Uluslararası Fen Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Science Research 2022, Vol. 6 (2), 23-33
https://doi.org/10.29329/ijiasr.2022.454.1

Original article

Investigation on Biosynthesis of Phytosterol Compounds in Ayvalık Olive Variety (*Olea europaea* L.) During Ripening under Conventional and Organic Cultivation Conditions

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Abstract

Olive oil obtained from the fruit of olive trees (*Olea europaea* L.) belonging to the Oleaceae family; basically consists of two parts: saponifiable and unsaponifiable parts. The saponifiable fraction represents about 98% by weight of the oil. On the contrary, the unsaponifiable fraction constitutes about 2% of the total oil weight.

Phytosterols constitute the largest part of the unsaponifiable fraction and are the most important parameter to establish the purity of olive oil. Olive oil quality is affected by many factors, the most important being the ripening stage. Phytosterol content changes with maturation. Because the metabolic events in its structure continue until the fruit ripens. This study was carried out in Ayvalık olive variety grown with organic and conventional growing techniques during the 2019 and 2020 seasons. Fruits obtained from Geyikli region of Çanakkale province were harvested in 8 different maturity stages (September– December) at 15-day intervals. Phytosterol contents of fruits and changes in phytosterols in olive ripening process were determined periodically by gas chromatography-mass spectrophotometer (GC-MS) technique.

At the end of the study, the total phytosterol content of the fruits harvested from both conventional and organic orchards during both production seasons in both years, depending on the maturity index. Phytosterol components were found to increase regularly until December, but it decrease after December. While the maximum total sterol amount was determining 2.008 g kg-1 on 08.12.2019, it was 2.520 g kg-1 on 06.12.2020. After this date, it was observed that the total sterol content decreased as a result of over-ripening.

Keywords: *Olea europaea* L., Maturity Index, Total sterol, Sterol components, β-sitosterol, chromatography.

Received: 20 February 2022 * **Accepted:** 19 June 2022 * **DOI:** https://doi.org/10.29329/ijiasr.2022.454.1

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INTRODUCTION

Olive is a plant species belonging to the genus Olea, which has many shrub-shaped species and subspecies of the Oleaceae family. Olive, which has found the best growing conditions in the Mediterranean climate zone, is one of the oldest cultivated plants in the World (Gundogdu et al., 2016). The beneficial effects of olive oil on human health are due to the presence of a wide variety of bioactive minor components. Among them, phytosterols have diversified amount of biological activity and physical properties (Boskou, 2006; Gundogdu and Seker, 2020; Pérez-Jiménez et al., 2007)

There are four forms of sterols in olive oil: (1) 4α-desmethyl sterols, (2) 4α-methyl sterols, (3) 4,4-dimethyl sterols, and (4) triterpene dialcohols. 75-90% of the total sterol in olive oil is β-sitosterol, 5-36% Δ-5-avenasterol, 3% campesterol and 1% stigmasterol (Boskou, 2002). The change in sterol composition during maturation is due to the cessation of the enzymatic activity of sterol biosynthesis, and the conversion of stanol and sterol ester to other sterol forms with the increase of reactions of hydrogenation, dehydration and dehydrogenation. The decrease in sterol concentration during ripening is due to the greater production of the saponification fraction (Sanchez Casas et al., 2004).

The composition of phytosterols is 0.5-1.5% in oil. These bioactive components are very important as quality criteria in terms of providing reliable information about the purity of the oil and determining its adulteration with other seed oils (Ben Temime et al., 2008). Total sterol amount in olive oil is an important criterion in both the International Olive Oil Council (IOOC, 2019) and the Turkish Food Codex (TGK, 2017) and should be at least 1000 mg kg-1.

The amount of these components; Since it varies according to diverse factors such as variety, climate, care, harvest, growing conditions and processing, many researches are needed on the components responsible for a quality olive oil and the factors affecting their formation. It is very important to understand the chemical structure of olive oil, especially today, where geographical registration gains importance.

In this study, the phytosterol content and composition changes of the Ayvalık olive variety grown by organic and conventional methods were investigated periodically for 2 years. Thus, the effect of cultivation conditions on the phytosterol content of Ayvalık olive variety was determined. Also, the maturity index was calculated when the phytosterol content was high.

