Influence of microwave heating on quality parameters and chemical characteristics of Turkish olive oils

D. Sevim[1*]
O. Köseoğlu[1]
M. Casale[2]
Y. Altunoğlu[1]
P. Kadiroğlu[3]

[1]Olive Research Institute
Ministry of Agriculture and Forestry
İzmir, Turkey

[2]Dipartimento di Farmacia
Università di Genova,
Genova, Italy

[3]Department of Food Engineering
Adana Science and Technology University
Adana, Turkey

(*) CORRESPONDING AUTHOR:
Olive Research Institute
Ministry of Agriculture and Forestry
İzmir, Turkey
University street no:43 35100
Bornova, İzmir, Turkey
Tel:+902324627073
Fax: +902324357042
E-mail: dcengeler@gmail.com

Microwave use has become quite common and microwave ovens are being used increasingly due to their fast heating technology. In this study, the effects of microwave heating on the deterioration of olive oil quality parameters like free fatty acidity, peroxide value, ultraviolet absorbance values (K_{232}, K_{270}) and also antioxidant compounds as chlorophyll and carotenoid content, α-tocopherol content, total phenolic content in addition oxidative stability and DPPH$^{\bullet}$ radical scavenging activity which are related to chemical characteristics of Turkish olive oils, were investigated, at different times of the microwave treatment, in 2015/16. All analytical data were processed through univariate and multivariate data analysis (Principal Component Analysis). It was determined that heating time significantly influences quality parameters and chemical characteristics. This heating treatment produces significant losses in olive oil quality parameters and also in bioactive components. Therefore, heating time should be reduced to minimum to preserve olive oil nutritional content.

Keywords: Microwave heating, Olive oil, Antioxidant activity, Oxidative stability, PCA.

INTRODUCTION

Microwave heating is a common and fast element for food preparation and manufacturing. The effect of microwave heating on the micro and macro components in foods can differ significantly from those produced by heating in a conventional oven [1]. In recent years, modern lifestyle has caused changes in eating and drinking processes and technologies. The usage of the microwave ovens showed an increment at homes and in industry owing to their advantages [2]. Due to the positive effects on human health owed to the antioxidant compounds found in olive oil, the interest in consumption of olive oil is increasing day by day [3, 4]. The content of these compounds is related to the olive variety, geographical origin, seasonality, agronomic factors, and technological conditions in the production of olive oil and olive oil storage conditions [5, 6]. Olive oil, like other vegetable oils, is used in deep-frying, pan-frying, roasting, microwave cooking, etc. Each thermal processing type has particular characteristics, namely regarding temperature and exposure time [7].
A few studies have been carried out to evaluate the effect of microwave heating on the degradation of quality parameters and chemical characteristics of virgin olive oils. Brenes et al. [8] evaluated phenolic content of extra virgin olive oil from different Spanish cultivars after microwave and conventional heating, Chiavaro et al. [9] reported the microwave heating effect on differential scanning calorimetry (DSC) thermal properties of three commercial categories of olive oil for direct human consumption, Abd El-Moneim Mahmoud et al. [1] compared oxidation of olive oils (Italian and Egyptian) during microwave and conventional heating for fast food preparation, Malheiro et al. [10] evaluated

effect of microwave heating with different exposure times on Portuguese Protected Designation of Origin (PDO) olive oils, Cossignani et al. [11] investigated the alterations in Italian olive oil composition with microwave heating, Yahyaoui et al. [2] have also studied the effect of microwave heating on different commercial Tunisian olive oils.

According to our knowledge there is no research regarding the effect of microwave heating on the degradation of quality parameters and chemical characteristics of important olive oils that obtained from Memecik and Ayvalık, Turkish olive cultivars. Therefore, the objectives of this research were to evaluate the influence of different microwave heating times (3, 5, 10, 15 min) on quality parameters (free fatty acidity, peroxide value, K_{232} and K_{270} values) and chemical characteristics that include chlorophyll and carotenoid, alpha tocopherol and total phenolic content and also oxidative stability and DPPH• Radical Scavenging Activity (RSA) of Memecik and Ayvalık olive oils.

