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The Variation of Fatty Acid Composition between Fresh and Stored Avocado (*Persea Americana*)

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Abstract
Avocado is one of the most popular subtropical/tropical fruits and its consumption has increased noticeably during the last century. However, the seeds of avocado are usually discarded after consumption. In order to evaluate new sources of oil for the growing population, avocado agricultural waste including seeds can be considered. Therefore, this study aimed to determine the fatty acid profile of fresh avocado seeds along with the seeds which have been stored at -20 C for two years. The oil was extracted by soxhlet and the percent of fatty acid methyl esters was identified through gas chromatography. The results indicated that total fat (2.88% fresh and 0.86% stored) decreased dramatically during storage and the composition of fatty acids was significantly changed. The major fatty acids in fresh seeds were monounsaturated, while saturated fatty acids were dominant in stored avocado seeds. The results demonstrated fresh seeds of avocado should be evaluated for their application in the energy, cosmetics, and pharmaceutical industry.

Key words
Avocado, Fatty acids, Storage, Oil quality.

Introduction
Avocado (Persea Americana) known also as alligator pear is one of the evergreen subtropical and tropical fruits that are grown in nearly 50 countries from all continents (Bayram et al., 2006). Due to its excellent nutritional and medicinal properties, its cultivation and consumption have dramatically increased in recent years. Avocado is a climacteric fruit and ripens after harvest, not on the tree (Orhevba and Jinadu, 2011). Persea Americana belongs to the Lauraceae family which includes approximately 150 species, the majority of which are the habitat of tropical America and are native to Central and South America. Mexico ranks first in the production of avocados (Villamil et al., 2018). Avocado contains a high amount of fats, fiber, and protein. Due to having low sugar content and digestible oil, avocado fruit is a rich source of energy and can be placed in diabetics' diets as an essential component. The nutritional value of avocado is due to containing high levels of vitamins such as C, E, K, and B groups and minerals such as magnesium, iron, phosphorus, sodium, zinc, and potassium (Orhevba and Jinadu, 2011; Oluwole et al., 2013 and Maitera et al., 2014). Moreover, the whole fruit including pulp, peel, and seed possesses many essential nutrients and phytochemicals with the potential to treat many diseases (Elsayed and Lobna, 2013; Asif, 2015; Adaramola et al., 2016; Yilmaz et al 2020). However, cultivar, storage conditions, ripeness, edaphoclimatic conditions, and geographical growth location affect the quantity and composition of the phytochemicals of avocados (Bora et al., 2001, Setyawan et al., 2021). Increasing the consumption of this preferred fruit brings the challenge of agricultural waste which is needed to be evaluated. Although seeds comprised most of the fruit they are non-edible parts of the fruit and are usually discarded as residues (Adaramola et al., 2016). The exploitation of agricultural waste of avocado fruit is a promising trend that may be profitable in both environmental and industrial aspects by reducing the production of waste and evaluating seeds in the pharmaceutical and energy industry due to containing high levels of valuable bioactive compounds such as antioxidants, antibacterial and fatty acids (Mensah and Golomeke, 2015; AlMatar et al., 2019).
On the other hand, technological advances in energy in the way of world development have increased energetic consumption obtained from fossils. There are serious concerns about the limitation of fossil fuels needed for the increasing population along with environmental issues such as global warming. Therefore, managing agricultural waste can be an alternative solution to these problems. One of the prominent characteristics of avocado seed that makes it a good candidate for pharmaceutical and bio-fuel applications is the oil quality (Reyes-Cueva et al., 2020, Bora et al., 2001). Many studies have proved that avocado seeds have analgesic, vasorelaxant, anti-inflammatory, hypotensive, anticonvulsant, antiviral, wound healing, antiulcer, antihepatotoxic, antioxidant, hypoglycemic, anti-cancer activity (Yasir et al., 2010; Dabas et al., 2013, Setyawan et al., 2021; Soledad, 2021). Some studies also reported that avocado seed oil is suitable for the production of biodiesel due to its characteristics (Hiwot, 2017; Sathishet al., 2021).
However, the oil content and the fatty acid composition of the seed oil are important criteria that specify the quality of seed oil to be evaluated as biodiesel and raw material for the pharmaceutical industry (Zarifikhosroshahi and Ergun, 2022). However, more studies are needed on the identification of fatty acids composition in seed oil because these parameters are so variable due to affecting factors. Therefore, this study aimed to compare the oil content and the fatty acids composition of seed oil of fresh and stored avocado seeds from the Mersin region in Turkey, considering the geographic location of cultivation and storage factors.