MATERIALS and METHODS

As plant material in the study; It was studied with Ayvalık olive variety. Samples were taken in the production years of 2019 and 2020 during the September-December period, which covers the development and especially ripening periods of the olive fruit. Fruit samples were obtained from Geyikli (Canakkale) town of Ezine district, where organic and conventional cultivation techniques were applied. Samples were harvested by hand at 8 different maturity stages at 15-day intervals. According to the method prescribed by the International Olive Oil Council, the "olive maturity index" was calculated from randomly 100 selected fruits (Gundogdu and Kaynas, 2020; IOOC, 2007). After calculating the maturity index, phytosterol contents and different phytosterol components were determined by Gas chromatography mass spectrometry device.

Phytosterol extraction: For this purpose, after weighing 1.5±0.001 g of oil sample, cholesterol standard (0.1%) to be dissolved in 2 ml of chloroform was added. Then, the chloroform was evaporated on a rotary evaporator. 6 ml of 6 M KOH and 10 ml of ethanol were added and kept in an oil bath at 90 °C for saponification for 1.5 hours. The unsaponifiable fraction was extracted with hexane. After the hexane was evaporated, 0.25 ml of pyridine and 0.3 ml of BSTFA (N,Obis(trimethylsilyl)trifluoroacetamide) were added; Derivatized in an oven at 80 °C for 30 minutes. 1ml of the derivatized extract was taken into a GC vial. The injection volume is 1 µL. (Gul and Seker, 2006).

GC/MS Conditions: Derivatized samples were determined by Shimadzu ® QP 2010 brand GC/MS instrument. Calculation; cholesterol internal standard was used. Then, the locations of other sterols were defined according to the location of the cholesterol and the concentration calculation was made according to the RF value of the internal standard. Hydrogen gas is 40 ml min-1, Helium gas is 45 ml min-1, and Air is 450 ml min-1. D5 capillary column (30 m x 0,25 mm x 0,32 µm) were used. Injection mode: Split; It is in the ratio of 1:20. Injection temperature is 220oC.

Thus, fruit and olive oil samples were analyzed with 3 replications on each ripening stage. Data were analyzed statistically utilizing One-way ANOVA by the SAS® software. Least Significant Differences (LSD) test was used to identify the significance of differences between treatments means by ($p<0.01$).

RESULTS and DISCUSSION

The phytosterol composition and contents of olive samples grown by different agricultural methods and harvested at different maturity stages were investigated during the 2019 and 2020 harvest seasons. Obtained findings are presented below.

Results obtained in 2019:

In Table 1. Phytosterol components and contents of olive oils belonging to different periods in which conventional and organic cultivation methods were applied are presented. Total phytosterol content increased regularly until the 08.12.2019 in both cultviation methods depending on the maturity index. It was observed that it decreased after peaking in 08.12.2019 (Fig. 1.). At the harvest date when total phytosterol peaked, the maturity index on olive fruits was calculated as 4.250 and 4.508 for grown with conventional and organic cultivation techniques, respectively. Lazzez et al., (2008) examining the sterol compositions of olive oils stated that the total amount of sterols increased with maturation. The highest total phytosterol amount was determined as 2.008 g kg-1 in the fruit samples harvested from the conventional orchard in the 08.12.2019. At the same time, a maturity index of 4.25 was calculated at this harvest date.

The most plentiful sterol in olive oil is β-sitosterol. In the 2019 production season, it was the highest detected sterol in both cultivation methods. This was traced by Δ-5-avenasterol and campesterol. The highest β-sitosterol fraction was detected as 1.830 g kg-1 in fruit samples harvested from conventional orchard in the 08.12.2019.

