Oxidative stability of olive oils with supercritical carbon dioxide extracts of olive leaf

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In this study, quality parameters and oxidative stability of olive oils added with olive leaf (OL), an agricultural by-product extracted by the supercritical carbon dioxide (SCD) method, were evaluated. The SCD OL extraction method was applied at 100°C, 100 bar and 80 minutes. Extracts containing phenolic substances obtained from olive leaves were added to refined olive oil (ROO) (E) at concentrations of 350 (A), 200 (B), 150 (C) and 60 mg CAE/kg oil (D). Olive oil samples were stored in clear glass bottles for 12 months under light (L) and dark (D) conditions. Samples were analysed at 0, 3, 6, 9 and 12 months of storage period. The results of the analyses revealed that the amount of free fatty acid values (FFAV), peroxide value (POV), total phenolic content (TPC), chlorophyll (TChl) and carotenoid (TCar) content and oxidative stability index (OSI) increased as the olive leaf extract (OLE) addition ratio increased. When the effect of OLE concentration on the amount of α-tocopherol was examined, it was determined that the amount of α-tocopherol was higher in ROO (E) and this amount decreased with the addition of OLE. In the samples which OLE was added, odours like alcohol, henna and dried herbs were detected as a result of sensory analysis. Principal component analyses (PCA) indicated that olive oils were discriminated clearly based on the concentrations of OLEs. As a result, it was determined that the TPC, TChl and TCar content and OSI values were the highest in the A sample, which was stored in the dark and had the highest OLE content. Sample A, which is rich in antioxidant components, can be said to be the best example enriched with OLE, apart from sensory properties. In terms of the content of AT, which is equivalent to vitamin E, we can say that the E sample stored in the dark has the best properties except for the sensory properties.

Keywords: Supercritical Carbon Dioxide, Olive leaf, Olive Oil, Antioxidant activity, Oxidative stability, PCA

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1. INTRODUCTION

Olive tree (*Olea europaea* L.) is one of the oldest trees with an edible fruit unique to the Mediterranean climate. It is known that olive trees are pruned periodically in January-February of each year. The olive leaves (OLs) obtained as a result of this pruning are used as animal feed and the branches are used as fuel. The amount of OLs obtained varies according to the age of the pruned tree and the type of pruning, and it is stated that it is 12-30 kg / tree on average [1]. Considering both the number of trees in our country and the amount of pruning waste leaves taken from a tree, it is clearly seen that a significant amount of olive tree leaves is waste. The positive effects of OLs on human health have been demonstrated by various studies [2]. In the food industry, it is important in terms of phenolic substances with antioxidant properties in OLs. The most abundant phenolic compound in OLs is oleuropein [3]. It is important to investigate the extraction of phenolic substances in two

different ways from the OLs, which is an agricultural by-product with an important phenolic substance content, and to investigate the possibilities of adding the extracts to the ROO.

There is a high amount of waste and by-product formation during the processing of agricultural products. Evaluating these agricultural by-products and bringing them into production is of great economic and environmental importance. OL is an agricultural by-product that cannot be evaluated as economically efficient today. In today's world, the evaluation and the awareness on this issue is increasing. It is also known that the agricultural by-product (OL) selected as a raw material in our study is quite rich in antioxidants.

The polyphenolic extract in OLs can be used to extend the shelf life of foods such as meat products and vegetable oils and to benefit from their functional properties. For this purpose, in our study, it is aimed to examine the oxidative stability of this product by adding it to ROO.

Olive oils that are not suitable for direct consumption and have FFAV of over expressed as oleic acid of more than 2.0 grams per 100 grams, oxidised, have bad physic-chemical and organoleptic characteristics need to be refined. This type of olive oil is called *lampante* virgin olive oil [4]. Undesirable compounds are removed from the oil by the refining process. However, this process also causes the removal of colour pigments and phenolic compounds that add characteristic features to olive oil. In our study, it is aimed to extend the shelf life of ROO by adding OLE to ROO.

Today, the stability and antioxidant content of ROOs are increased by adding synthetic antioxidants. The most widely used synthetic antioxidants are BHA (Butylated Hydroxy Anisol), BHT (Butylated Hydroxy Toluene), PG (Propyl Gallate) and TBHQ (Tertiary Butyl Hydroxyquinone). Synthetic antioxidants have created serious health concerns. In some countries, its use in foods is prohibited or restricted due to the suspicion that it may have a carcinogenic effect [5]. Instead, antioxidants obtained from natural products can be used to increase the shelf life of ROOs. Studies have shown that oleuropein, one of the important phenolic compounds in olive leaves, has a higher induction value than synthetic antioxidants such as Trolox, BHA, BHT, TBHQ [6]. In the study in which 0.5-2% olive leaf extract was added to the refined olive oil, it was determined that the stability of the refined oils gave them similar or superior antioxidant properties to the commercial refined olive oil used as a reference [7]. Paiva-Martins et al. [8] stated that when OLE is added to ROO, it has the same stability as extra virgin olive oil, and the extract obtained from 1 kg OL increases the stability of extra virgin olive oil when added to 50-320 litres of ROO. By adding OLE to ROO, both the nutritional value and stability character of the oil have been brought to the quality of extra virgin olive oil [8]. It was determined by Salta et al. [9]. that the antioxidant capacity (DPPH•) and oxidative stability of commercial sunflower oil, olive oil, canola and palm oils increased with the addition of OLE rich in oleuropein and hydroxytyrosol. The antioxidant capacities (DPPH•) of sunflower oil, palm oil, olive oil and canola oil increased by 79%, 67%, 86% and 84%, respectively. The study by Bouaziz et al. [10] showed that the shelf life of refined olive oil and crude oils can be extended and stabilised by olive leaf hydrolysate extract, which has high antioxidant activity. Olive leaf can be considered as a potential source of antioxidants of natural origin. The incorporation of such extracts into the food industry can significantly contribute to the health benefit of consumers and extend the shelf life of food products.

SCD differs from other extraction methods with its features such as being environmentally friendly, fast and more selective towards the extracted material. Carbon dioxide is a supercritical solvent, and it is widely used because of its cheapness [11]. In the SCD method, process parameters such as temperature, pressure, extraction time, particle size, cosolvent properties - flow rate and carbon dioxide flow rate can be selected as constant, dependent or independent variables. This selection varies depending on the purposes of the extraction to be performed. Sahin et al. [12] compared SCD and soxhlet methods based on extracted oleuropein in OLE and determined that oleuropein obtained by using methanol by soxhlet method was 37.8 mg/g dry matter and this value was higher than the value found with SCD. Vassiliki and Gerothanassis [13] examined the antioxidant activity and phenolic content of OLEs and then added this extract (200 mg/kg oil) to sunflower oil and concluded that OLE was successful in improving its oxidative stability. In another study carried out by applying SCD, it was determined that the phenolic substance extraction efficiency was 45% under conditions of 334 bar 100°C, 2 ml/min cosolvent (10% methanol) flow rate [14].

In this study, ROOs were enriched through the addition of phenolic extracts from OLEs. It was aimed to use phenolic substances obtained from OLs, the waste after pruning of olive trees, as a natural antioxidant source. The objective of the study was to investigate the quality criteria and the changes in some antioxidant compounds in ROO under dark and light storage conditions by adding the different concentrations of OLEs. In this context, the SCD method was used to obtain the extract from OLs. The optimum condition for this was determined as 100°C, 100 bar and 80 minutes. OLE was added to ROO as 350, 200, 150 and 60 mg CAE (Caffeic Acid Equivalent)/kg oil in ROO, and the samples were stored in clear glass bottles at room conditions for 12 months under light and dark conditions. Samples were analysed at 0, 3, 6, 9 and 12 months of storage period.