To evaluate the effect of microwave heating on the deterioration of quality parameters and chemical characteristics of Turkish olive oils, all analytical data were processed through univariate and multivariate data analysis.

EXPERIMENTAL PART

OLIVE OIL SAMPLES

Two Turkish olive cultivars were harvested (10 kg), "Memecik" from the South Aegean and "Ayvalık" from the North Aegean of Turkey, in 2015/16 crop season. Oil samples were obtained with Abencor (Mc2 Ingenieria y Sistemas, Sevilla, Spain) which is laboratory scale oil mill equipped with hammer crusher, malaxer, and centrifuge. The temperature of malaxation and duration was 30°C and 30 min, respectively. Olive oil samples were stored in dark brown coloured glass bottles at +4°C until analysis (Each oil samples contains 1000 mL).

MATURITY INDEX (MI)

To eliminate the effect of the maturation stage on the quality olive oils, the fruits were collected at the same maturity stage. The maturity index was detected with the method recommended by International Olive Council [12] that is based on the colour of the olive fruit skin.

MICROWAVE HEATING TREATMENT

A laboratory scale microwave oven was used for olive oil treatment (MARSX press, CEM, USA). Two aliquots (90 ml) of each oil were placed in opened 150 ml flasks on the rotatory turntable plate of the oven at equal distance and exposed at a frequency of 2450 Hz at medium power (800W). The oil samples were subjected to microwave for 3, 5, 10 and 15 min. The two 90 ml aliquots of each oil were combined after microwaving, to obtain a homogeneous sample used for analysis. Unheated olive oil was used as control.

QUALITY PARAMETERS

The quality parameters (free fatty acidity (FFA) and the peroxide values (PV)) of olive samples were detected according to Food Codex of Turkey (13). Briefly, for FFA determination, the olive oil sample was weighed and dissolved into solvent mixture of ethanol and diethylether at 1:1 volume ratio. Then, titration of the mixture was performed under constant stirring, against a 0.1 M KOH solution using phenolphthalein as an indicator. The results were given in percentage oleic acid. For the PV determination, the sample was dissolved by stirring rapidly with the addition of 10 ml of chloroform. Acetic acid (15 ml) and 1 ml of potassium iodide solution were added and shaken for one minute and left for five minutes in a dark ambience at 15-25°C. Seventy-five ml of distilled water was added. The free iodine that was formed in the flask was titrated with the sodium thiosulphate solution by shaking vigorously, using starch solution as the indicator. The result was expressed as milliequivalents of active oxygen per kilogram ($meqO_2/kg$). The ultraviolet spectrophotometric values (K_{232} and K_{270}) were determined according to the methods given by International Olive Council [14].

TOCOPHEROL ANALYSIS

α-tocopherol analysis was evaluated with the procedure recommended by Carpenter [15] and IUPAC [16]. One gram of the oil sample was weighed with the addition of 10 mL of hexane, then filtered through a 0.45-µm filter and 20 µL was injected into the HPLC (Agilent Technologies 1100 series). A flow rate is 1 mL/min. The UV detector was set to 292 nm wavelength. A Waters µ-porasil column (250 mm × 4.6 mm × 5 µm) (Waters Corporation, MA) was used. The temperature of the column was at 25°C. The total run time was 10 min.

TOTAL PHENOLIC CONTENT (TPC)

The TPC of the oils was evaluated according to the method of Gutfinger [17] introducing modifications of the method of HrnciriK and Fritsche [18]. Olive oil sample (2.5 g) was weighed and 5 ml of hexane and then 5 ml of methanol/water (60:40 v/v) was added. The solution was shaken for 2 min for the extraction of phenolic compounds. The phases were separated from each other by centrifuging at 3500 rpm for 10 min. 0.2 ml of methanolic phase was diluted with

deionised water to 5 mL, and then 0.5 mL of Folin-Ciocalteu reagent (Merck, Germany) was added. 3 mins later, 1 mL of sodium carbonate solution (35%, w/v) was added and completed with 10 mL of water. The absorbance of the solution was measured at 725 nm with a Spectrophotometer (UV-1700 Shimadzu PharmaSpec, Japan) after 2 h. The results were expressed as mg caffeic acid equivalent per kg (mgCAE/kg).