Materials and Methods
Plant Material
Avocado fruits for two applications with three replicates were collected from a farm located in Mersin, Turkey in 2019. Mersin is located in the Mediterranean region of Turkey with a subtropical climate and has hot, humid summers and mild, wet winters. The seeds were removed manually from the fruits in the same maturation status. The fresh seeds were cut into small pieces and ground in a grinder. The other samples were kept at -20 for two years. Thereafter, they were also grounded for further analysis.

Oil Extraction
The extraction of oil was carried out using 100 grams of dried seed via an automatic soxhlet device "Gerhardt GmbH & Co. KG" using. The solvent was GC-grade Hexane (Merck, Germany). The yield of oil was calculated by weighting the extracted oil and expressed as g100g-1 of dry samples.

Determination of Fatty Acids

Potassium hydroxide was used for the esterification of fatty acids (David et al., 2003). The methylated fatty acids were analyzed by gas chromatography equipped with a flame ionization detector (FID). DB-Wax column (30 m × 0.25 mm ID, 0.25 µm) was used for the separation of fatty acids. The oven temperature was 50°C for 1 min, raised to 25°C min-1 to 200°C, then 3°C min-1 to 230°C which was held for 18 min, while the injector and the detector temperatures were set at 280°C and 250°C, respectively. The results were expressed in GC area % as a mean value and ± standard deviation (David et al., 2003).

Results and Discussion

The oil content and fatty acid composition of oil from fresh and stored avocado seeds are given in Table 1. The total fat significantly decreased during storage at -20ºC (2.89% and 0.86% in fresh and stored samples, respectively). The oil yield obtained in this study is lower than the results reported by Oluwole et al., (2013) in unripe and ripe seeds of avocado (9.27% and 9.47%, respectively) and also reported (%8.10) by Adaramola et al., (2019). However, Bora et al (2002) obtained a lower oil yield (1.87%) from avocado seeds compared to the results of this study in fresh seeds despite a higher amount than stored seeds. However, so many factors such as plant species and cultivars, environmental conditions, the climate of cultivation location, ripening stage, harvesting time, the oil extraction methods, and solvent used for extraction (Zarifikhosroshahi and Ergun, 2021; Keles, 2020; Guney 2020; Koc et al 2019, Marfo et al., 1986). Although Akinoso and Raji (2010) reported that seeds with oil yield higher than 17% are considered oil seeds, Bwade et al. (2013) reported that the quality of agricultural products is determining criterion for whether or not oil can be industrially processed from them. One of the oil quality parameters which is used for the evaluation of oil in industry and medicine is the composition of fatty acids. The results showed that oleic acid, linoleic acid, palmitoleic acid, palmitic acid, and stearic acid are the main fatty acids in fresh seed oil; respectively while linoleic, oleic, palmitic acid and stearic acid are the main ones in stored seeds, respectively. Saturated fatty acids constituted about 17.65% of the total fatty acids of the fresh seed oil while was the most part of stored avocado seed oil (46.32%). However, PUFA (Poly Unsaturated Fatty Acid) consisted of the same ratio of seed oil in both fresh and stored seeds. However, linoleic acid was detected in different isomeric forms in fresh and stored seeds (gamma in fresh and alpha in stored seeds oil). The amount of total SFA (Saturated Fatty Acid) was high following the total PUFA. In contrast to total PUFA, fresh seeds had lower (17.65%) saturated fatty acids. The stored seeds had high levels of palmitic acid (24.25%) and stearic acid (8.04%) (Fig. 1). Although unsaturated fatty acids consisted the main part of avocado seed oil (80.36% and 51.67% in fresh and stored seed oil, respectively) total MUFA (Mono Unsaturated Fatty Acid)was twice (46.86%) higher in fresh seeds oil. Hiwot (2017) reported that oleic acid was the main fatty acid of avocado seed oil (71.65%) leading to high SFA along with high palmitic acid (13.58%). However, the researcher found a lower amount of linoleic acid (12.48%) than the amount obtained for linoleic acid (27.69%) in this study. These results are compatible with obtained results in this study from avocado fresh seed oil. Contrary, Bora et al (2001) reported that linoleic acid (38.89%) was the major fatty acid of avocado seed following palmitic acid (20.84%) and oleic acid (17.41%) which are different from the results of this study and study by Hiwot (2017). However, all studies proved that saturated fatty acids were dominant in avocado seed oil. The ratio of Oleic/Linoleic acid is a parameter responsible for the oil shelf life. Based on the results, fresh seed oil of avocado has higher shelf life than stored one. The oil obtained from both fresh and stored seeds had favorable C18:2/C18:3 (approximately 5.5). This ratio of oil could be an assessment criterion for reducing the triglycerides and HDL (high-density lipoprotein) in blood plasma (Werman et al., 1991). seed and However, the results of the study showed that long storage of avocado seed caused severe changes both in oil quantity and the composition of fatty acids. During storage, the main change is in the ratio of MUFAs to SFAs. This may be due to an interruption in the conversion of palmitic acid to palmitoleic acid and oleic acid in biosynthetic pathways of plant C18 unsaturated fatty acids (Karki et al., 2019) However, the composition of fatty acids in seed oil is influenced.