2. MATERIAL AND METHODS

2.1 SUPERCRITICAL CARBON DIOXIDE (SCD) EXTRACTION

SCD extraction device (Waters, USA) was used for SCD extraction studies. The device; consists of main body, computer, cooling unit and CO_2 cylinders. SCD extraction process was applied at 100°C, under the pressure of 100 bar for 80 minutes. For SCD extraction in olive leaf, the amount of dried olive leaves is 3 g, CO_2 flow rate is 36 g/min and cosolvent flow rate 9 mL/min was kept constant.

2.2 EXTRACTION OF OLIVE LEAVES

OLs are agricultural wastes generated during olive harvesting and pruning. The OLs (5 kg) used in our study were obtained from the leaves of olives collected from Memecik trees in the gardens of Directorship of Olive Research Institute of Ministry of Agriculture and Forestry of Turkey. OLs, branches, olives, soil, etc. were cleaned and washed, the excess water was removed, and then it was packaged using laminated packages that are not permeable to light and moisture. Packaged OLs were stored at -24°C until drying. The drying process of OLs was carried out at 50°C, considering the optimum conditions specified in literature [15]. OLs with an initial moisture content of 40% were dried until the final moisture content was 5.04% ± 0.29. All dried OLs were packed using air- and moisture-proof sealed laminated packaging and stored at -24°C until the grinding time. Dried OLs were ground using a Brook Crompton 2000 Series hammer mill and sieved with a particle diameter of 0.5-1.0 mm with the help of a sieve adapted to the mill. The sieved leaves were packed in laminated packages and stored at -24°C. The dried and ground OLs were subjected to SCD extraction.

2.2.1 Addition of phenolic extract from OL to ROO

The samples used in analyses were prepared with ROO (E). The stock solution (2300 mg CAE/kg oil) was prepared by adding the obtained OLEs to refined olive oil. 90mL, 60mL, 30mL and 15mL were taken from this prepared stock solution and added to 900mL of refined oil, and the samples in which the extract was added were coded as A, B, C, D, respectively. ROO was coded as E. OLEs were added to ROO so that 350 mg CAE/kg oil in sample A, 200 mg CAE/kg oil in sample B, 150 mg CAE/kg oil in sample C, and 60 mg CAE/kg oil in sample D. Olive oil samples were stored in dark and light conditions for 12 months. Samples were analysed at 0, 3, 6, 9 and 12 months.
Two independent replications were carried out for each trials.

2.3 METHODS

2.3.1 Quality parameters analyses

The FFAV, POV and UV spectrophotometric analyses were done according to the Turkish Food Codex [16]. For FFAV (% in oleic acid) 5±0.02 grams of olive oil weighing into 250 mL erlen, 50 mL alcohol-diethyl ether (50:50) was added and shaken. After added 1 mL of phenol phthalein indicator to the solution, it was titrated with 0.1 N potassium hydroxide solution in ethanol. POV expressed in milliequivalents of active oxygen per kilogram (meq O_2/kg) (POV); analysis was done with 0.01 N sodium thiosulfate ($Na_2S_2O_3$). UV spectrophotometric indices (K_{232} and K_{270} absorption coefficients) were measured spectrophotometrically (UV-Spectrophotometer-Shimadzu, Japan) at 232 and 270 nm 0.25 g was weighed into 25 mL balloon joje and diluted with cyclohexane.

2.3.2 Total phenol content (TPC) analysis

The extraction and the determination of TPC of the samples were determined by the Folin-Ciocalteu method at 725 nm [17] using caffeic acid calibration curve (R^2=0.99) with a spectrophotometer (UV-1700, Shimadzu, Japan). The TPC were expressed as mg equivalent of caffeic acid per kilogram of oil (mg CAE/kg oil).

2.3.3 α-tocopherol content (ATC) analysis

AT that was major tocopherol in olive oil was evaluated according to the method developed by Carpenter [18] and IUPAC [19]. Sample (1 g) was diluted with hexane containing 1% isopropyl alcohol (1/10) and solution was filtered with Econofilter 25/0.45µm RC (Agilent Technologies). And then a solution of olive oil was analysed by HPLC system (Agilent 1100), UV detector was used at 292 nm. A µ-porasil column (250mm*4.6mm*5µm) (Waters, Ireland) was used. A flow rate of hexane/2-propanol (99:1) was 1 ml/min. The injection volume was 20 µL. The ATC was expressed as mg/kg.

2.3.4 Total chlorophyll (TChl) and carotenoid (TCar) analyses

TChl and TCar of the samples were analysed by spectrophotometer (UV-1700, Shimadzu, Japan) at 670 and 470 nm, respectively. 7.5 g of sample was weighed to a 25 mL volumetric flask and diluted with cyclohexane. The results were expressed as mg/kg oil [20].

$TChl = (A_{670} \times 10^6)/(613 \times 100 \times d)$

$TCar = (A_{470} \times 10^6)/(2000 \times 100 \times d)$

2.3.5 Oxidative stability index (OSI) analysis

The OSI of the olive oil was conducted by using Rancimat apparatus (Model 743 Metrohm Ltd., Herisau, Sweden) [21]. 3 g sample weighed and placed in a heating block at 120°C under a constant air flow of 20 l/h, and then, the conductivity variation of water (60ml) due to the increase in oxidation compounds was calculated. The results were expressed as hours (h).

2.3.6 Fatty acid composition (FAC) analysis

The analysis of FAC was determined using gas chro-

matography system (HP 6890, U.S.A) with flame ionisation detector (FID) [22]. FAC analysis were done using the capillary column (DB-23, 30m*0.25 mm*0.250 µm, Agilent J&W GC Columns, U.S.A.), at 250°C injector temperature. The oven temperature was set as an increment of 2°C/min from 170 to 210°C and holding at 210°C for 10 min.

2.3.7 Sensory analysis

Sensorial analyses carried out in accordance with the method of the COI/T.20/DOC.15/Rev.10 [23] using ROOs with different concentrations of OLEs.

2.4 STATISTICAL ANALYSIS

The analytic evaluations were written as mean±standard deviation (SD). Analysis of variance (ANOVA) was applied to show the among the samples using the Fisher's least significant difference test at $p<0.05$ significance level. Principal component analysis (PCA) was performed using Minitab® 17 programme (Minitab Inc., State College, PA, USA) in order to evaluate the classification pattern of the samples according to quality parameters of olive oils with different concentrations of OLEs and stored under different conditions.

3. RESULTS AND DISCUSSION

3.1 QUALITY PARAMETERS ANALYSES

OLs are important by-products obtained during the olive harvest and usually make up 10% of the total olive weight. However, OLs are also attained during pruning of olive trees [24]. These leaves are raw materials that do not have any production or purchasing costs, and studies have shown that the OL phenolic composition, especially the oleuropein content, is quite high. OL, which is the most nutritious part of the olive tree after its fruit, contains many phenolic compounds and tocopherols that show antioxidant properties [25]. According to recent studies, it has been noted that OLE protects olive oil against oxidation, and it has been determined that the oxidative stability of 50-320 L of ROO enriched with the extract obtained from 1 kg of OL is the same as that of crude olive oil [8].

