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Analytical Methods

Effects of malaxation temperature and harvest time on the chemical characteristics of olive oils

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ABSTRACT

The aim of the study was to determine the effects of harvest time and malaxation temperature on chemical composition of olive oils produced from economically important olive varieties with a full factorial experimental design. The oils of Ayvalik and Memecik olives were extracted in an industrial two-phase continuous system. The quality parameters, phenolic and fatty acid profiles were determined. Harvest time, olive variety and their interaction were the most significant factors. Malaxation temperature was significant for hydroxytyrosol, tyrosol, p-coumaric acid, pinoresinol and peroxide value. Early and mid-harvest oils had high hydroxytyrosol and tyrosol (maximum 20.7 mg/kg) and pigment concentrations (maximum chlorophyll and carotenoids as 4.6 mg/kg and 2.86 mg/kg, respectively). Late harvest oils were characterized with high peroxide values (9.2–25 meq O_2/kg), stearic (2.4–3.1%) and linoleic acids (9.3–10.4%). Multivariate regression analysis showed that oxidative stability was affected positively by hydroxytyrosol, tyrosol and oleic acid and negatively by polyunsaturated fatty acids.

1. Introduction

The quality of virgin olive oil is equally affected by every step of production: agronomical (state of olive grove and olive fruits), technological (extraction system and malaxation conditions), and environmental (temperature and light during storage of olive oil). Use of healthy olive fruits at the time of extraction is a necessity in the production of virgin olive oils having considerable degree of chemical, nutritional and sensory characteristics. High quality raw material must be followed by the right operational choices in the production stage. Even healthy olive fruits are affected by adverse extraction conditions and result in poor quality olive oil. Type of extraction system and temperature-time combination in the malaxer, where the olives are crushed to form the oil part out of paste, are therefore significant parameters to be adjusted. Milling operation may seem to be a simple mechanical crushing process; however, it involves the act of several enzymes of olive, which play role in the overall quality of the final product (Clodoveo, 2012; Fregapane & Salvador, 2013).

A strategic choice of appropriate agronomical parameters and processing conditions of olive fruits determine the overall degree of acceptability of olive oils. The parameters to be adjusted are various. There are studies about some of the factors to evaluate their effects on the quality of olive oil. In the study of Monteleone, Caporale, Carlucci, and Pagliarini (1998), the olive ripening stage and storage, malaxation time and temperature effects on the total phenol and oxidative stability of olive oils were evaluated. In one study, the effect of three different extraction systems, maturity of olives and kneading temperature on the sterol composition was reported (Koutsaftakis, Kotsifaki, & Stefanoudaki, 1999). Ben-David et al. (2010) investigated the effect of olive type, temperature, time, talc addition and different irrigation systems for a laboratory scale mill and determined oil yield, total phenol content and free fatty acidity. In other studies, researchers especially looked for the effect of oxygen in the head-space of malaxer and processing temperature with different cultivars (Catania, Vallone, Farid, & De Pasquale, 2015; Servili, Selvaggini, Taticchi, Esposto, & Montedoro, 2003).

Nutritionally, olive oil has chemical compounds, responsible for its comparatively higher quality values than other vegetable oils (Frankel, 2011). The presence of high percentage of monounsaturated oleic acid makes olive oil much less susceptible to oxidation and contributes to high stability and long shelf life. Olive oil polyphenols, the other factor for its unique characteristics, belong to different classes based on their molecular weights and structures: phenolic acids, phenyl ethyl alcohols (hydroxytyrosol and tyrosol), flavonoids, lignans and secoiridoids are the most characterized among others. Lately, the claims related to the support of

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oleic acid and phenolics of olive oil for healthy cholesterol levels and cardiovascular system were reported (EFSA, 2011, 2012).

In this study, it was aimed to determine the effects of olive variety, harvest time and malaxation temperature on phenolics, fatty acid profiles and oxidative stability of oils. To our knowledge, there are no scientific reports related to the combined effects of these three factors on minor components and quality characteristics of olive oils obtained in an industrial two-phase system. Unsupervised and supervised multivariate analysis were used in the evaluation of the data of full-factorial design to show the effect of quality variables in the differentiation of olive oils and explanation of oxidative stability index.

