



Harvesting time substantially affects the squalene content in olive oils of Gemlik and Kilis Yaglik varieties

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Abstract

Olive (*Olea europaea* L.) is a significant crop especially in Mediterranean countries. In Turkey olives and olive oil have a major agricultural importance. Olives and olive oil have been used not only nutrient and as a flavor enhancer in Mediterranean dishes, but also a medicine because of having essential roles for human health. The pharmaceutical value and uses are related to biochemical content and compounds available. Mainly four chemical sources: mono-unsaturated fatty acids, polyphenols, carotenoids and squalene are functional bioactives in olive oil particularly for pharmaceutical affects. The present study was designed to examine changes of squalene contents of olive oil gained Kilis Yağlık and Gemlik olive cultivars' fruits harvested in tree different time along the two years. Squalene content in olive oils varied according to harvest time, cultivar and cultivation year. Accordingly, the variety affected the squalene content, in Gemlik cultivar squalene was more than Kilis Yaglik cultivar. In terms of its cultivated year, in the high yield year, an increase in squalene content was observed. In olive oil, squalene content decreased first then increased by the fruit maturation. Therefore, harvest time effected biochemical content of oil by the fruit ripening stages.

Key Words: *Olea europaea* L., olive oil, squalene, harvest time, olive varieties

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1. Introduction

Olive is an important agricultural plant grown in the Mediterranean basin. Turkey is also one of the important olive producing countries. According to 2019 data, Turkey ranks 4th in the world olive production with 1525000 tons. Spain is in the first rank with 5965080 tons, followed by Italy (2194110 tons) and Morocco (1912238 tons) in the second and third ranks, respectively (Faostat, 2019). The olive plant has been used as food and medicine in this region for centuries. The

olive plant was distributed to Mediterranean countries by eastern civilizations (Sanchez-Rodriguez et al., 2019). Olive oil, which is used by humans in diets, has positive effects on physiological and cellular activities, has been produced since ancient times. (Martakos et al., 2020). Olive oil is acquired by crushing olive fruits and is a useful and valuable component of the Mediterranean diet. In recent years, due to the increasing importance of the Mediterranean diet in terms of health and nutrition, studies on olive and olive oil have increased.

It is considered one of the healthiest plant-based oils by consumers around the world, as there are many arguments for the role of olive oil, particularly in preventing heart and blood vessel ailments. (Fernández-Cuesta et al., 2013). Olive oil includes of fatty acids with a carboxyl group (COOH) and a carbon atom in this carboxyl group. The main fatty acid with beneficial health effects in olive oil is oleic acid, which is an unsaturated fatty acid and create more than half of the total fatty acids (Alowaiesh et al., 2018). Oleic acid is accompanied by numerous biologically active minor components, including a wide variety of sterols, squalene, phenolic compounds and tocopherols (Fernandez-Cuesta et al., 2013).

Squalene is a inherently consisting terpenoid aliphatic hydrocarbon that is produced by all animals and plants, including humans, and represents almost all of the hydrocarbons, and represents almost all of the hydrocarbons (Martakos et al. 2020, Cayuela and Garcia., 2018). Squalene is mainly derived from shark liver oil, but squalene, which is produced from plants, has become more important over time (Fernández-Cuesta et al., 2013). Squalene is found in nature, primarily in olive oil, and in vegetable oils such as rice bran oil, wheat germ oil, palm oil, or amaranth oil. On the other hand, squalene (C₃₀H₅₀), known as microalgae oil, which can also be obtained from microalgae, is one of the oils obtained from squalene algae with a double hydrogen bonded isoprene frame (Figure 1.). It has been stated that it can be designed to be used as a fuel for jets while being used as a biofuel, as it creates a hydrocarbon source equivalent to heavy oil (Oya, et al., 2015, Kimura, et al., 2018). Olive oil is one of the sources that contain the most squalene, which makes up more than half of the non-soap-forming substance. It has been reported that the concentrations of pigment, tocopherol and squalene in olive oil differ based on location, olive fruit varieties, growing parameters, ripening stage and oil production parameters (Martakos et al., 2020).

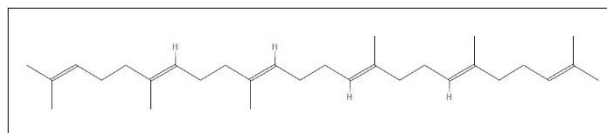


Figure 1. Chemical Structure of Squalene (Tansey, T. R. and Shechter, I. 2000).