DPPH• RADICAL SCAVENGING ACTIVITY (RSA)

DPPH• RSA analysis of the oil samples was determined according to method given by Lavelli [19] and Jiang et al. [20]. 1 g oil was dissolved in 5 mL methanol and vigorously shaken for 1 hour at room conditions and then centrifuged at 3500 rpm for 10 min [19]. The antioxidant activity was determined at the methanolic phase. 100 µl of the extracts were mixed with either 1900 µl of 100 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) or 1900 µl of methanol to obtain the blank solution and kept at dark and room temperature for 15 min. The spectrophotometer was used at 517 nm to get the absorbance values (Shimadzu UV-1700 PharmaSpec, Japan). The results were expressed as µmol Trolox/100g oil. The standard curve of adjusted concentrations of Trolox (R^2 = 0.9925) was used to calculate the trolox equivalent (TE) of the DPPH• RSA.

OXIDATIVE STABILITY

The oxidative stability of the olive oils was determined according to the method given by Tura et al. [21] by using Rancimat 743 (Metrohm Ltd., Herisau, Sweeden). The conductivity variation was measured at 120°C with an air flow of 20 L/h. The results were expressed in hours (h).

CHLOROPHYLL AND CAROTENOID CONTENT

Cholorophyll and carotenoid content of the samples were evaluated by measuring in cyclohexane at 670 nm and 470 nm, respectively, using the specific extinction values, with a spectrophotometer (Shimadzu UV-1700 PharmaSpec, Japan) [22]. The total chlorophyll and carotenoid content were expressed in mg per kilogram of oil (mg/kg oil).

STATISTICAL ANALYSIS

UNIVARIATE DATA ANALYSIS

The analytical measurements were given as mean±-standard deviation (SD). Statistical differences were determined with JMP (Version 5.0.1, SAS Institute Inc, Cary, NC, 1989-2007). JMP was used to carry out one-way analysis of variance (ANOVA) with the Student's t-test. Differences were regarded as significant at p <0.05.

MULTIVARIATE DATA ANALYSIS

Principal Component Analysis (PCA) represents a powerful tool in exploratory data analysis, useful for the identification of general data features, to find similarities among the samples and to detect anomalies or errors [23]. In this study, a chemometric evaluation of analytical data obtained by PCA means, was performed with the aim of evaluating the effect of microwave heating on the degradation of quality parameters and chemical characteristics of Turkish olive oils.

RESULTS AND DISCUSSION

MATURITY INDEX (MI)

MI of the olive fruits were evaluated for Memecik and Ayvalık. MI of Memecik olive was determined 2.20. Maturity index value of Ayvalık olive was found 2.81.

QUALITY PARAMETERS

As can be seen in Table I, ANOVA analysis showed that there were significant differences between the olive oil samples depending on FFA, PV, K_{232} and K_{270} (p <0.05). Prior heating, Memecik and Ayvalık olive oils were classified as extra virgin olive oil, with FFA value of 0.22% and 0.72%, respectively. In both for the treated and untreated olive oil samples of Memecik and Ayvalık varieties, significant differences were observed in FFA values being slightly higher values due to microwave heating (p <0.05). Cossignani et al. [11] found that significant differences were observed for FFA of six Italian extra virgin olive oil with microwave heating treatment. Our results are compatible with the researchers. Malheiro et al. [10], Albi et al. [24] and Yahyaoui et al. [2] reported that no statistical changes were determined between the olive oil samples in FFA values with the heating time. The obtained results are contradictory with the results obtained by researchers. On the other hand, Cerretani et al. [4] reported that the lipolysis of the oil was significantly noticeable only at the longer treatment times (12 and 15 min), being more pronounced in extra virgin olive oil as compared to the olive oil (blend virgin olive oil with refined olive oil) and pomace oil (blend virgin olive oil with refined pomace oil).