In Table I, it is seen that the FFAV of the samples to which OLE was added changed between 0.19-0.23 (% oleic acid) before 12 months of storage. When the changes in the FFAV were examined according to the amount of OLE added to the ROO, it was determined that there was a statistically significant

Table I - FFAV of the samples during storage (% in oleic acid)

Samples	0. Month Light (L)	3. Month L	6. Month L	9. Month L	12. Month L
A	0.23±0.00^{a}	0.25±0.01^{a}	0.28±0.01^{a}	0.28±0.00^{a}	0.55±0.01^{a}
B	0.22±0.01^{b}	0.24±0.01^{a}	0.26±0.01^{b}	0.27±0.01^{a}	0.33±0.01^{b}
C	0.21±0.01^{b}	0.22±0.01^{b}	0.23±0.00^{c}	0.25±0.01^{b}	0.28±0.01^{c}
D	0.19±0.00^{c}	0.20±0.00^{b}	0.22±0.01^{c}	0.23±0.01^{c}	0.20±0.01^{d}
E	0.15±0.01^{d}	0.18±0.01^{c}	0.18±0.01^{d}	0.21±0.01^{d}	0.18±0.00^{d}
	0. Month D	**3. Month D**	**6. Month D**	**9. Month D**	**12. Month D**
A	0.23±0^{a}	0.24±0.01^{a}	0.25±0.25^{a}	0.26±0.01^{a}	0.35±0.01^{a}
B	0.22±0.007^{b}	0.23±0.01^{ab}	0.25±0.24^{a}	0.25±0.01^{ab}	0.29±0.01^{b}
C	0.21±0.007^{b}	0.21±0.00^{bc}	0.22±0.21^{b}	0.23±0.01^{bc}	0.27±0.01^{c}
D	0.19±0^{c}	0.21±0.21^{c}	0.23±0.22^{b}	0.23±0.00^{c}	0.25±0.01^{d}
E	0.15±0.007^{d}	0.16±0.16^{d}	0.17±0.16^{c}	0.18±0.01^{d}	0.20±0.01^{e}

($p < 0.05$) a-e: Different letters in the same column concerning all samples significantly different values. L: Light, D:Dark. A: 350 mg CAE/kg oil in ROO, B: 200 mg CAE/kg oil in ROO, C: 150 mg CAE/kg oil in ROO, D: 60 mg CAE/kg oil ROO, E: ROO.

Table II - POV of the samples during storage (meq O_2/kg oil)

Samples	0. Month L	3. Month L	6. Month L	9. Month L	12. Month L
A	4.69±0.02^{a}	4.78±0.04^{a}	4.85±0.02^{a}	5.21±0.02^{a}	7.17±0.02^{a}
B	4.58±0.04^{b}	4.67±0.05^{b}	4.75±0.02^{a}	5.04±0.04^{b}	6.44±0.26^{b}
C	4.48±0.01^{c}	4.55±0.02^{c}	4.64±0.05^{a}	4.88±0.04^{c}	5.60±0.08^{c}
D	4.16±0.04^{d}	4.22±0.04^{d}	4.38±0.01^{a}	4.74±0.05^{d}	5.40±0.52^{c}
E	1.90±0.04^{e}	3.43±0.02^{e}	3.50±0.02^{a}	3.61±0.04^{e}	4.34±0.02^{d}
	0. Month D	**3. Month D**	**6. Month D**	**9. Month D**	**12. Month D**
A	4.65±0.02^{a}	4.72±0.007^{a}	4.80±0.02^{a}	5.07±0.07^{a}	6.18±0.07^{a}
B	4.58±0.04^{b}	4.65±0.05^{ab}	4.68±0.01^{b}	4.93±0.02^{b}	5.52±0.36^{b}
C	4.48±0.01^{c}	4.58±0.04^{bc}	4.63±0.06^{b}	4.77±0.04^{c}	5.31±0.19^{bc}
D	4.16±0.04^{d}	4.21±0.02^{c}	4.22±0.03^{c}	4.43±0.02^{d}	4.90±0.10^{c}
E	1.90±0.04^{e}	3.26±0.03^{d}	3.39±0.03^{d}	3.51±0.02^{e}	4.20±0.09^{d}

($p < 0.05$) a-e: Different letters in the same column concerning all samples significantly different values. L: Light, D: Dark. A: 350 mg CAE/kg oil in ROO, B: 200 mg CAE/kg oil in ROO, C: 150 mg CAE/kg oil in ROO, D: 60 mg CAE/kg oil ROO, E: ROO.

difference (P<0.05) between the samples, but there was no difference determined between the B and C samples, and the lowest FFAV was calculated in the ROO (E sample). At the end of 12 months of storage, it was determined that the FFAV of all samples did not exceed the IOC [4] ROO limit of 0.3% (oleic acid), except for samples A and B in light conditions and sample A in dark conditions.

Table II shows that the POV varies between 1.90-4.69 (meqO_2/kg oil) before storage. Considering the changes in the POV of the samples during storage according to the amount of OLE added to the ROO, it was determined that there was a statistically significant difference (P<0.05) between the samples in the months except the 6th, and the POV increased as the leaf extract concentration increased. It was determined that the POV of all samples did not exceed the ROO limit of 5 (meqO_2/kg oil) according to the IOC standard during the 6 months of storage in light and dark conditions. However, it was determined that this value exceeded in samples A and B stored for 9 months, and samples A, B, C and D stored for 12 months in light conditions, and in samples A at 9 months and samples A, B and C at 12 months of storage in dark conditions.

When the results of FFA and POV were evaluated, it was determined that these values increased as the OLE ratio increased. When the A and E samples were compared, it was found that the FFA amount of the A sample was 58% higher than that of the E sample under light conditions, this increase was 205% at the end of 12 months of light storage, and 77% in dark storage. At the end of 12 months, when we look at the storage in light conditions, it was determined that the increase in sample A was 139%, and in sample E 24%, while in dark conditions it was 50% in sample A and 34% in sample E. When the POVs of A and E samples were compared, it was determined that the POV of sample A was 147% higher than that of sample E, this increase was 65% after 12 months of light storage, and 47% in dark storage. At the end of 12 months, the increase in storage in light conditions was 53% in A sample, 128% in E sample, and 31% in A sample and 121% in E sample in dark conditions. Bouaziz et al. [10] determined that the POV of ROO and olive pomace oil samples with added OLE increased considerably after 6 months at 50°C of storage. It was indicated that the POV of the samples to which OL and hydrolysate extract were added increased from 14 meqO_2/kg oil to approximately 680 and 328 meqO_2/kg oil, respectively. Malheiro et al. [26] in the study conducted on olive oils obtained by adding OLs, it was presented that the addition of leaves increased the FFA and POV of the samples. Similar results were obtained in our study.