2. Materials and methods

2.1. Olive oil samples

Thirty six olive oil samples were obtained from Ayvalik (A, also called Edremit) and Memecik (M) varieties, which were extracted according to a general full factorial design in a two-phase continuous olive plant (Polat Machinery, Aydin, Turkey), located at Izmir Institute of Technology. The extractions were done at three malaxation temperatures (27, 37 and 47 °C) with the olive fruits harvested at their early, mid and late maturation stages, with two replications. The olives were harvested in the west coast of Anatolia. Olives of Ayvalik variety were obtained from Edremit Bay area in north of Izmir. Memecik olives were obtained from Aydin region in south of Izmir. The maturity levels of olive fruits were determined according to IOC method (International Olive Council, 2011). Maturity index of early, mid and late harvest olives were between 1.08–2.45, 3.23–3.57, and 4.21–6.43, respectively. Olive oil samples were kept in glass containers and head spaces were flushed with nitrogen prior to refrigerated storage (4 °C). In the text, abbreviations e, m, and f are used for early, mid and late harvest olive oils, respectively.

2.2. Chemicals

Analytical grade reagents of Riedel-deHaen (Germany), Sigma-Aldrich (Germany) and Merck (Germany) were used in the analysis. Caffeic, p-coumaric, 3-hydroxyphenylacetic, 4-hydroxyphenylacetic, 2,3-dihydroxylbenzoic, ferulic and gallic acids, hydroxytyrosol, tyrosol, luteolin, vanillin and pinoresinol standards were products of Extrasynthese (France) and Fluka (Germany). Fatty acid methyl ester (FAME) mixture containing C4–C24 (Supelco #47885-U) was used as a reference standard for GC-FID analysis.

2.3. Oxidative stability index (OSI)

Automated oxidative stability test was performed using Rancimat system (873 Biodiesel, Metrohm, Switzerland). The reaction vessels containing 3 g of oil samples were covered and connected to the conductivity cells containing deionized water as volatile absorbent. The vessels were kept at 120 °C within the heating blocks. The air flow rate was maintained at 20 L/h. The time taken for conductivity to experience a sharp increase was termed as the induction time (h). The measurements were duplicated.

2.4. Total phenol content (TPC)

Total phenol content of the oil samples were determined by Folin-Ciocalteu spectrophotometric method as given in Montedoro, Servili, Baldioli, and Miniati (1992). The results were expressed in terms of gallic acid equivalent (mg GA/kg oil).

2.5. Chlorophyll & carotenoid content

A procedure given in Mínguez-Mosquera, Rejano-Navarro, Gandul-Rojas, Sanchez Gomez, and Garrido-Fernandez (1991) was used in the measurement of total chlorophyll and carotenoid contents of olive oils. The absorbances corresponding to chlorophyll (A_{670}) and carotenoids (A_{470}) were measured with a UV spectrophotometer (Shimadzu UV-2450 Tokyo, Japan) at 1.0 cm optical path (d). Chlorophyll and carotenoids were expressed in mg/kg of oil:

$$\text{Chlorophyll (mg/kg)} = (A_{670} \times 10^6)/(613 \times 100 \times d)$$

$$\text{Carotenoids (mg/kg)} = (A_{470} \times 10^6)/(2000 \times 100 \times d)$$

2.6. Peroxide value (PV) and free fatty acidity (FFA)

PV and FFA analyses were done according to European Official Method of Analysis (European Union Commission, 1991). PV was expressed as meq O_2/kg and FFA was expressed as % oleic acid.

2.7. Color

The CIE color parameters (L^*, a^* and b^*), chroma and hue (C and H) were calculated by using the standard illuminant D65 and 10° observation angle from the UV–Visible spectra of the olive oil samples (Shimadzu UV-2450, Kyoto, Japan). Transmittance was taken over the range of 400–700 nm at 120 nm/min scan speed in a plastic cell with 1.0 cm optical path length. The color parameters were calculated by the Shimadzu UVPC color analysis software (ver. 2.7). Values for each sample were obtained as the average of three replicates.