Prior to microwave heating, the PV of the olive oils were below the maximum permitted levels for their classification in each category (20 meq O_2/kg oil) [25]. PV of the olive oil samples significantly increased with microwave heating exposure. After the heating treatment, PV values of Memecik and Ayvalık olive oils did not exceed the permitted limit. Cossignani et al. [11] reported that PV increased in six extra virgin olive oils after microwave heating treatment during for 10 min 8 min, Yahyaoui et al. [2] mentioned that the PV increased in Chemlali and Zalmati olive oils after the mi-

Table I - Quality parameters of olive oil samples subjected to different microwave heating times

Time (min)	Free Fatty Acidity (oleic acid %)	Peroxide Values (meqO2/kg)	K_{232}	K_{270}
Memecik				
M0	0.22±0.00^{g}	6.64±0.03^{f}	1.63±0.04^{f}	0.14±0.01^{ab}
M3	0.19±0.00^{g}	11.39±0.14^{e}	1.64±0.04^{ef}	0.10±0.00^{c}
M5	0.26±0.01^{f}	13.66±0.12^{d}	1.71±0.04^{cdef}	0.13±0.02^{b}
M10	0.29±0.01^{ef}	16.31±0.24^{b}	1.81±0.07^{abcd}	0.13±0.00^{b}
M15	0.29±0.01^{e}	19.78±0.01^{a}	1.95±0.17^{a}	0.13±0.01^{ab}
Ayvalık				
A0	0.72±0.02^{d}	6.50±0.12^{f}	1.70±0.01^{def}	0.14±0.00^{ab}
A3	0.86±0.01^{c}	15.79±0.20^{c}	1.88±0.00^{ab}	0.13±0.01^{b}
A5	0.86±0.01^{bc}	15.90±0.05^{c}	1.85±0.04^{abc}	0.13±0.00^{ab}
A10	0.89±0.00^{ab}	15.64±0.07^{c}	1.78±0.00^{bcde}	0.14±0.01^{ab}
A15	0.91±0.02^{a}	16.51±0.21^{b}	1.78±0.01^{bcde}	0.15±0.00^{a}

$^{a-g}$ Different letters in the same column concerning all samples significantly different ($p < 0.05$)

crowave heating exposure during 15 min, Vieira And Regitano-D'arce [26] also found higher PV with short periods (4-6 min) of microwave heating, Malheiro et al. [10] reported that PV of Azeite de Moura PDO olive oil increased until 15 min, on the other hand they mentioned that Azeite de Tras-os-Montes PDO and Azeite da Beira Interior PDO, two Portuguese olive oils, PV values showed a decline - when treated with microwave heating for up to 10 min. Microwave energy exposure was reported to favour the free radical formation [27]. Additionally, peroxides usually degrade at both high and low temperatures [4]. In this study, results are supporting the previous reports.

The specific extinction coefficients of K_{232} and K_{270} are commonly indicative of the conjugation of trienes and the presence of carbonyl compounds, respectively [2,10]. All these UV parameters were within the legal limits before microwave heating exposure [25]. Before microwave heating exposure of Memecik and Ayvalık olive oils, K_{232} values were varied between of 1.63 and 1.70, while K_{270} values were the same for both olive oils. Microwave heating caused important differences in the K_{232} and K_{270} values of all analysed olive oils. K_{232} values of Memecik olive oil presented a positive correlation with the exposition time. Ayvalık olive oil values increased until 5 min and decreased afterwards. Both the K_{232} and K_{270} values of all the treated and untreated samples were within the legal limits which were given as <2.4 and <0.20, respectively for the corresponding class of oil. Our results are in accordance with those obtained by Malheiro et al. [10], Yahyaoui et al. [2] and Caponio et al. [28].

CHEMICAL CHARACTERISTICS

As shown in Table II, it was determined that there were significant differences between the olive oil samples depending on α-tocopherol, oxidative stability, total chlorophyll, total carotenoid, total phenol content and DPPH$^{\bullet}$ RSA (p <0.05).