In Table III it is seen that the K_{232} and K_{270} values of the samples varied between 2.04 and 2.25 and varied between 0.87-0.96 before 12 months of storage, respectively. It was determined that there was a sta-

Table III - K_{232} and K_{270} values of the samples during storage (meq O_2/kg oil)

	K_{232}					K_{270}				
Samples	**0. Month L**	**3. Month L**	**6. Month L**	**9. Month L**	**12. Month L**	**0. Month L**	**3. Month L**	**6. Month L**	**9. Month L**	**12. Month L**
A	2.25±0.02^{a}	2.33±0.02^{a}	2.35±0.00^{a}	2.37±0.01^{a}	2.46±0.007^{a}	0.96±0.01^{a}	0.97±0.01^{a}	0.99±0.01^{a}	1.01±0.01^{a}	1.03±0.01^{a}
B	2.20±0.02^{a}	2.23±0.06^{ab}	2.26±0.02^{b}	2.28±0.04^{b}	2.35±0.02^{b}	0.94±0.02^{b}	0.97±0.00^{a}	0.98±0.01^{a}	1.00±0.01^{a}	1.01±0.00^{b}
C	2.19±0.02^{a}	2.21±0.05^{ab}	2.24±0.04^{b}	2.27±0.02^{b}	2.30±0.01^{c}	0.90±0.00^{c}	0.94±0.01^{a}	0.95±0.02^{a}	0.98±0.01^{a}	0.99±0.01^{c}
D	2.04±0.04^{b}	2.17±0.05^{b}	2.21±0.04^{b}	2.22±0^{bc}	2.28±0.01^{cd}	0.87±0.01^{d}	0.94±0.02^{a}	0.96±0.02^{a}	0.97±0.01^{a}	0.97±0.01^{d}
E	2.10±0.04^{b}	2.15±0.02^{b}	2.18±0.01^{b}	2.19±0.007^{c}	2.25±0.02^{e}	0.90±0.01^{c}	0.93±0.02^{a}	0.94±0.00^{a}	0.95±0.02^{a}	0.96±0.00^{e}
	0. Month D	**3. Month D**	**6. Month D**	**9. Month D**	**12. Month D**	**0. Month D**	**3. Month D**	**6. Month D**	**9. Month D**	**12. Month D**
A	2.25±0.02^{a}	2.27±0.02^{a}	2.28±0.01^{a}	2.30±0.04^{a}	2.43±0.01^{a}	0.96±0.01^{a}	0.98±0.00^{a}	0.98±0.01^{a}	1.00±0.00^{a}	1.02±0.01^{a}
B	2.20±0.02^{a}	2.21±0.02^{ab}	2.22±0.02^{b}	2.25±0.02^{ab}	2.31±0.01^{b}	0.94±0.02^{b}	0.94±0.02^{ab}	0.96±0.01^{ab}	0.97±0.01^{ab}	0.98±0.01^{b}
C	2.19±0.02^{a}	2.20±0.00^{abc}	2.20±0.01^{b}	2.22±0.01^{b}	2.27±0.01^{c}	0.90±0.00^{c}	0.93±0.02^{b}	0.95±0.02^{ab}	0.95±0.02^{ab}	0.96±0.01^{c}
D	2.04±0.04^{b}	2.14±0.03^{bc}	2.19±0.00^{b}	2.20±0.01^{b}	2.23±0.00^{d}	0.87±0.01^{d}	0.91±0.00^{b}	0.93±0.02^{b}	0.94±0.02^{b}	0.95±0.00^{d}
E	2.10±0.04^{b}	2.12±0.04^{c}	2.13±0.01^{c}	2.19±0.02^{b}	2.21±0.01^{e}	0.90±0.01^{c}	0.90±0.01^{b}	0.92±0.00^{b}	0.93±0.00^{b}	0.92±0.00^{e}

(p < 0.05) a-e: Different letters in the same column concerning all samples significantly different values. L: Light, D: Dark. A: 350 mg CAE/kg oil in ROO, B: 200 mg CAE/kg oil in ROO, C: 150 mg CAE/kg oil in ROO, D: 60 mg CAE/kg oil ROO, E: ROO.

Table IV - TPC (mg CAE/kg oil) and ATC (mg/kg) of the samples during storage

	TPC					AT				
Samples	**0. Month L**	**3. Month L**	**6. Month L**	**9. Month L**	**12. Month L**	**0. Month L**	**3. Month L**	**6. Month L**	**9. Month L**	**12. Month L**
A	338.33±1.58^{a}	218.36±2.52^{a}	102.94±3.80^{a}	35.72±1.71^{a}	27.16±1.20^{a}	218.50±4.03^{a}	172.60±3.46^{b}	149.45±3.11^{a}	132.50±2.61^{a}	124.28±2.75^{a}
B	194.45±5.93^{b}	103.70±2.93^{b}	52.23±2.79^{b}	29.83±1.66^{b}	24.77±0.19^{a}	230.93±3.93^{b}	160.60±1.76^{a}	157.34±0.50^{ab}	145.74±4.05^{b}	138.27±2.93^{b}
C	129.15±4.67^{c}	71.095±3.61^{c}	45.69±1.18^{b}	20.40±1.19^{b}	18.51±1.42^{b}	244.71±3.05^{c}	164.09±0.16^{a}	159.50±2.61^{b}	133.46±2.19^{a}	136.70±2.15^{b}
D	59.20±8.41^{d}	32.95±3.25^{d}	25.43±0.81^{c}	17.58±1.50^{c}	14.00±1.07^{c}	253.72±0.55^{d}	193.96±5.44^{c}	181.53±5.89^{c}	170.44±2.95^{c}	164.39±1.32^{c}
E	35.65±4.45^{e}	20.63±2.92^{e}	18.23±2.85^{d}	13.23±1.38^{d}	11.05±1.27^{d}	260.94±0.84^{d}	228.30±1.48^{d}	224.60±2.75^{d}	196.71±4.10^{d}	172.69±2.06^{d}
	0. Month D	**3. Month D**	**6. Month D**	**9. Month D**	**12. Month D**	**0. Month D**	**3. Month D**	**6. Month D**	**9. Month D**	**12. Month D**
A	338.33±1.58^{a}	240.46±2.96^{a}	135.75±1.68^{a}	51.56±1.33^{a}	45.68±0.32^{a}	218.50±4.03^{a}	202.48±3.06a	189.57±2.36^{a}	154.53±2.53^{a}	135.08±2.46^{a}
B	194.45±5.93^{b}	121.80±5.03^{b}	60.43±1.23^{b}	47.31±0.04^{b}	36.86±0.49^{b}	230.93±3.93^{b}	211.22±3.79a	196.41±2.62^{b}	160.31±2.40^{ab}	148.82±2.01^{b}
C	129.15±4.67^{c}	102.63±4.62^{c}	77.58±2.72^{c}	34.93±0.96^{c}	27.78±0.18^{c}	244.71±3.05^{c}	230.41±5.01b	209.90±2.75^{c}	167.51±1.68^{b}	164.76±0.76^{c}
D	59.20±8.41^{d}	48.59±4.78^{d}	35.53±1.71^{d}	29.23±1.44^{d}	23.81±1.13^{d}	253.72±0.55^{d}	246.48±1.93c	215.11±2.07^{c}	188.43±4.42^{c}	178.33±0.48^{d}
E	35.65±4.45^{e}	27.81±2.58^{e}	24.64±3.79^{e}	21.28±1.45^{e}	19.20±0.27^{e}	260.94±0.84^{d}	253.31±2.92c	234.89±1.28^{d}	199.45±2.90^{d}	183.50±0.79^{e}

($p < 0.05$) a-e: Different letters in the same column concerning all samples significantly different values. L: Light, D: Dark. A: 350 mg CAE/kg oil in ROO, B: 200 mg CAE/kg oil in ROO, C: 150 mg CAE/kg oil in ROO, D: 60 mg CAE/kg oil ROOl, E: ROO.

tistically significant difference ($P<0.05$) between A, B, C, D and E with respect to K_{232} values of the olive oil samples. Considering the change in the K_{232} value of the samples during the storage period, it is seen that the highest value was in the A samples stored in light conditions and stored for 6 and 12 months in dark conditions. There is no limit for the K_{232} value of ROO in the IOC standard. When the changes in the K_{270} values of the samples were examined, it was determined that there was a statistically significant difference ($P<0.05$) between the samples except for the C and E samples. During storage in light and dark conditions, it was determined that the K_{270} value of all samples did not exceed 1.25, which is the limit for ROO in the IOC standard.