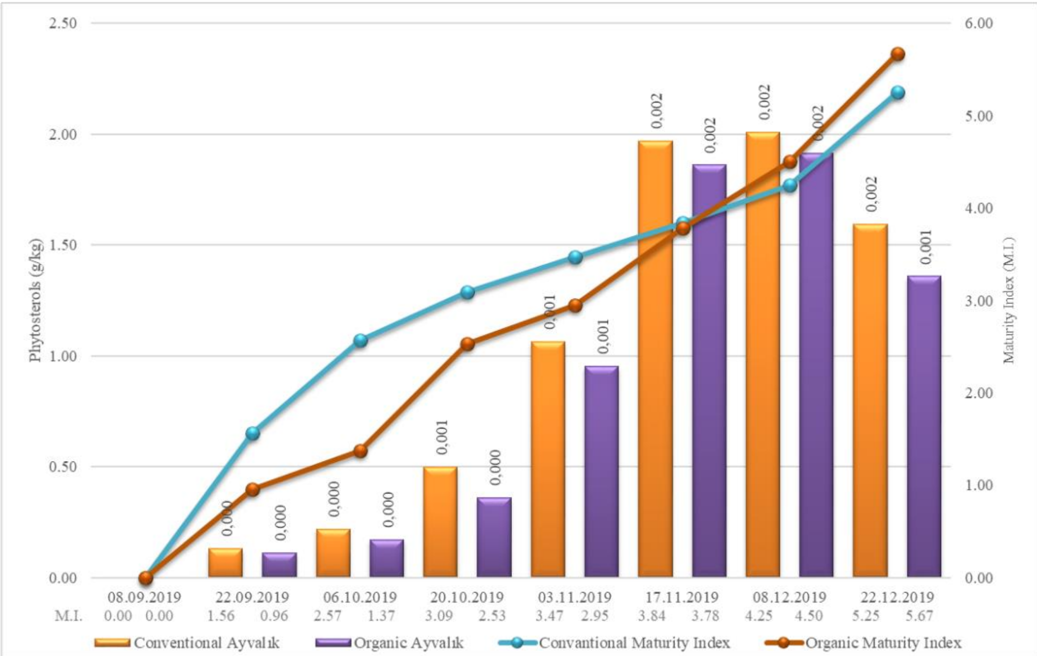

Fig. 1. Total phytosterol contents of olive oils from different periods obtained under conventional and organic conditions (g kg^{-1}) 2019

Results obtained in 2020:

In Table 2. the highest total phytosterol content obtained in 2020 was determined as 2.520 g kg-1 in fruits harvested from the conventional orchard. At this harvest date, maturity indexes of conventional and organic grown fruits were calculated as 4.56 and 4.25, respectively. In the production season of 2020, unlike 2019, the highest β-sitosterol content was obtained from fruits harvested from organic orchards at 2.215 g kg-1.

In olive oil specimens obtained from Ayvalık olive fruits grown with different agricultural methods; Total phytosterol increased regularly in the second year up to the 06.12.2020 depending on the maturity index. It was observed that it decreased after peaking on this date (Fig. 2).

In this direction, it was determined that the total phytosterol content of Ayvalık olive oils grown by conventional cultivation methods was higher than the total phytosterol content of the oils obtained by organic growing methods in both years (p<0.01). Moreover, the total phytosterol content obtained in the 2020 production season was determined to be higher than the data obtained in 2019 (p<0.01).