Tocopherol is known as vitamin E in olive oil. α, β, γ and δ tocopherols have inhibitory effect on LDL oxidation and they have several nutritional benefits [3]. Alpha tocopherol contents of Memecik and Ayvalık unheated olive oils are 356.10 mg/kg and 233.80 mg/kg, respectively. For both olive oil samples, significant differences were observed on the α-tocopherol content. The content of Memecik olive oil decreased until 3 min of microwave heating, followed by an increase until 5 min, and, then again, a decrease was observed until 10 min, after that an increase was determined until 15 min. Ayvalık olive oil α-tocopherol content decreased drastically until 3 min, after that a slight decrease was observed with increasing microwave heating time. Malheiro et al. [10] reported that microwave heating affects the α-tocopherol content that clearly decreased with the increment of the exposure times, Brenes et al. [8] mentioned that microwave heating causes a decrease whereas Albi et al. [24] reported a complete disappearance of α-tocopherol content in olive oil. The obtained results were expected and are in accordance with the results obtained by researchers.

TPC is affecting the antioxidant potential and sensory quality of olive oils [3]. Before microwave heating, the total phenols content of Memecik and Ayvalık are determined 389.95 mg CAE/kg oil and 296.57 mg CAE/kg oil, respectively. TPC decreased in the olive oils with increasing microwave heating time. TPC of Memecik olive oil showed a drasticall reduction to 297.40 mg CAE/kg oil at 5 min of exposure, then a gradual decrease to 289.45 mg CAE/kg oil at the highest level of heating time. TPC of Ayvalık olive oil decreased gradually until 10 min, after that an increase was determined until 15 min. TPC of olive oils were also evaluated by some researchers previously with a general report of microwave heating exposure causing losses on phenolic content [2, 4, 17]. The obtained results are in accordance with the authors.

Chlorophylls and carotenoids in olive oils are in charge of the greenish and yellow colouring of certain olive oils, respectively [2, 10]. These pigments are

Table II - Chemical characteristics of olive oil samples subjected to different microwave heating times

Time (min)	Alpha Tocopherol (mg/kg)	Oxidative stability (h)	Total Chlorophyll (mg/kg)	Total Carotenoid (mg/kg)	Total Phenol (mgCAE/kg)	DPPH• RSA (µmol TE/100 g oil)
Memecik						
M0	356.10±2.43^{a}	9.06±0.01^{ab}	1.35±0.12^{bc}	1.19±0.03^{bcde}	389.95±1.18^{a}	131.03±0.77^{a}
M3	240.16±0.92^{e}	8.19±0.01^{c}	2.13±0.01^{a}	1.38±0.01^{ab}	364.41±0.59^{b}	123.44±1.28^{b}
M5	300.01±3.50^{b}	7.95±0.08^{c}	1.53±0.13^{b}	1.22±0.06^{abcd}	297.40±6.51^{c}	120.54±2.81^{b}
M10	246.64±0.91^{d}	7.30±0.04^{d}	1.88±0.15^{a}	1.41±0.05^{a}	295.73±1.78^{c}	117.65±2.30^{bc}
M15	273.74±0.76^{c}	6.82±0.09^{e}	1.56±0.15^{b}	1.35±0.19^{abc}	289.45±3.55^{c}	114.21±0.51^{c}
Ayvalık						
A0	233.80±0.3^{f}	9.30±0.04^{a}	0.63±0.06^{d}	1.00±0.02^{e}	296.57±2.96^{c}	86.17±0.77^{d}
A3	204.37±0.45^{h}	7.41±0.18^{d}	1.18±0.22^{c}	1.06±0.06^{de}	276.88±10.66^{d}	80.57±0.51^{de}
A5	204.59±0.35^{h}	7.51±0.06^{d}	1.15±0.17^{c}	1.22±0.19^{abcd}	233.33±0.00^{f}	78.40±4.60^{ef}
A10	206.31±1.10^{h}	8.73±0.13^{b}	1.15±0.20^{c}	1.14±0.09^{cde}	227.47±2.37^{f}	74.60±5.37^{f}
A15	210.39±0.43^{g}	9.18±0.45^{a}	1.27±0.09^{bc}	1.22±0.02^{abcd}	263.07±0.59^{e}	77.31±1.53^{ef}