Malheiro et al. [26] stated that the K_{232} and K_{270} values of the olive oils obtained by adding 1, 2.5, 5 and 10% OLs to the Cobrançosa olive variety were higher than the control group with the significant increment in oils with 10% leaf. It was stated in the research that K_{232} and K_{270} values may exceed the determined international legal limits depending on the OL addition ratio.

3.2 TOTAL PHENOL CONTENT (TPC) AND α-TOCOPHEROL CONTENT (ATC) ANALYSIS

The TPC of the samples before storage varied between 35.65-338.33 (mg CAE/kg oil) (Tab. IV). It was determined that there was a statistically significant difference ($P<0.05$) in the total amount of phenols during storage. In general, as the leaf extract addition rate increased, the total amount of phenol also increased. During the storage period, it was determined that the highest value in the TPC in light conditions was in samples A and B at 12 months, in samples A in other months, and the lowest value was in samples E. In dark conditions, it was determined that the highest value was in A samples and the lowest value was in E samples. It is seen that the TPC in the samples stored in dark conditions is higher than the samples stored in light conditions, the TPC decreases during 12 months of storage, and this decrease is more in the samples stored in light conditions.

Bouaziz et al. [10], it was stated that the antioxidant activity of ROO and pomace oil enriched with 400 ppm OL and hydrolysate extracts increased due to the increase in the phenolic antioxidant content. Salta et al. [9] reported that with the addition of OLE containing 195 mg CAE/kg oil to sunflower oil, palm oil and olive oil, the content of sunflower oil and palm oil, which initially had an undetectable total phenol content, changed to 155 and 157 mg CAE/kg oil, respectively. In olive oil, it was determined that the total phenol content was 94 mg CAE/kg oil at the beginning and this value increased to 299 mg CAE/kg oil with the addition of OLE. Tarchoune et al. [27], it was stated that the total amount of phenol increased significantly with the addition of leaves in olive oils obtained by adding 3% OL to Neb Jmel and Oueslati Tunisia olive varieties. Similar results were obtained in

our study. Morello et al. [28], Cinquanta et al. [29] and Sevim [25] stated that there was a decrease in the total amount of phenol in olive oils during the storage period, and they stated that this decrease in the total amount of phenol was due to the increased oxidation and hydrolytic activity during storage.

ATC of the samples to which different amounts of OLE were added before 12 months of storage ranged from 218.5 to 260.94 mg/kg (Tab. IV). During the storage period, it was determined that the highest amount of AT in light conditions was in E samples, in dark conditions, the highest value was in D and E samples at 3 months, and in E samples in other months.

When the effect of the addition of OLE to the ROO on the change in the amount of α-tocopherol was examined, it was determined that the amount of α-tocopherol was higher in the E sample. It was determined that this amount decreased with the addition of OLE. The amount of α-tocopherol of the samples stored in dark conditions is higher than the samples stored in light conditions.

In the study in which tocopherol components were extracted from OLs by supercritical liquid extraction method, it was determined that the amount of α-tocopherol was approximately 90, 60 and 60 mg/kg at 25, 35 and 45 MPa pressures, respectively. According to the particle size of the leaf, the amount of α-tocopherol was determined to be approximately 100, 90 and 60 mg/kg at 0.25, 0.15 and 1.5 mm, respectively [30].

3.3 TOTAL CHLOROPHYLL (TChl) AND CAROTENOID (TCar) ANALYSES

TChl and TCar of the olive oil samples was shown in Table V. The TChl and TCar values of the samples before storage was ranging from 0.34 to 9.99 mg/kg and from 0.19 to 2.97 mg/kg, respectively. It was determined that the TChl and TCar values of the samples changed statistically significantly ($P<0.05$), and the TChl and TCar values increased as the leaf extract addition rate increased. During the storage period, under light and dark conditions the highest and the lowest TChl and TCar values were determined in A samples and in E samples, respectively.

Di Giovacchino et al. [31], Di Giovacchino et al. [32] and Di Giovacchino and Preziuso [33] reported in their studies that the chlorophyll content of the olive oil obtained increased as the leaf addition rate to the olives increased. Morello et al. [28], Ranalli et al. [34] and Gomez-Alonso et al. [35], Sevim et al. [36], it was determined that the amount of chlorophyll decreased during the storage period. There is no limit set in national and international standards for chlorophyll and carotenoid values.

3.4 OXIDATIVE STABILITY INDEX (OSI) ANALYSIS

Table VI shows that the OSI of the samples before storage ranged between 5.63-6.97 h. It was determined that the OSI of the samples changed statis-

Table V - TCh and TCa of the samples during storage (mg/kg)

	TCh					TCa				
Samples	**0. Month L**	**3. Month L**	**6. Month L**	**9. Month L**	**12. Month L**	**0. Month L**	**3. Month L**	**6. Month L**	**9. Month L**	**12. Month L**
A	9.99±0.08^{a}	8.45±0.31^{a}	8.07±0.05^{a}	7.07±0.08^{a}	6.85±0.01^{a}	2,97±0,01^{a}	1,55±0,03^{a}	1,42±0,00^{a}	1,33±0,02^{a}	1,20±0,01^{a}
B	6.77±0.08^{b}	6.14±0.03^{b}	5.29±0.06^{b}	4.38±0.32^{b}	2.64±0.04^{b}	1,75±0,01^{b}	1,06±0,02^{b}	1,10±0,01^{b}	1,10±0,01^{b}	1,00±0,01^{b}
C	3.32±0.08^{c}	3.11±0.01^{c}	2.92±0.04^{c}	2.19±0.08^{c}	2.05±0.02^{c}	0,71±0,00^{c}	0,59±0,01^{c}	0,56±0,02^{c}	0,46±0,01^{c}	0,36±0,00^{c}
D	2.11±0.05^{d}	1.61±0.03^{d}	1.49±0.05^{d}	0.88±0.04^{d}	0.35±0.04^{d}	0,53±0,02^{d}	0,37±0,01^{d}	0,34±0,01^{d}	0,26±0,01^{d}	0,23±0,01^{d}
E	0.34±0.04^{e}	0.31±0.02^{e}	0.28±0.01^{e}	0.28±0.007^{e}	0.26±0.01^{e}	0,19±0,01^{e}	0,17±0,00^{e}	0,16±0,00^{e}	0,16±0,00^{e}	0,14±0,01^{e}
	0. Month D	**3. Month D**	**6. Month D**	**9. Month D**	**12. Month D**	**0. Month D**	**3. Month D**	**6. Month D**	**9. Month D**	**12. Month D**
A	9.99±0.08^{a}	9.09±0.10^{a}	8.82±0.02^{a}	8.26±0.01^{a}	7.93±0.02^{a}	2,97±0,01^{a}	2,03±0,04^{a}	1,56±0,03^{a}	1,50±0,01^{a}	1,44±0,00^{a}
B	6.77±0.08^{b}	6.40±0.12^{b}	6.08±0.09^{b}	5.98±0.01^{b}	5.81±0.04^{b}	1,75±0,01^{b}	1,25±0,03^{b}	1,19±0,01^{b}	1,10±0,01^{b}	1,07±0,01^{b}
C	3.32±0.08^{c}	3.20±0.03^{c}	3.00±0.01^{c}	2.87±0.04^{c}	2.76±0.02^{c}	0,71±0,00^{c}	0,68±0,01^{c}	0,62±0,01^{c}	0,56±0,01^{c}	0,48±0,01^{c}
D	2.11±0.05^{d}	1.81±0.08^{d}	1.76±0.01^{d}	1.63±0.02^{d}	1.41±0.03^{d}	0,53±0,02^{d}	0,48±0,00^{d}	0,41±0,01^{d}	0,34±0,01^{d}	0,28±0,01^{d}
E	0.34±0.04^{e}	0.32±0.02^{e}	0.31±0.01^{e}	0.31±0.01^{e}	0.30±0.01^{e}	0,19±0,01^{e}	0,19±0,01^{e}	0,19±0,00^{e}	0,19±0,00^{e}	0,19±0,01^{e}