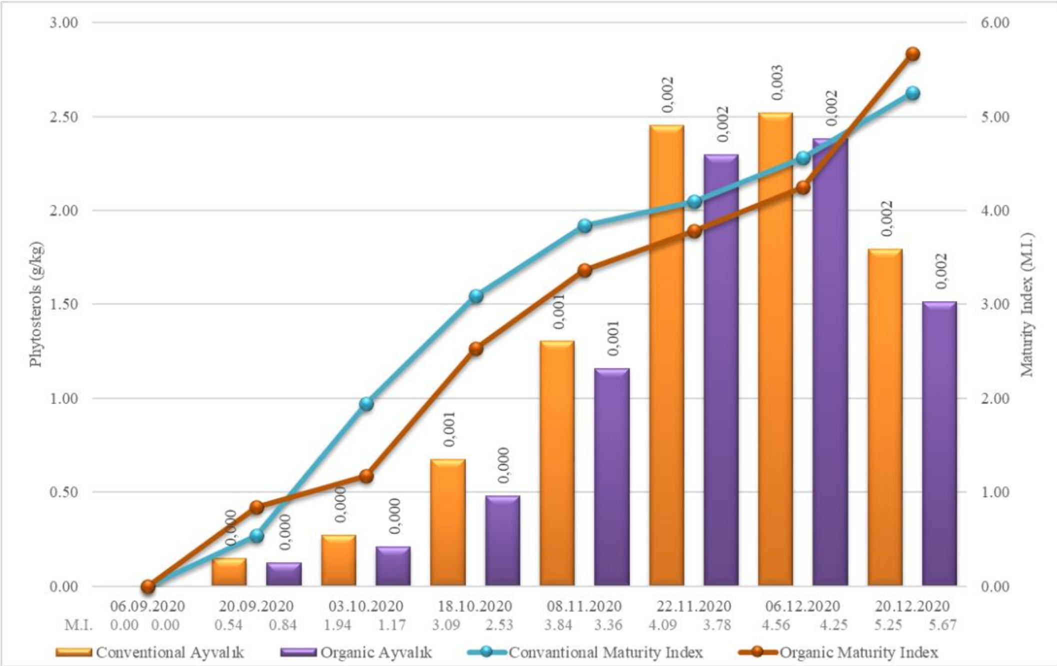

Fig. 2. Total phytosterol contents of olive oils from different periods obtained under conventional and organic conditions (g kg^{-1}) 2020.

These differences rely on activities of enzymatic reactions and environmental parameters such as presence of water and climate. In addition, it is thought that it is caused by the use of traditional methods such as agricultural fertilization used in the growing of olive varieties in which conventional cultivation methods are used. According to the literature, it has been reported by different researchers that phytosterol fraction and total sterol amount have an impact on the chemical structure of olive oil, depending on the crop year, cultivation conditions and maturity, in addition to the significant effects of genetic factors (Boskou et al., 2006; Gul & Seker, 2006; Seker et al., 2007).

According to the two annual results, sterols which constituting an important part of the unsaponifiable part of olive oils: campesterol, brassicasterol, β-sitosterol, stigmasterol, Δ-5-avenasterol and total phytosterol increased with maturation and the change between periods was found to be statistically nificant at the p<0.01 probability level.

Campesterol ratio increased regularly in both crop years as the maturity progressed. The highest rate of campesterol was 0.063 g kg-1 in organic orchards in 2019 and and 0.091 g kg-1 in conventional orchards in 2020. Later, it was observed that it decreased due to over-mature fruits.

Sönmez (2015), in the study examining the minor components of the oils obtained at different maturity levels (September, October, November and December) in the organically grown Ayvalık olive variety, the campesterol content of olive oil showed a linear change during the ripening process. Campesterol content, which was determined as 3.40% in September, decreased to 2.81% in December.

Stigmasterol is one of the essential sterol compounds of olive oil. In our investigative, it was observed that there were fluctuations in the amount of stigmasterol in the olive oils obtained periodically. Studies have shown that the level of stigmasterol fluctuates as maturity progresses.

Sonmez, (2015); In the study in which the minor components of the oils obtained at different maturity levels (September, October, November and December) of the organically grown Ayvalık olive variety were examined, it was determined that the stigmasterol content of the oils showed fluctuations during the ripening process.

Anastasopoulos et al., (2011) reported that the stigmasterol content change depending on maturation in the study conducted on organic and convantional Koroneiki cultivars in 2000 and 2004 harvest years.

The β-sitosterol value, which constitutes the largest part of the sterol composition in terms of amount, was obtained in December with the highest content in our study and showed a linear increase. Studies have also shown that β-sitosterol levels increase as ripening progresses.

Sonmez, (2015); In the study examining the minor components of the oils obtained at different maturity levels in Ayvalık olive variety, the β-sitosterol content showed a linear change during the ripening process. Similar to the values we found in the study, β-sitosterol content increased as fruit maturity increased.

In the research conducted by Finotti et al., (2001), the unsaponifiable matter composition of Buza and Lastovka olive cultivars was investigated in 3 different ripening periods in the 1998 harvest season. It has been reported that as the degree of maturity increases, the amount of β-sitosterol increases.

Yorulmaz et al. (2013), in the study in which they determined the impact of ripening degree on the composition of sterol of Ayvalık olive oil, which is one of our common domestic varieties; the components most affected by maturation; β-sitosterol, Δ-5-avenasterol, campesterol, and total phytosterol contents were determined. It has been determined that Δ-5-avenasterol also increases by showing fluctuations as maturation progresses.