$^{a-h}$ Different letters in the same column concerning all samples significantly different ($p < 0.05$)

also important for providing olive oil stability owing to their antioxidant attributes in the dark and pro-oxidant activity in the light [2]. According to the preliminary results, chlorophyll contents were calculated as 1.35 mg/kg for Memecik olive oil and 0.63 mg/kg for Ayvalık olive oil. The level of carotenoids in the unheated oils ranged between 1.19 mg/kg and 1.00 mg/kg for Memecik and Ayvalık olive oils, respectively. At the end of the microwave heating (at 15. min) the total chlorophyll and carotenoid levels were higher than those of unheated samples of Memecik and Ayvalık. The increment of the microwave heating time caused the reduction of the total chlorophylls and carotenoids contents in compliance with previous reports [2, 10].

The oxidative stability of Memecik and Ayvalık olive oils were measured using the Rancimat method and given in Table II. First, the microwave heating treatment oxidative stability of Memecik and Ayvalık olive oils was determined as 9.06 (h) and 9.30 (h), respectively. Significant differences were observed between the samples for the oxidative stability for both olive oil samples (Table II). The oxidative stability of Memecik olive oil showed a marked decrease whereas the oxidative stability of Ayvalık olive oil decreased drastically until 3 min, after that it stayed constant until 5 min of heating and then a slight increase was determined until 15 min. This was probably because of the decrease in total phenol and α-tocopherol contents of the Memecik olive oil compared to Ayvalık olive oil. Some researchers reported that olive oil has a high resistance to oxidative degradation due to its fatty acid composition, characterised by high monounsaturated-to polyunsaturated fatty acid ration, and to the presence of minor compound such as phenolic and tocopherols [3, 29]. Albi et al. [24] mentioned that polyphenols, which have well-known antioxidant properties, were noticed to partially prevent thermos-oxidation of extra virgin olive oil and olive oil when subjected to different thermal exposure, e.g. microwave.

At the beginning of the analysis, DPPH• RSA was determined 131.03 µmol TE/100g oil for Memecik olive oil and 86.17 µmol TE/100g oil for Ayvalık olive oil. DPPH• RSA significantly decreased as the microwave heating time increased for both olive oils (Table II), possibly due to the losses of phenolic and tocopherol content. The antioxidant activity of olive oils affected the total phenolic and α-tocopherol content [20, 30, 31, 32]. Sevim et al. [30] reported that the phenolic content can break the free radical chain reaction and their antioxidant properties are from their hydrogen-donating capacities, Kiritsakis et al. [33] mentioned that the antioxidant activity depends on the phenolic profile, and that there is a positive correlation between the antioxidant capacity and the phenolic content.

QUALITY PARAMETERS AND CHEMICAL CHARACTERISTICS: MULTIVARIATE DATA ANALYSIS

Lastly, the quality parameters (FFA, PV, K_{232}, K_{270}) and chemical characteristics (alpha tocopherol, oxidative stability, total chlorophyll, total carotenoid, total phenol and DPPH• RSA) were considered together and processed through a principal component analysis. Figure 1a) and b), Figure 2a) and b) show the score and loading plots obtained for Memecik and Ayvalık olive oils, respectively. Especially for Memecik olive oil, the first PC seemed associated to the microwave heating time, in fact, control samples had positive scores on PC1 and the oils heated for 15 minutes at negative value of PC1 were on the opposite site. Looking at the loading plot, it was possible to see that especially FFA, PV, K_{232} and total carotenoid decreased in the olive oils with increasing microwave heating time. Control samples were characterised by high values of oxidative stability, total phenols, alpha tocopherol and DPPH• RSA. As far as Ayvalık olive oil is concerned, even if the trend of the scores along microwave heating time was more 'circular',