2.8. HPLC analysis of phenolic compounds

Phenolic profiles of olive oils were determined based on the procedure given in Alkan, Tokatli, and Ozen (2012). Amounts of individual phenolic compounds in olive oil were determined by an HPLC system (Agilent 1200, Santa Clara, CA, USA) equipped with photodiode array detector (DAD). A C18 column (250 mm, 4 mm, 5 μm, SGE 8211, Australia) was used in the analyses. Column temperature was maintained at 35 °C, injection volume was 20 μL and mobile phase flow rate was adjusted to 1 mL/min. Mobile phases were water/acetic acid (99.8:0.2 v/v) and methanol. Initially, the mobile phases were 90% for water/acetic acid and 10% for methanol and the concentrations were changed according to a gradient profile during 85 min. Gallic acid was used as the internal standard. Phenolic compounds were determined by using their commercial standards at two different wavelengths of 280 and 320 nm. Five-point calibration curves for each standard were plotted and the results were expressed in terms of mg/kg oil. Total phenolic acids (TPA) was defined as the summation of caffeic, p-coumaric, ferulic, 4-hydroxyphenylacetic, 3-hydroxyphenylacetic and 2,3 dihydroxylbenzoic acids.

2.9. GC analysis of fatty acids

Fatty acid methyl ester analyses were carried out according to European Official Methods of Analysis (European Union Commission, 1991). Esterified oil samples were examined with a GC-FID system (Agilent 6890, USA) including a split/splitless (1:50) injector. HP 88 capillary column (100 m * 0.25 mm * 0.2 mm, Agilent, USA) was used. Helium with 2 mL/min constant flow rate was the carrier medium. Injection volume and temperature was 1 mL and 250 °C. Oven temperature was set to 140 °C

initially and was maintained there for 10 min. Then it was increased to 220 °C with a rate of 3 °C/min and kept at this temperature for another 5 min. The detector temperature was maintained at 280 °C. Peaks in standard mixture were compared with those of samples in the chromatogram and the results were expressed as percentage of FAME. The percentages of individual fatty acids, total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA) and the ratio of oleic to linoleic acids (C18:1/C18:2) were reported.

2.10. Statistical analysis

The significance of factor effects on the chemical compounds was determined by Analysis of Variance (ANOVA) at 5% probability level (Minitab 16.0, Minitab Inc., State College, USA). In the multivariate classification and regression analysis, data matrix **X** of size (36×40) contained 36 olive oil samples (n observations) and 40 measured variables (k variables) were used in the multivariate analysis. The variables include: eleven individual phenols, total phenolic acids (TPA), summation of hydroxytyrosol and tyrosol as oleuropein derivatives (O-der), total phenol content (TPC), five quality parameters, five color parameters, eleven fatty acids, SFA, MUFA, PUFA, MUFA/PUFA and oleic to linoleic acid ratio (C18:1/C18:2). The data were analyzed first by using PCA (Principal Component Analysis) to examine the natural data clustering. As a supervised technique, discriminant analysis by orthogonal projections to latent structures (OPLS-DA) was used in the classification of oil samples, in which case Y is a user-created variable representing the classes of samples (such as 1 for early, 2 for mid and 3 for late harvest). The OPLS method provides a model which can separate the systematic variation in **X** matrix in two parts; a predictive part correlated to **Y** variable (class information in this case), an orthogonal part uncorrelated to **Y** (Galindo-Prieto, Eriksson, & Trygg, 2015). The multivariate models were described with their number of components (PC), R^2 as the total variance explained and R^2_{CV} as the total variance explained in the leave-one-out cross validation. The principal components of OPLS models were given as $P_p + P_o$, where p and o stand for the number of predictive and orthogonal components, respectively. In the OPLS models, the insignificant variables were eliminated with the variable importance plots (VIP) as a feature of the SIMCA software (ver. 13.0, Umetrics, Umea, Sweden), then the models were rebuilt with the most significant ones.

3. Results and discussion

3.1. Chemical parameters

The quality parameters and chemical compositions of oil samples are given in Tables 1–3. The most significant factors with respect to chemical composition and quality were found as olive type, maturity level (harvest time) and their interaction ($p < 0.05$), based on ANOVA results of full factorial experimental data. The malaxation temperature and its interactions, on the other hand, were found significantly effective only for some phenolic compounds such as hydroxytyrosol, tyrosol, pinoresinol, p-coumaric acid and PV, and insignificant for fatty acid compositions. The changes in OSI, TPC and linoleic acid were not significant. The results of univariate analysis in terms of p-values are given as Supplementary data (Tables S1–S3) for all chemical characteristics.