Table 1. The fatty acid composition of seed oils of fresh and stored avocado [%]

Fatty acid methyl esters	Fresh Seed	Stored Seed	Mean difference (fresh-stored)
Total oil (g/100g)	2.89±0.21	0.86±0.04	-2.03
Caproic acid (C6:0)	0±0	0.7±0.04	0.70
Caprylic acid (C8:0)	0±0	0.81±0	0.81
Capric acid (C10:0)	0±0	1.08±0.02	1.08
Lauric acid (C12:0)	0.12±0.01	2.32±0.05	2.20
Myristic Acid (C14:0)	1.68±0.01	7.39±0.16	5.71
Pentadecanoic acid (C15:0)	0.26±0.01	0.56±0.02	0.30
Palmitic acid (C16:0)	11.42±0.04	24.25±0.06	12.83
Margaric Acid (C17:0)	0.09±0	0.3±0.01	0.21
Stearic acid (C18:0)	3.75±0.01	8.04±0.11	4.29
Arachidic acid (C20:0)	0.15±0.01	0.32±0.04	0.17
Behenic acid (C22:0)	0.08±0	0.36±0.03	0.28
Lignoseric acid (C24:0)	0.1±0.01	0.23±0.01	0.13
Σ SFA*	**17.65±0.02**	**46.32±0.05**	**28.67**
Palmitoleic acid (C16.1)ω−7	12.85±0	0.94±0.01	-11.92
Oleic acid (C18:1n9c)ω−9	33.89±0.04	19.28±0.11	-14.61
Eicosenoic acid (C20:1n9c)ω−9	0.13±0.01	0±0	-0.13
Erucic acid (C22:1n9)	0±0	0.03±0.01	0.03
Σ MUFA*	**46.86±0.04**	**20.24±0.11**	**-26.62**
Linoleic acid (Cl 8:2n6c) ω−6	27.69±0.01	26.95±0.2	-0.74
g-Linolenic acid (C18:3n6) ω−6	0.05±0.01	3.91±0.21	3.86
a-Linolenic acid (C18:3n3) ω−3	4.97±0.03	0±0	-4.97
cis-11.14-Eicosatrienoic acid (C20:2)	0.06±0	0±0	-0.06
cis-8.11.14-Eicosatrienoic acid (C20:3n6) ω−6	0.41±0.01	0±0	-0.41
cis-11,14,17-Eicosatrienoic acid (C20:3n3) ω−3	0.22±0	0.58±0.06	0.36
cis-13.16-docosadienoic acid (C22:2)	0.11±0.01	0±0	-0.11
Σ PUFA*	**33.5±0.01**	**31.43±0.07**	**-2.07**
Unsaturated/Saturated fatty acid ratio	**4.55**	**1.15**	
Polyunsaturated/Saturated fatty acid ratio	**1.90**	**0.68**	
Oleic/Linoleic acid ratio	**1.22**	**0.72**	

*SFA: Saturated Fatty Acid; MUFA: Mono Unsaturated Fatty acid; PUFA: Poly Unsaturated Fatty acid