Squalene plays an significant role in the quality of olive oil. Many scientific articles have been written to reveal its benefits to human health and its anti-cancer properties (Newmark, 1999, Rodriguez-Rodriguez and Simonsen, 2012). In extra virgin olive oil, squalene, the main component of small compound fractions, is a known biological response modifier related to oxygen, immune system and steroid synthesis providing anti-aging, anti-inflammatory and hypocholesterolemic activities. The scavenging and repairing ability against foreign products, together with the results in experiments performed in vitro associated with cardiovascular diseases, support a possible protective role of squalene against oxidative stress and free radicals, indicating the high potential of this molecule for use in medicine, cosmetics and nutrition (Mastralexi and Tsimidou, 2021).

For olive oil stability the role of squalene was investigated for various concentrations and experimental conditions (Psomiadou and Tsimidou, 1999). Olive oil has proven moderate antioxidant activity due to the higher concentration. It has been stated that the moderate antioxidant activity of squalene in olive oil can be explained by the different existing lipids competing with each other, leading to a decrease in the oxidation rate.

Warleta et al., (2010) studied cell proliferation, scavenging properties, apoptosis, cellular cycle profile, reactive oxygen species (ROS) level and oxidative effect of squalene on DNA damage using human breast cells. According to the results obtained, squalene exhibited antitumor properties against different types of cancer and a protective effect on breast cancer.

In this study, the relationship of squalene content, an important compound that determines the quality of olive oil, were investigated with olive harvest time, as well as the effect of cultivars and crop load.

2. Material and Methods

In the research olive fruits of Kilis Yaglik and Gemlik cultivars grown in Kilis region were used. Kilis Yaglik variety was obtained from Kilis Dogancay area, and Gemlik variety was obtained from Kilis 7 Aralik University Faculty of Agriculture Mercedabik Campus. While the crop load of trees was high in 2018, it was low in 2019.

2.1. Harvest and Oil Extraction: The fruits of the selected olive trees were harvested in mid-October, November and December in 2018 and 2019. Olives were extracted without waiting (1 day after harvest) in an oil extraction device (Olio Mio Mini, Italy) with a two-phase decanter system. The olives were first crushed in the mill section, and the resulting olive paste was kneaded in a malaxer at 30 °C for 1 hour and then separated into oil and pulp (pomace+black water) in the decanter. Obtained oils were stored in dark glass containers in a dark, dry and cool environment until analysis.

2.2. Analysis of Oils: According to the European Standard NF EN ISO 12966-2, about 0.1 g of olive oil in 10 ml of hexane was trans-methylated with a 0.5 ml solution of KOH in methanol (2 M). Fatty acid methyl esters (FAMES) were analyzed in accordance with European Standard NF EN ISO 5508. Analyzes were performed using a Shimadzu brand GC-2010 Plus instrument equipped with a flame ionization detector (GC-FID) (T 320° C) using a capillary.

2.3. Statistical Analysis: JUMP7 statistical program was used to determine statistical significance levels by employing the Tukey's multiple comparison test and the differences

between individual averages were considered to be statistically important at $p < 0.05$. The results were expressed as mean.

3. Results and Discussion

The squalene content of the olive oils obtained from Kilis Yaglik and Gemlik olive varieties harvested at different times are shown in Table 1 and Figure 2. Accordingly, while there was more squalene in Gemlik cultivar, it first decreased and then increased in both cultivars periodically. While the highest squalene was obtained from Gemlik variety as 2.76% in December, it was obtained from Gemlik variety at least in November.

While the squalene content was higher in 2018, less squalene was obtained in 2019 when the yield was low. According to the results obtained, it was seen that the variety affected the amount of squalene, and fluctuations in the squalene content occurred with the periodical maturation. The product load also affected the squalene content, and more squalene accumulation occurred in the year when the yield was high. The amount of squalene in olive oil is affected by the variety, environmental and climatic data, year and crop load. Olive varieties found around the world have varied widely in squalene content (Martínez-Beamonte et al., 2019) According to the study conducted in Spanish cultivars, large differences were observed between 'Arbequina' and 'Picual' cultivars (Beltrán et al., 2016).

In another study, cultivars 'Drobnica', 'Nocellara de Belice', 'Oblica' and 'Souri' were found to have the highest squalene content (Fernández-Cuesta et al., 2013). In addition, it was observed that there were differences even within the same cultivars. This wide range has also been demonstrated by studies conducted in different countries (Manzi et al., 1998; Baaccouri et al., 2008; Ambra et al., 2017; Queslati et al.; 2009; Uluata et al., 2016).

Depending on the variety, maturity levels are important for obtaining high oil yields (Baccouri et al., 2007; Anastasopoulos et al., 2011; Bodoira et al., 2015; Laroussi-Mazghani et al., 2016). Besides this, the ripeness of the fruit is affected by the irrigation, climate, growing area and conditions, temperature and harvest time.