It was observed that FFA values of Ayvalik oils slightly increased with the malaxation temperature unlike Memecik oils. High temperatures do not necessarily lead to an increase in hydrolytic activity of lipase enzyme and subsequent increase in FFA. Decrease in FFA of olive oils at temperatures above 35 °C was also reported elsewhere (Boselli, Di Lecce, Strabbioli, Pieralisi, & Frega, 2009; Clodoveo, Hbaieb, Kotti, Mugnozza, & Gargouri, 2014; Panzanaro, Nutricati, Miceli, & De Bellis, 2010). Statistically, olive oils of the two varieties were significantly different in PV with Ayvalik having lower peroxide value range (7.74–17.06 meq O_2/kg) than Memecik (13.36–25.33 meq O_2/kg). Although no correlation was detected between PV and FFA values, Memecik oils generally had higher values of both parameters. The change of PV with respect to olive type and temperature was found significant (p-value <0.01 and p-value <0.05, respectively). Temperature of malaxation influenced PV of the oil as there was an increasing trend with temperature. This observation is more evident especially for the mid and late harvest Memecik oils. Carotenoid and chlorophyll pigments of the oil samples showed differences with respect to olive variety and harvest time. The findings supported the earlier reports about decreasing pigmentation with harvest time (Criado, Motilva, Goni, & Romero, 2007). Chlorophylls and carotenoids were in the ranges of 1.50–4.55 mg/kg and 1.11–2.86 mg/kg in Memecik oils and 1.28–2.57 mg/kg and 1.01–1.61 mg/kg for Ayvalik oils, respectively. The decrease in the pigment concentrations of Ayvalik oils with ripening was not as sharp as in other samples. Similarly, color parameters of oils depend mainly on harvest time and olive variety. Memecik oils increased in lightness (L^*) with harvest time as the chromatic parameters a^* and b^* decreased.

The fatty acid compositions of the olive oils were significantly affected by olive type and harvest time. Oleic acid (C18:1) content of the olive oil samples was observed in the range of 70.97–75.16%. An increase in oleic acid during olive fruit maturation was reported in another study (Beltran, Del Rio, Sanchez, & Martinez, 2004). Such trend was also observed in Memecik oils, as the oleic acid content in the early harvest was found lower than the mid and late harvest oils. Linolenic acid (C18:3) content changed significantly with respect to olive type and harvest time. Ayvalik olive oils had linolenic acid in the range of 0.57–0.72%, in which the late harvest oils had higher values. Conversely, Memecik olive oils had higher linolenic acid contents (0.72–0.94%) with no particular differences among harvests. Lipoxygenase enzyme prefers linolenic acid as one of its substrates during hydroperoxide generation (Tamborrino et al., 2014), which implies that oils of high polyunsaturated fatty acids may likely have elevated PV. This can also explain higher PV of Memecik oils. Palmitic acid (C16:0) responsible for over 90% of saturated fatty acid composition of olive oil, ranged between 11.50 and 14.87%. Ayvalik oils had significantly higher saturated fatty acids than Memecik olive oils, similar to the previous observations (Gurdeniz, Ozen, & Tokatli, 2010). As reported by Manai-Djebali et al. (2012), olive oils of high SFA contents are expected to be less prone to oxidation than those high in PUFA. However, the OSI values of olive oils were not found significantly different, even though the FFA and PV of Ayvalik oils were lower than Memecik oils.