($p < 0.05$) a-e: Different letters in the same column concerning all samples significantly different values. L: Light, D: Dark. A: 350 mg CAE/kg oil in ROO, B: 200 mg CAE/kg oil in ROO, C: 150 mg CAE/kg oil in ROO, D: 60 mg CAE/kg oil ROO, E: ROO.

tically significantly (P<0.05), and the OSI increased as the leaf extract addition rate increased. During the storage period, it was determined that the OSI was highest in A samples and lowest in E samples under light and dark conditions.

It was determined that the OSI of edible vegetable oils increased as a result of the addition of OL or enrichment with OLE Farag et al. [5], Salta et al. [9] and Bouaziz et al. [10]. Considering that phenolic compounds are responsible for approximately 50% of the OSI of olive oils [37], the results obtained in our study were found to be compatible with the studies conducted. Salta et al. [9] stated that the OSI values (at 110°C) of the oils to which the extract was added increased by 54% in sunflower oil, 20% in palm oil and 50% in olive oil with the addition of OLE. Bouaziz et al. [10] stated that ROO and olive pomace oils enriched with 400 ppm OL and hydrolysate extracts are highly resistant to oxidative degradation. At the end of 6 months, there was a decrease of 32% and 42% in ROO and pomace oil, respectively, in the OSI, and a decrease of 18% and 17%, respectively, in the samples to which the extract was added. In the study investigating the effect of Neb Jmel and Oueslati OLs on the amount of α-tocopherol in olive oil, they stated that the addition of 3% of the OLs caused a slight increase in the amount of α-tocopherol in olive oil obtained from Neb Jmel variety, while it did not cause any change in olive oil obtained from Oueslati variety [27]. This is because the ATC of Neb Jmel (82.37 µg/g dry weight) OL is higher than that of the Oueslati (10 µg/g dry weight) variety, and the Oueslati OL has no effect due to the very low amount of α-tocopherol. Koseoglu et al. [38] stated that the OSI of olive oils decreased during the storage period, and this decrease was due to the change in total phenol, α-tocopherol, chlorophyll and carotenoid amounts. The OSI of olive oils is particularly affected by the content of phenolic compounds [39].

PCA analyses were performed to determine the classification groups of olive oils with OLE according to their quality parameters such as TPC, OSI, ATC, K_{232}, K_{270}, POV, FFAV, TCar, and TChl. PCA score plot was constructed using 3 principal components revealing 80.6% of the total variance. The first principal component accounts for 40.9% of the total variance while the second principal component described 28.5% of the total variance. Score plots were constructed regarding samples with different concentrations of OLE (A, B, C, D, E) (Fig. 1), and storage conditions 0 (not stored), D (stored in the dark), L (stored in the light)

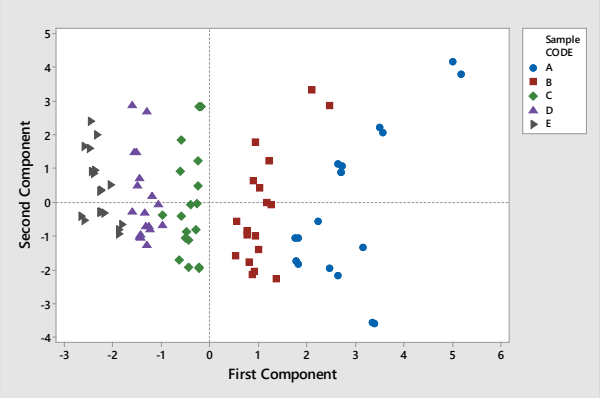

Figure 1 - PCA score plot according to storage conditions of olive oils

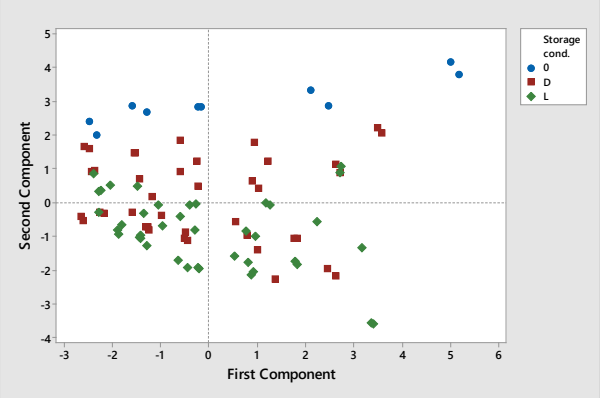

Figure 2 - PCA score plot according to storage conditions of olive oils

Table VI - OSI of the samples during storage (h)

Samples	0. Month L	3. Month L	6. Month L	9. Month L	12. Month L
A	6.97±0.02^{a}	5.65±0.05^{a}	5.42±0.02^{a}	5.20±0.04^{a}	5.48±0.04^{a}
B	6.67±0.05^{b}	5.50±0.02^{b}	5.17±0.02^{b}	4.90±0.01^{b}	5.21±0.09^{b}
C	6.28±0.04^{c}	5.30±0.05^{c}	5.12±0.01^{bc}	4.80±0.02^{c}	5.06±0.01^{c}
D	6.05±0.01^{d}	5.27±0.02^{c}	5.07±0.02^{c}	4.75±0.00^{c}	4.96±0.01^{cd}
E	5.63±0.04^{e}	5.10±0.02^{d}	4.90±0.04^{d}	4.60±0.02^{d}	4.85±0.07^{d}
	0. Month D	**3. Month D**	**6. Month D**	**9. Month D**	**12. Month D**
A	6.97±0.02^{a}	6.27±0.05^{a}	6.17±0.05^{a}	5.11±0.02^{a}	5.28±0.02^{a}
B	6.67±0.05^{b}	5.80±0.02^{b}	5.74±0.01^{b}	5.03±0.02^{a}	5.08±0.02^{b}
C	6.28±0.04^{c}	5.58±0.01^{bc}	5.43±0.02^{c}	4.93±0.04^{b}	4.99±0.02^{c}
D	6.05±0.01^{d}	5.35±0.03^{c}	5.19±0.05^{d}	4.71±0.04^{c}	4.89±0.02^{d}
E	5.63±0.04^{e}	5.39±0.24^{c}	5.02±0.02^{e}	4.12±0.01^{d}	4.66±0.01^{e}

($p < 0.05$) a-e: Different letters in the same column concerning all samples significantly different values. L: Light, D: Dark. A: 350 mg CAE/kg oil in ROO, B: 200 mg CAE/kg oil in ROO, C: 150 mg CAE/kg oil in ROO, D: 60 mg CAE/kg oil ROO, E: ROO.

