Lettre*, *4*(1), 234–239. Retrieved from https://www.scholarsresearchlibrary.com/abstract/evaluation-of-antacid-and-carminative-properties-of-cucumis-sativus-under-simulated-conditions-8584.html
Soetjipto, H., Anggreini, T., & Cahyanti, M. N. (2018). Profil asam lemak dan karakterisasi minyak biji labu kuning (Cucurbita moschata D.). *Jurnal Kimia Dan Kemasan*, *40*(2), 79. https://doi.org/10.24817/jkk.v40i2.3797
Soltani, R., Hashemi, M., Farazmand, A., Asghari, G., Heshmat-Ghahdarijani, K., Kharazmkia, A., & Ghanadian, S. M. (2017). Evaluation of the effects of Cucumis sativus seed extract on serum lipids in adult hyperlipidemic patients: A Randomized Double-Blind Placebo-Controlled Clinical Trial. *Journal of Food Science*, *82*(1), 214–218. https://doi.org/10.1111/1750-3841.13569
Stevenson, D. G., Eller, F. J., Wang, L., Jane, J. L., Wang, T., & Inglett, G. E. (2007). Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. *Journal of Agricultural and Food Chemistry*, *55*(10), 4005–4013. https://doi.org/10.1021/jf0706979
Tang, J., Meng, X., Liu, H., Zhao, J., Zhou, L., Qiu, M., … Yang, F. (2010). Antimicrobial activity of sphingolipids isolated from the stems of cucumber (Cucumis sativus L.). *Molecules*, *15*(12), 9288–9297. https://doi.org/10.3390/molecules15129288