Comparison of TPC and oleuropein derivatives as hydroxytyrosol and tyrosol is shown in Fig. 1. The decrease in total phenol content with respect to harvest time is seen in Memecik oils. There is no significant difference between hydroxytyrosol contents of oils obtained from both varieties (0.08–2.29 mg/kg Ayvalik and 0.24–2.65 mg/kg Memecik) as shown in Tables 2 and 3, but difference is highly significant with respect to harvest time and malaxation temperature. High hydroxytyrosol content of oils obtained at 47 °C is in agreement with the statement, which claimed that oleuropein degradation was enhanced during malaxation and hydrolytic enzyme β-glucosidase and esterase were released leading to the production of hydroxytyrosol and tyrosol (Taticchi et al., 2013). Protection of LDL particles from oxidative damage and maintenance of normal blood HDL-cholesterol concentration has been linked to a daily consumption of 5 mg hydroxytyrosol and/or its derivatives per 20 g of olive oil by European Food Safety Authority

Table 1
Quality parameters (mean ± SD) of Ayvalik and Memecik olive oils at three harvest times (early, mid and late) and malaxation temperatures (27 °C, 37 °C and 47 °C).

Responses	Early harvest			Mid harvest			Late harvest		
	27 °C	37 °C	47 °C	27 °C	37 °C	47 °C	27 °C	37 °C	47 °C
Ayvalik olive oils									
TPC	116.7 ± 23.0	101.3 ± 29.3	84.12 ± 0.35	94.65 ± 14.6	98.96 ± 8.3	189.8 ± 96.6	191.3 ± 111	181 ± 104	83.4 ± 3.80
FFA	1.0 ± 0.01	0.83 ± 0.0	1.60 ± 0.08	0.63 ± 0.0	0.56 ± 0.01	1.04 ± 0.31	1.96 ± 0.23	0.98 ± 0.01	1.15 ± 0.36
PV	7.74 ± 2.57	6.50 ± 0.13	15.19 ± 1.99	12.72 ± 3.01	11.35 ± 4.3	13.36 ± 3.9	17.06 ± 4.8	9.16 ± 0.01	11.93 ± 3.31
OSI	5.75 ± 0.71	6.96 ± 0.21	4.25 ± 0.57	4.60 ± 0.04	5.20 ± 0.24	5.89 ± 1.08	5.37 ± 0.25	5.20 ± 0.01	5.52 ± 0.01
Chl	2.12 ± 0.76	2.57 ± 0.31	2.39 ± 0.14	1.67 ± 0.91	1.31 ± 0.06	1.51 ± 0.11	1.30 ± 0.35	1.28 ± 0.11	1.34 ± 0.34