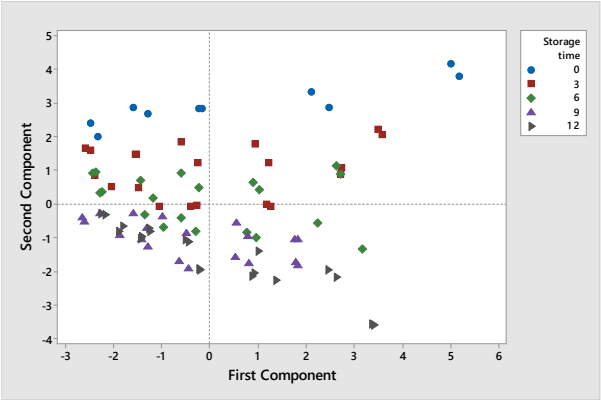

Figure 3 - PCA score plot according to storage conditions of olive oils

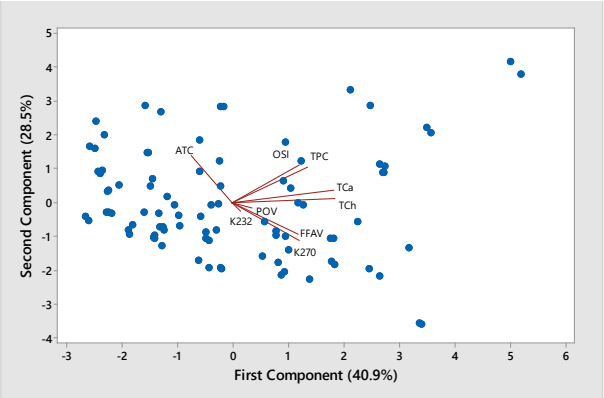

Figure 4 - PCA score plot according to storage conditions of olive oils

(Fig. 2) and storage time (0, 3, 6, 9, 12 months) (Fig. 3). The score plot according to samples with different concentrations of OLE indicated that the samples were separated successfully on the plot. The score plot constructed for the storage times and storage conditions showed that the samples were not discriminated clearly. According to PCA biplot analysis (Fig. 4), C samples stored at dark condition were characterised by ATC. B samples stored at dark were characterised by TPC and OSI. TCar and TChl characterised B samples stored at light storage condition. The main quality properties that discriminated samples A and B stored at light and dark conditions were determined as FFAV, POV, K_{232} and K_{270} values.

Table VII - FAC of the samples during storage (%)

	Samples	A	B	C	D	E
OA	0. Month L	69.82±0.12^{a}	68.67±0.01^{b}	72.12±0.00^{e}	72.49±0.01^{e}	72.48±0.00^{e}
	3. Month L	71.38±0.43^{c}	71.31±0.06^{d}	72.38±0.18^{f}	72.67±0.01^{f}	71.33±0.05^{b}
	6. Month L	71.44±0.01^{c}	70.58±0.16^{d}	71.48±0.00^{bc}	71.56±0.01^{d}	71.56±0.04^{cd}
	9. Month L	70.68±0.13^{b}	70.61±0.07^{c}	71.39±0.01^{b}	71.39±0.01^{bc}	71.31±0.26^{b}
	12. Month L	71.44±0.00^{c}	70.28±0.02^{c}	71.67±0.05^{cd}	69.88±0.00^{a}	71.63±0.05^{cd}
	3. Month D	70.98±0.07^{b}	70.36±0.01^{d}	71.29±0.01^{b}	71.40±0.01^{bc}	71.51±0.04^{bc}
	6. Month D	71.57±0.11^{c}	70.55±0.01^{d}	71.33±0.02^{b}	71.46±0.01^{c}	71.60±0.02^{cd}
	9. Month D	71.47±0.01^{c}	70.51±0.04^{d}	71.04±0.04^{a}	71.36±0.08^{b}	71.75±0.01^{d}
	12. Month D	71.50±0.00^{c}	66.72±0.06^{a}	71.79±0.06^{d}	71.42±0.10^{bc}	69.51±0.11^{a}
		A	**B**	**C**	**D**	**E**
LA	0. Month L	10.29±0.11^{a}	9.99±0.01^{b}	10.71±0.01^{c}	10.68±0.00^{e}	10.83±0.01^{f}
	3. Month L	10.90±0.01^{b}	10.41±0.01^{d}	10.72±0.01^{cd}	10.74±0.01^{f}	10.49±0.00^{b}
	6. Month L	10.83±0.00^{b}	10.37±0.00^{c}	10.60±0.00^{b}	10.60±0.02^{c}	10.59±0.01^{c}
	9. Month L	11.56±0.06^{f}	10.6±0.03^{g}	10.82±0.04^{e}	10.85±0.00^{g}	10.66±0.03^{de}
	12. Month L	11.11±0.00^{d}	10.56±0.00^{f}	10.53±0.00^{c}	10.23±0.00^{a}	10.63±0.01^{cd}
	3. Month D	11.35±0.02^{e}	10.77±0.01^{h}	10.82±0.00^{e}	10.74±0.01^{f}	10.65±0.01^{d}
	6. Month D	10.80±0.02^{b}	10.52±0.01^{e}	10.71±0.01^{cd}	10.64±0.00^{d}	10.61±0.01^{c}
	9. Month D	10.91±0.01^{bc}	10.61±0.00^{g}	10.58±0.01^{b}	10.90±0.01^{h}	10.70±0.04^{e}
	12. Month D	11.03±0.00^{cd}	9.11±0.01^{c}	10.75±0.01^{d}	10.51±0.00^{b}	9.85±0.01^{a}
		A	**B**	**C**	**D**	**E**
LnA	0. Month L	0.71±0.00^{a}	0.70±0.00^{b}	0.76±0.00^{d}	0.76±0.00^{e}	0.77±0.00^{f}
	3. Month L	0.76±0.01^{f}	0.74±0.01^{e}	0.76±0.00^{d}	0.76±0.00^{e}	0.71±0.01^{b}
	6. Month L	0.76±0.00^{de}	0.73±0.01^{d}	0.74±0.01^{c}	0.75±0.00^{d}	0.75±0.00^{de}
	9. Month L	0.75±0.01^{d}	0.72±0.00^{c}	0.74±0.00^{c}	0.74±0.00^{c}	0.74±0.01^{cd}
	12. Month L	0.73±0.00^{b}	0.69±0.00^{b}	0.70±0.00^{a}	0.69±0.00^{a}	0.74±0.01^{cd}
	3. Month D	0.76±0.00^{de}	0.74±0.00^{de}	0.75±0.01^{cd}	0.76±0.00^{e}	0.76±0.00^{ef}
	6. Month D	0.76±0.00^{de}	0.74±0.00^{de}	0.76±0.00^{d}	0.75±0.00^{d}	0.75±0.00^{de}
	9. Month D	0.74±0.01^{c}	0.72±0.00^{c}	0.72±0.00^{b}	0.74±0.00^{c}	0.73±0.00^{c}
	12. Month D	0.72±0.00^{b}	0.60±0.00^{a}	0.72±0.00^{b}	0.70±0.00^{b}	0.67±0.00^{a}

OA: Oleic Acid, LA: Linoleic Acid, LnA: Linolenic Acid; ($p < 0.05$) a-h: Different letters in the same column concerning all samples significantly different values. L: Light, D: Dark. A: 350 mg CAE/kg oil in ROO, B: 200 mg CAE/kg oil in ROO, C: 150 mg CAE/kg oil in ROO, D: 60 mg CAE/kg oil ROO, E: ROO.