The total phyosterol amount of olive oil obtained from Ayvalık variety olives harvested from conventional and organic orchards during the production seasons increases regularly during the ripening process. Phytosterols started to be synthesized from the second half of September and the total amount of phytosterol reached the highest value in the at the beginning of December. Lazzez et al., (2008) examining the sterol compositions of olive oils stated that the total amount of sterols increased with maturation.

In another study, the sterol composition of olive oils acquired from Koroneiki variety olives collected at different harvest periods from different regions of Southern Greece were analyzed. They stated that the values of campesterol, stigmasterol and β-sitosterol increased with increasing maturity (Varzakas et al., 2010).

Lukic et al., (2013), found that Buza, Crna and Rosinjola olive cultivars have highest total amount of sterol value in green ripening stages than color changed and matured stages in months of October and November.

Table 1. Phytosterol components and contents determined in different periods of Ayvalık olive variety grown conventionally and organically (g kg^{-1}) 2019

Harvest periods	Cultivation conditions	Phytosterols (g kg^{-1}) 2019					
		BRS1	CAS	STIS	SITS	AS	TSIO
08.09.2019	Conventional	0.00 d2	0.00 f	0.00 d	0.00 i	0.00 e	0.00 h
	Organic	0.00 d	0.00 f	0.00 d	0.00 i	0.00 e	0.00 h
22.09.2019	Conventional	0.00 d	0.00 f	0.00 d	0.120 i	0.011 e	0.131 gh
	Organic	0.00 d	0.00 f	0.00 d	0.103 hi	0.008 e	0.111 gh
06.10.2019	Conventional	0.001 cd	0.005 ef	0.004 cd	0.180 gh	0.030 de	0.220 fg
	Organic	0.001 cd	0.004 ef	0.002 cd	0.124 hi	0.040 cde	0.171 fgh
20.10.2019	Conventional	0.003 bcd	0.011 cdef	0.002 cd	0.450 f	0.034 de	0.500 e
	Organic	0.001 cd	0.009 def	0.001 cd	0.324 fg	0.025 de	0.360 ef
03.11.2019	Conventional	0.007 abcd	0.030 bcde	0.010 bc	0.923 e	0.095 bcd	1.064 d
	Organic	0.012 ab	0.037 abc	0.013 bcd	0.825 e	0.066 bcde	0.953 d
17.11.2019	Conventional	0.013 a	0.055 ab	0.018 ab	1.710 ab	0.175 a	1.971 a
	Organic	0.011 ab	0.063 a	0.028 a	1.641 b	0.118 ab	1.861 a
08.12.2019	Conventional	0.009 abcd	0.041 ab	0.014 bc	1.830 a	0.114 abc	2.008 a
	Organic	0.012 ab	0.056 ab	0.020 ab	1.717 ab	0.110 abc	1.915 a
22.12.2019	Conventional	0.010 abcd	0.048 ab	0.014 bc	1.385 c	0.137 ab	1.594 b
	Organic	0.008 abcd	0.033 bcd	0.010 bcd	1.218 d	0.090 bcd	1.359 c
LSD ($p<0.01$)		0.0094	0.0262	0.0131	0.1636	0.0759	0.2154

BRS: Brassikasterol, CAS: Campesterol, STIS: Stigmasterol, SITS: β-Sitosterol, AS: Δ-5-avenasterol, TSIO: Total Sterol

[2]Values followed by different letters in the same column are significantly different on at $p<0.01$

Table 2. Phytosterol components and contents determined in different periods of Ayvalık olive variety grown conventionally and organically (g kg^{-1}) 2020