Car	1.43 ± 0.35	1.61 ± 0.42	1.45 ± 0.33	1.17 ± 0.31	1.01 ± 0.21	1.29 ± 0.03	1.04 ± 0.08	1.33 ± 0.31	1.05 ± 0.12
L*	87.5 ± 2.57	88.4 ± 3.08	91.1 ± 2.64	92.34 ± 0.92	94.97 ± 3.53	90.84 ± 2.09	87.14 ± 0.74	87.68 ± 0.46	87.77 ± 5.77
a*	−1.88 ± 1.24	−2.53 ± 1.05	−3.02 ± 0.11	−3.14 ± 1.12	−4.30 ± 0.18	−3.20 ± 0.24	−0.82 ± 0.61	−3.76 ± 0.24	−3.56 ± 0.98
b*	71.51 ± 12.8	72.82 ± 0.27	65.36 ± 1.77	63.17 ± 23.1	49.4 ± 0.83	59.9 ± 5.76	77.05 ± 6.02	50.32 ± 1.35	54.27 ± 2.61
C	71.54 ± 12.7	72.82 ± 0.27	65.36 ± 1.77	63.26 ± 23.0	49.58 ± 0.81	60.0 ± 5.73	77.14 ± 5.96	50.32 ± 1.34	54.39 ± 2.67
H	91.63 ± 1.28	92.0 ± 0.83	92.66 ± 0.16	93.25 ± 2.21	94.99 ± 0.30	93.09 ± 0.52	91.75 ± 1.12	94.30 ± 0.30	93.74 ± 0.85
Memecik olive oils									
TPC	154.5 ± 30.2	206.1 ± 81.8	141.0 ± 54.6	117.1 ± 18.7	124 ± 13.7	194.3 ± 10.2	115.03 ± 74.8	103.9 ± 16.3	78.45 ± 5.13
FFA	3.77 ± 1.53	1.78 ± 0.30	1.53 ± 0.14	0.87 ± 0.13	2.17 ± 1.65	1.09 ± 0.22	1.41 ± 0.53	0.79 ± 0.06	0.84 ± 0.06
PV	18.6 ± 1.41	16.86 ± 1.10	15.18 ± 0.95	13.64 ± 1.29	15.10 ± 0.59	18.81 ± 9.26	13.36 ± 0.05	18.07 ± 3.10	25.33 ± 11.9
OSI	4.29 ± 1.07	5.40 ± 0.08	6.22 ± 0.30	5.89 ± 0.46	5.25 ± 2.06	7.06 ± 0.55	5.35 ± 0.59	5.63 ± 0.63	5.50 ± 1.44
Chl	4.10 ± 0.63	3.31 ± 0.23	4.55 ± 0.73	2.72 ± 0.66	2.21 ± 0.63	2.74 ± 0.86	1.67 ± 0.06	1.50 ± 0.13	1.78 ± 0.02
Car	2.35 ± 0.53	2.38 ± 0.52	2.86 ± 0.59	2.07 ± 0.02	1.77 ± 0.64	2.14 ± 0.43	1.30 ± 0.18	1.11 ± 0.35	1.20 ± 0.17
L*	82.3 ± 2.73	82.6 ± 5.34	79.21 ± 0.34	81.1 ± 5.25	86.13 ± 1.40	83.26 ± 1.40	89.95 ± 1.32	92.33 ± 3.84	89.97 ± 4.65
a_*	0.64 ± 2.40	1.28 ± 1.10	2.05 ± 0.36	−0.51 ± 0.40	−1.24 ± 1.56	1.13 ± 0.69	−3.56 ± 0.13	−3.21 ± 0.43	−2.87 ± 0.25
b*	91.36 ± 21.1	91.28 ± 0.77	99.86 ± 1.99	83.88 ± 10.6	82.13 ± 15.1	91.64 ± 6.38	53.84 ± 3.47	55.66 ± 0.75	57.99 ± 0.60
C	91.38 ± 21.2	91.29 ± 0.76	99.89 ± 2.00	83.21 ± 10.61	82.89 ± 15.1	91.65 ± 6.38	53.96 ± 3.47	55.76 ± 0.77	58.06 ± 0.59
H	89.77 ± 1.47	89.19 ± 0.70	88.82 ± 0.18	90.39 ± 0.32	90.98 ± 1.25	89.27 ± 0.48	93.37 ± 0.49	93.30 ± 0.40	92.84 ± 0.28