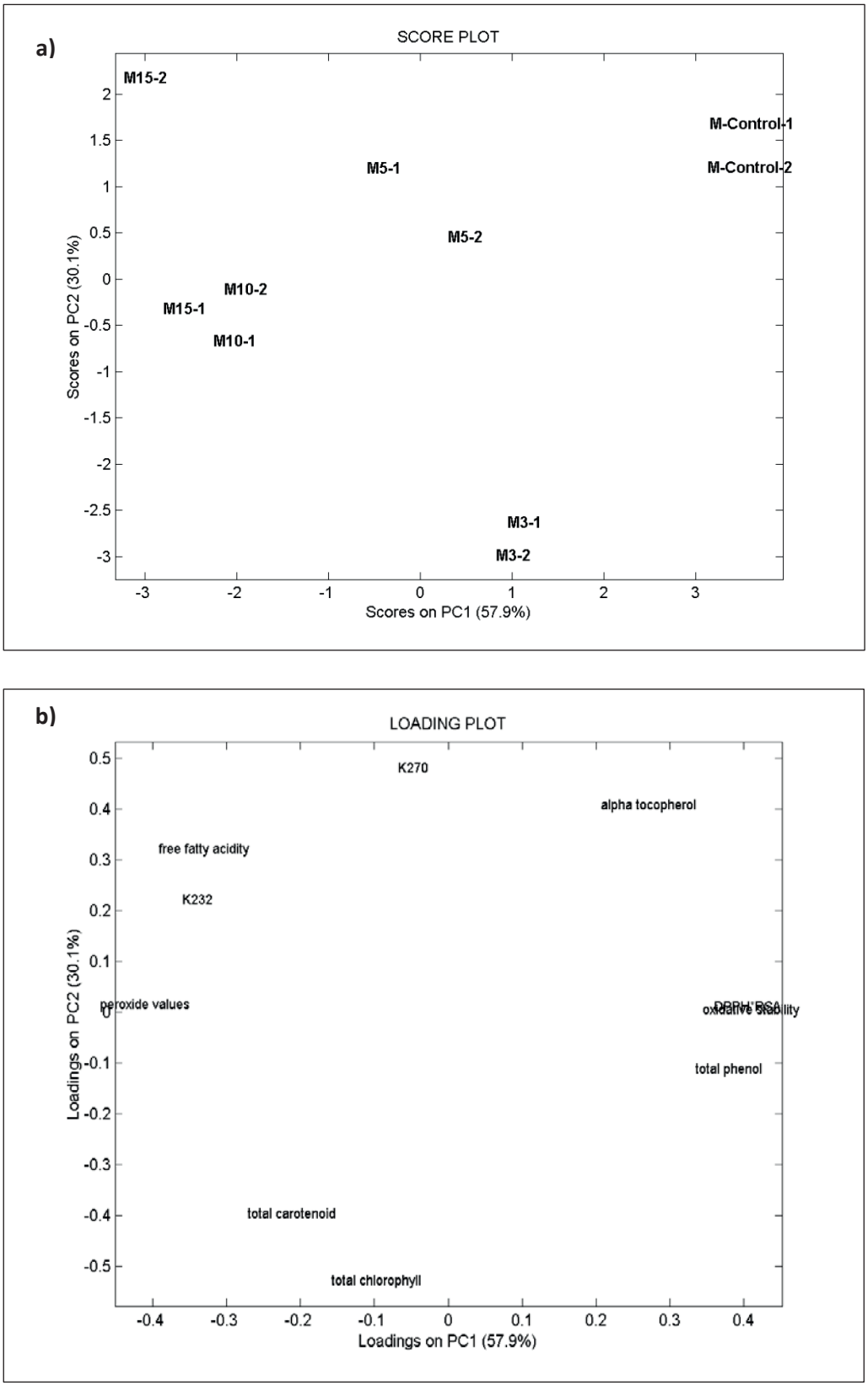

Figure 1 - a): Score plot. b): Loading plot obtained from PCA performed on quality parameters and chemical characteristics of Memecik olive oil.