Harvest periods	Cultivation conditions	Phytosterols (g kg^{-1}) 2020					
		BRS[1]	CAS	STIS	SITS	AS	TSIO
06.09.2020	Conventional	0.00 c[2]	0.00 f	0.00 c	0.00 g	0.00 e	0.00 g
	Organic	0.00 c	0.00 f	0.00 c	0.00 g	0.00 e	0.00 g
20.09.2020	Conventional	0.00 c	0.00 f	0.00 c	0.124 g	0.023 de	0.147 fg
	Organic	0.00 c	0.00 f	0.00 c	0.116 g	0.008 de	0.124 g
03.10.2020	Conventional	0.002 c	0.007 ef	0.007 c	0.164 fg	0.090 bcde	0.270 fg
	Organic	0.001 c	0.005 ef	0.006 c	0.160 g	0.036 de	0.208 fg
18.10.2020	Conventional	0.005 c	0.016 def	0.004 c	0.606 ef	0.042 de	0.673 ef
	Organic	0.003 c	0.014 def	0.002 c	0.438 fg	0.024 de	0.481 fg
08.11.2020	Conventional	0.019 abc	0.044 bcd	0.020 bc	1.035 de	0.185 ab	1.303 cd
	Organic	0.005 c	0.037 de	0.010 bc	1.030 de	0.076 cde	1.158 de
22.11.2020	Conventional	0.026 ab	0.075 abc	0.030 ab	2.108 a	0.215 a	2.454 a
	Organic	0.017 bc	0.077 ab	0.013 bc	2.022 ab	0.169 abc	2.298 ab
06.12.2020	Conventional	0.039 a	0.091 a	0.045 a	2.106 a	0.239 a	2.520 a
	Organic	0.008 bc	0.045 bcd	0.012 bc	2.215 a	0.104 bcd	2.384 a
20.12.2020	Conventional	0.009 bc	0.032 def	0.017 bc	1.590 bc	0.145 abc	1.793 bc
	Organic	0.018 bc	0.042 cd	0.017 bc	1.296 cd	0.142 abc	1.515 cd
LSD ($p<0.01$)		0.0208	0.0345	0.0208	0.4448	0.0985	0.5429

[1]BRS: Brassikasterol, CAS: Campesterol, STIS: Stigmasterol, SITS: β-Sitosterol, AS: Δ-5-avenasterol, TSIO: Total Sterol

[2]Values followed by different letters in the same column are significantly different on at $p<0.01$

Conclusions

Olive oil is considered as a healthy nutrient in the human diet. Although majority of olive oil composed of the saponifiable chemicals, the unsaponifiable components of olive oil is also important for their health benefits and their aromatic purposes. In this research, changes in phytosterol content during the fruit maturation were determined. As a result of this study, it was found that the concentration of phytosterols increased until December but it decreased after December. It was determined that the total sterol content of the oils obtained increased as the fruits matured in the Ayvalık olive variety grown by conventional and organic technichs. Although the fruits harvested in different periods were grown under the same ecological conditions, differences were detected in the total sterol content of fruits due to the application of different agricultural methods. Similarly in both years, as of December 20, the phytosterol content of over-mature fruits begins to decreases. The reason for this is that the amount of sterols decreases as the ripening progresses in the olive fruit. Because the fruit starts to produce more oil.

Ayvalık is an variety with high oil content and it is normally recommended to harvest early due to its high polyphenol content, but in terms of phytosterol synthesis, the fruits should be expected to mature a little more. Because Similarly in both years, phytosterol synthesis starts on September 15 at the earliest. It takes December for the total sterol content to reach its maximum.

The functionality of monovarietal olive oil stems from the selection of a single type of olive, an olive for oil, such as Ayvalık. In order to produce monovarietal olive oil with unique properties, it is important to understand components such as maturity index and phytosterol structure, apart from selecting the appropriate varieties.

The finding of this study demonstrated that dates for harvesting olives in Ayvalik cultivar might require being determined for each cultivation techniques for optimum taste and quality of olive oil.

Furthermore this research is a source for determining the period when olive fruits are rich in phytosterol by comparing 8 different maturity stage in which olive fruits are harvested. In addition, olive oil contains a significant amount of β-sitosterol, since sterols give the characteristic features of vegetable oils. It was the highest sterol in all periods.

Acknowledgements

This research was produced from the Ph.D. Thesis completed by the first author under the supervision of the second author and also supported by TUBITAK (Project numbers: 118O405) and Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit (BAP) (Project Grant Number: FDK-2020-3282). In addition, I would like to thank the "Higher Education Council" for supporting my PhD education within the scope of "100/2000 YÖK Ph.D. Scholarship" Program for in priority areas "sustainable agriculture".

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