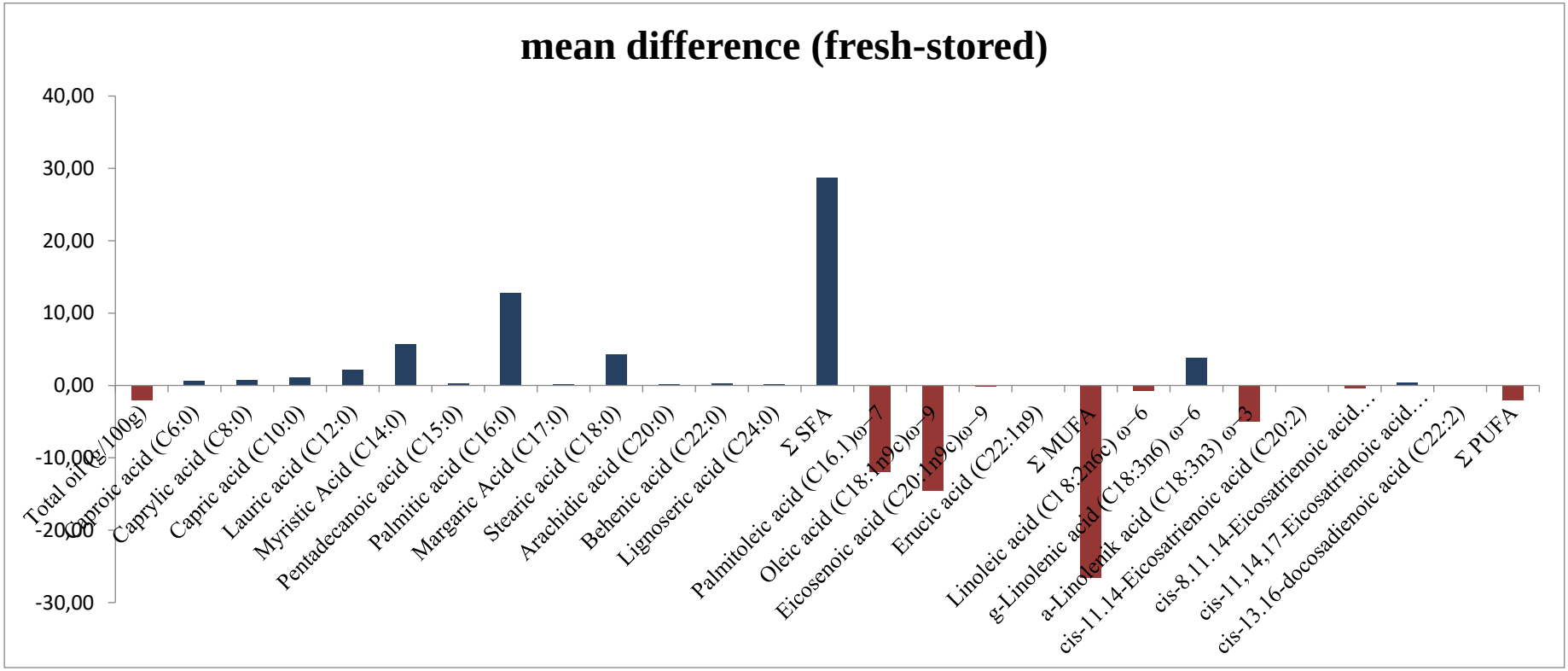

Figure 1. The distribution of fatty acid in fresh and stored seeds of avocado (The increase in SFA and decrease in MUFA during storage are remarkable)

Conclusion

The avocado fruit is very preferred in modern society due to its nutritional characteristics and its outstanding position in a healthy diet. On the other hand, its application in cosmetics, medicine, and energy makes it a precious product for the industry. However, the seeds are considered food waste and most of the time are discarded despite many remarkable properties which can also be evaluated in pharmacology and production of biofuel. This study compared the oil content and fatty acid composition of seed oil in fresh and stored seeds. The results indicated significant changes in oil yield and the composition of fatty acids and offered that long storage of seed in lower temperatures was not suitable. Consequently, avocado seeds can be a good source of oil for industrial applications in pharmacology and energy fields.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author's Contributions

The authors contributed equally to this manuscript.

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