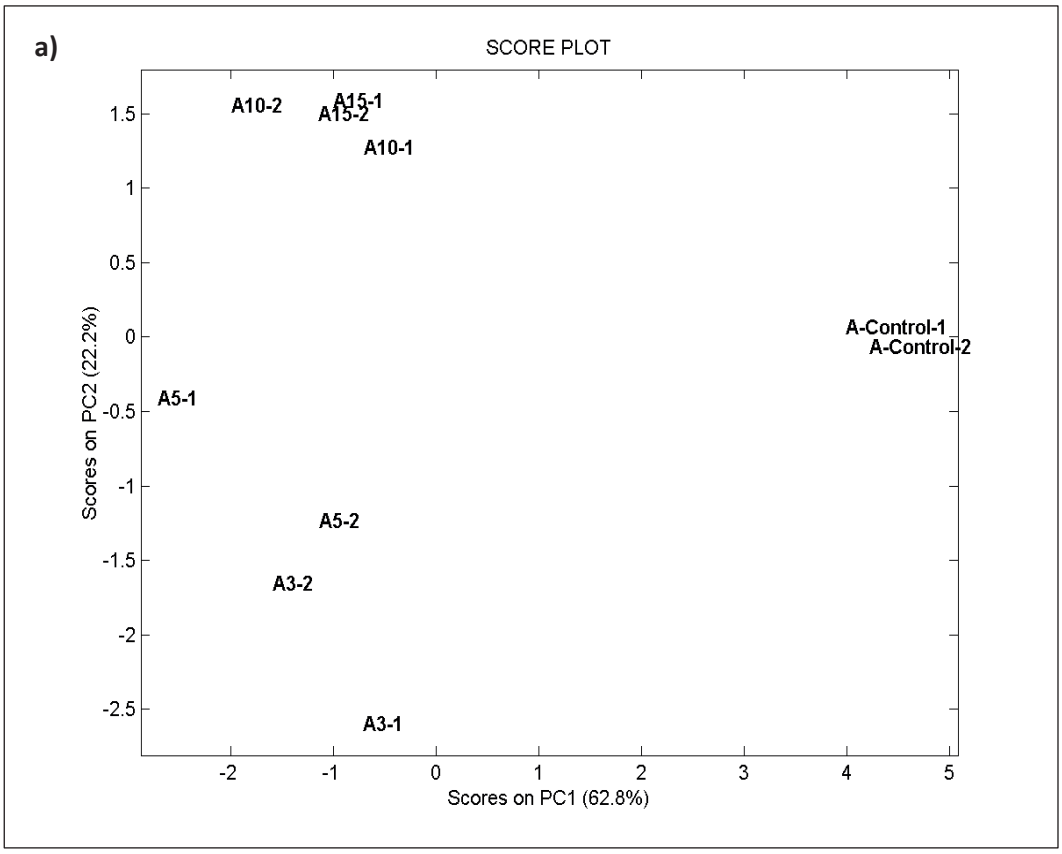

Figure 2 - a): Score plot. b): Loading plot obtained from PCA performed on quality parameters and chemical characteristics of Ayvalık olive oil.

similar considerations could be made, in fact control samples were characterised by high values of alpha tocopherol, oxidative stability, total phenols and DPPH• RSA; samples heated for 10 or 15 minutes showed positive scores on PC2 and are characterised by high values especially of total carotenoid, free fatty acids and K_{270}, but also PV and total chlorophyll. These multivariate results agreed with the univariate considerations made previously.

CONCLUSION

This study was performed to determine the impact of microwave heating with different exposure times (0, 3, 5, 10 and 15 min) on FFA, PV, ultraviolet absorbance values at 232 and 270 nm, chlorophyll and carotenoid content, α-tocopherol content, total phenolic content, oxidative stability, and DPPH• Radical Scavenging Activity of Memecik and Ayvalık olive oils. Considering the results obtained in this study, it was possible to conclude that the quality parameters utilised in this study allowed revealing the effect of the microwave heating on two Turkish olive oils. This heating treatment caused important quality and nutritional value losses in olive oils. It can be concluded that pigments such as chlorophylls and carotenoids are determined as thermal labile. According to the compiled studies, heating time should be decreased to minimum to preserve the nutritional value of virgin olive oils. Because, most of olive oils bioactive components, including phenolic compounds, are gradually loss under prolonged thermal processing. Therefore, it is preferable to add olive oil as final seasoning in fresh salads and soups etc.

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