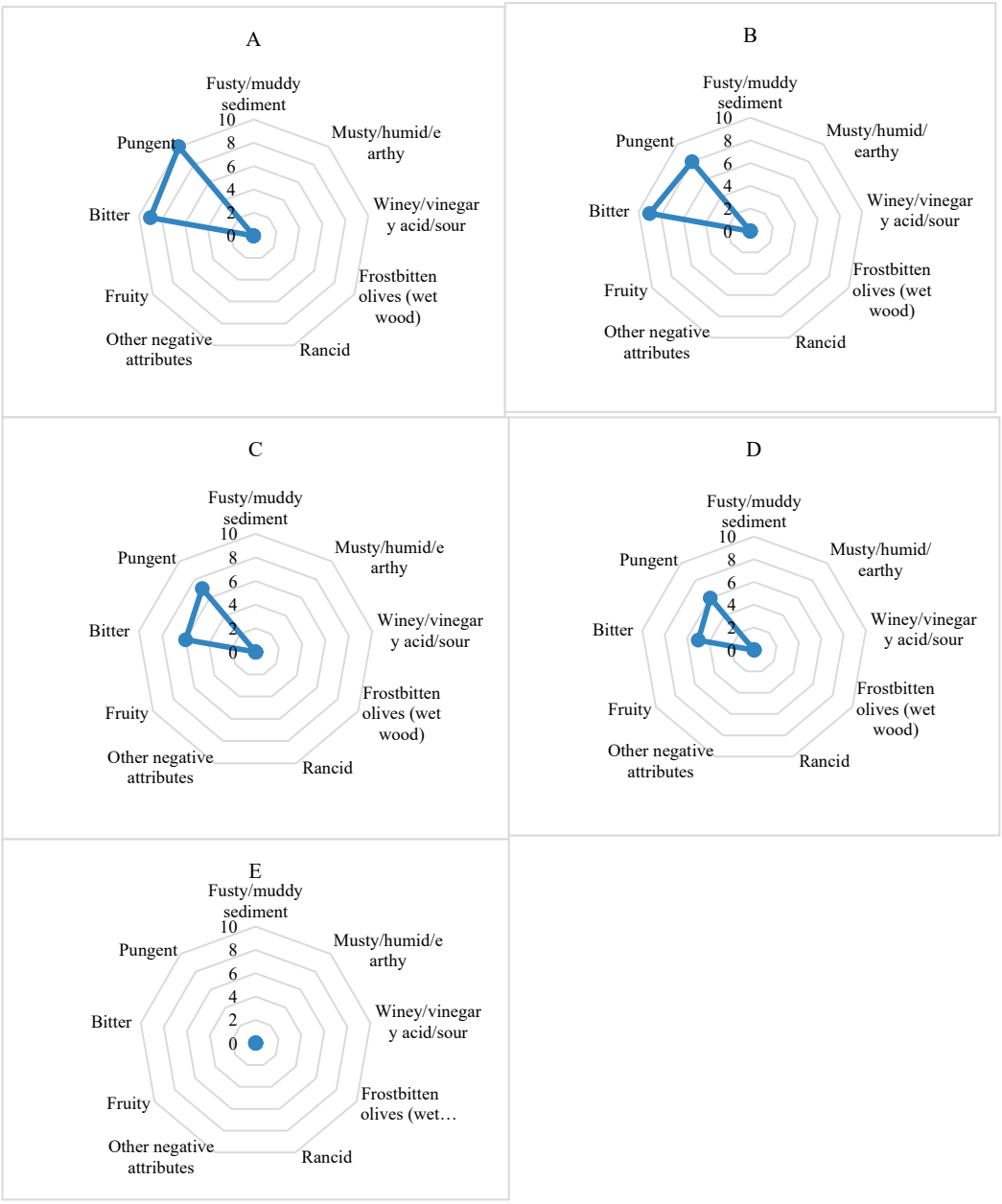

Figure 5 - Sensory analysis results of A, B, C, D and E samples at the beginning of the storage.
A: 350 mg CAE/kg oil in ROO, B: 200 mg CAE/kg oil in ROO, C: 150 mg CAE/kg oil in ROO, D: 60 mg CAE/kg oil ROO, E: ROO.

3.5 FATTY ACID COMPOSITION (FAC) ANALYSIS

OA, LA and LnA content of the samples before storage ranged between 68.67-72.48%, 9.99-10.83% and 0.70-0.77%, respectively (Tab. VII). It was determined that the FAC of the samples changed statistically significantly ($P<0.05$) during the 12 months.
In the study conducted by Sevim [19], it was determined that the addition of leaves to olives did not have a fixed effect on the FAC of the oil obtained. Malheiro et al. [26] in the study conducted on olive oils obtained by adding OLs, it was determined that the addition of leaves had no effect on the FAC of the samples obtained in the 2009 harvest year, while the addition of 10% leaf in the 2010 harvest year caused a decrease in the OA content and an increase in the LA content.

3.6 SENSORY ANALYSES

Sensory analyses were performed on A, B, C, D and E samples by the trained panellist group according to the International Olive Council sensory analysis standard. Sensory analysis results of A, B, C, D and E samples at the beginning of the storage were given in Figure 5. In the A, B, C, D samples with OLE added, intense alcohol, odours like henna and dried herbs, and an intense quinine or drug-like bitterness were

detected, and the samples were not found suitable for consumption by the panellists. Sudjana et al. [40], OLE was stated as a dark brown coloured liquid with a bitter taste, and similar results were obtained with our study.

4. CONCLUSION

This study was performed to determine the impact of OLs extracted by SCD extraction on the OSI of olive oils. With this purpose extracts containing phenolic substances obtained from OLs were added to ROO (E) at concentrations of 350 mg CAE/kg oil (A), 200 mg CAE/kg oil (B), 150 mg CAE/kg oil (C) and 60 mg CAE/kg oil (D). Samples were stored in clear glass bottles for 12 months under light and dark conditions and the samples were analysed at every 3 months for a year. The results of the analyses revealed that the amount of FFAV, POV, TPC, TChl and TCar content and OSI values increased as the OLE addition ratio increased. When examined the effect of the concentration of OLE on the amount of α-tocopherol, it was seen that the amount of α-tocopherol was higher in ROO (E) and this amount decreased with the addition of OLE. The sensory analysis results were examined, and it was determined that alcohol, henna and dried herb-like odours were detected in the samples to which OLE was added. PCA analyses indicated that olive oils were discriminated clearly based on the concentrations of OLEs.

As a result, it was determined that the TPC, TChl and TCar content and OSI values were the highest in the A sample, which was stored in the dark and had the highest OLE content. Sample A, which is rich in antioxidant components, can be said to be the best example enriched with OLE, apart from sensory properties. In terms of the content of AT, which is equivalent to vitamin E, we can say that the E sample stored in the dark has the best properties except for the sensory properties.

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