Accordingly, the biochemical content of the oil also changes (Beltran et al., 2016; Manzi et al., 1998). Due to limited information about

the 'Koroneiki' variety, it could not be determined that the squalene content of olive oil is a change from traditional or organic production of the applied agricultural regime (Anastasopoulos et al., 2011). Baccouri et al. (2007), in their study, compared the groups fed with rain water and fed with irrigation water in the cultivar 'Chetoui Tunisia'. It was determined that the squalene content in the oils obtained from olive plants fed with irrigation water was 1/3 lower than those fed with rain water.

Table 1. Changes squalene content of olive oil obtained Kilis Yağlık and Gemlik varieties by the harvest time and years.

Harvest Time	Years							
	2018 (1,74 a)			2019 (1,50 b)				
	Gemlik		Kilis Yağlık		Gemlik		Kilis Yağlık	
October	1,42	def	1,79	bc	1,86	b	1,38	ef
November	1,21	f	1,35	ef	1,49	de	1,88	b
December	2,76	a	1,88	b	1,61	cd	0,78	g
Cultivars Average	Gemlik 1,73 a				Kilis Yağlık 1,51 b			

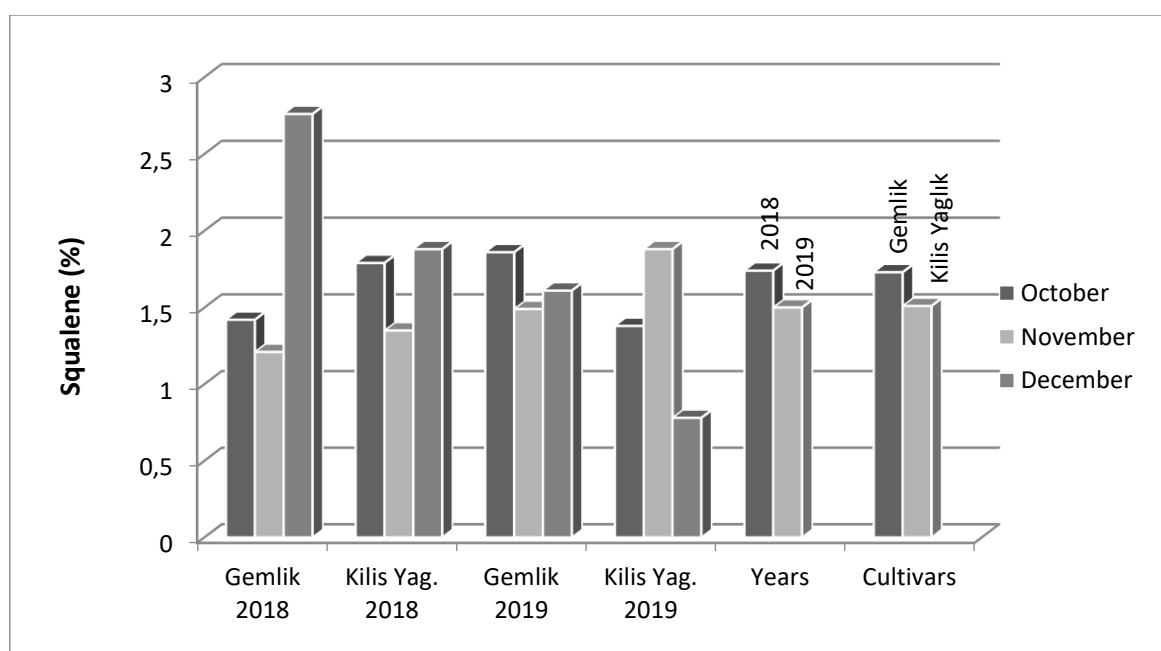


Figure 2. Effect of harvest time on squalene content of oils obtained from Kilis Yağlık and Gemlik varieties

In many studies revealed that the main reason for the variability in the biochemical structure of the oil is the maturity level, as well as the region where it grows, soil structure, harvest time, irrigation status and harvest years affect the content. (Ben Mansour et al., 2015; Çetinkaya, 2017; Lazzez et al., 2011). The content of squalene may be changed by the maturity that was found that the squalene first increased and then decreased in Tunisian varieties (Sakouhi et al., 2011).

4. Conclusion

Major and minor components in olive oil are affected by variety, harvest time and crop load. In the study, Gemlik cultivar synthesized more squalene than Kilis Yaglik cultivar. Squalene content also changes with harvest time. More squalene was synthesized in the year when the yield was high. In order to obtain a stable oil with optimum quality characteristics in olive oil, it is of great importance to determine the appropriate variety and harvest time for the purpose. In addition, considering the effect of variety and harvest time on other components and each other, optimum conditions for squalene synthesis should be provided.

Author Contribution

Hakan CETİNKAYA conceived and designed the experiments. İbrahim Samet GOKCEN performed the experiments. Hakan CETİNKAYA wrote the paper and İbrahim Samet GOKCEN contributed to writing the paper.

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Conflict of Interest

There were no conflicts of interest in the course of this research.

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