SD: Standard deviation of two replicates, TPC: total phenol content (mg/kg), FFA: Free fatty acid (% Oleic acid), PV: Peroxide value (meq O_2/kg), OSI: Oxidative stability index (h), Chl: Chlorophylls (mg/kg), Car: Carotenoids (mg/kg), CIE color parameters: L* (lightness-darkness), a* (greenness-redness), b* (blueness-yellowness), C (Chroma), H (Hue angle).

Table 2
Phenolic and fatty acid profiles (mean ± SD) of Ayvalik olive oils at three harvest times (early, mid and late) and malaxation temperatures (27 °C, 37 °C and 47 °C).

Responses	Early harvest			Mid harvest			Late harvest		
	27 °C	37 °C	47 °C	27 °C	37 °C	47 °C	27 °C	37 °C	47 °C
Phenolics (mg/kg)									
Hyt	0.16 ± 0.10	0.13 ± 0.02	0.08 ± 0.01	0.56 ± 0.20	0.29 ± 0.11	2.29 ± 0.49	0.89 ± 0.14	0.28 ± 0.08	0.23 ± 0.06
Tyr	8.00 ± 0.87	6.64 ± 2.58	9.26 ± 1.53	3.46 ± 1.59	2.48 ± 0.76	5.63 ± 1.70	2.22 ± 0.42	1.19 ± 0.34	1.57 ± 0.22
4Hpa	15.4 ± 3.35	16.4 ± 3.44	11.9 ± 2.86	1.48 ± 0.28	2.66 ± 0.08	2.02 ± 0.50	3.40 ± 0.29	1.44 ± 0.13	1.34 ± 0.40
3Hpa	1.55 ± 0.87	0.86 ± 1.03	0.88 ± 0.93	0.30 ± 0.10	0.55 ± 0.40	0.65 ± 0.62	0.27 ± 0.08	0.16 ± 0.08	0.19 ± 0.12
Caf	0.81 ± 0.37	0.76 ± 0.13	0.46 ± 0.05	0.17 ± 0.03	0.11 ± 0.13	0.11 ± 0.10	0.12 ± 0.04	0.07 ± 0.02	0.07 ± 0.01
Pin	1.86 ± 0.08	0.86 ± 0.17	1.59 ± 0.23	7.30 ± 6.06	2.04 ± 0.42	4.45 ± 1.43	1.82 ± 0.58	0.82 ± 0.30	0.49 ± 0.01
Dba	0.36 ± 0.16	0.24 ± 0.01	0.19 ± 0.08	0.11 ± 0.05	0.15 ± 0.04	0.18 ± 0.00	0.12 ± 0.06	0.07 ± 0.00	0.10 ± 0.01
Vnl	0.19 ± 0.06	0.17 ± 0.06	0.19 ± 0.21	0.18 ± 0.12	0.14 ± 0.06	0.15 ± 0.06	0.08 ± 0.05	0.07 ± 0.03	0.06 ± 0.01
pCu	0.77 ± 0.05	0.49 ± 0.19	0.40 ± 0.06	0.41 ± 0.21	0.18 ± 0.02	0.54 ± 0.09	0.36 ± 0.16	0.10 ± 0.02	0.14 ± 0.01
Fer	0.21 ± 0.08	0.18 ± 0.01	0.13 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.03	0.05 ± 0.01	0.02 ± 0.00	0.03 ± 0.01
Lut	4.75 ± 0.93	3.65 ± 0.27	4.00 ± 0.51	5.24 ± 0.88	4.08 ± 1.07	3.47 ± 0.67	2.74 ± 0.95	2.28 ± 0.26	1.39 ± 0.04
O-der	8.16 ± 0.97	6.76 ± 2.60	9.34 ± 1.53	4.02 ± 1.79	2.77 ± 0.87	7.91 ± 1.22	3.11 ± 0.28	1.47 ± 0.25	1.80 ± 0.28
TPA	19.13 ± 3.13	18.94 ± 4.55	13.94 ± 1.76	2.52 ± 0.52	3.69 ± 0.16	3.57 ± 1.14	4.30 ± 0.52	1.84 ± 0.10	1.85 ± 0.57
Fatty acids (%)									
C16:0	13.63 ± 0.17	13.74 ± 0.35	14.87 ± 0.74	12.68 ± 0.42	13.06 ± 0.04	12.08 ± 0.24	12.46 ± 0.29	12.10 ± 0.03	11.87 ± 0.41
C16:1	0.81 ± 0.11	0.82 ± 0.06	0.82 ± 0.03	0.72 ± 0.06	0.75 ± 0.03	0.58 ± 0.10	0.64 ± 0.02	0.66 ± 0.03	0.67 ± 0.01
C17:0	0.11 ± 0.03	0.11 ± 0.04	0.12 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.11 ± 0.07	0.15 ± 0.02	0.15 ± 0.02	0.14 ± 0.02
C17:1	0.19 ± 0.04	0.19 ± 0.06	0.20 ± 0.03	0.22 ± 0.01	0.21 ± 0.02	0.16 ± 0.07	0.20 ± 0.02	0.20 ± 0.02	0.20 ± 0.02
C18:0	2.17 ± 0.30	2.17 ± 0.24	2.31 ± 0.11	2.60 ± 0.03	2.55 ± 0.13	2.41 ± 0.98	3.08 ± 0.03	3.02 ± 0.11	2.96 ± 0.26
C18:1	72.75 ± 1.46	72.81 ± 0.69	71.99 ± 0.85	71.87 ± 0.54	72.34 ± 0.25	72.80 ± 1.53	73.35 ± 2.30	71.99 ± 0.47	72.29 ± 0.80
C18:2	9.08 ± 0.41	8.78 ± 0.28	8.36 ± 0.29	10.41 ± 1.31	9.55 ± 0.05	10.58 ± 0.48	10.41 ± 0.23	10.27 ± 0.04	10.23 ± 0.08
C18:3	0.61 ± 0.03	0.62 ± 0.07	0.59 ± 0.03	0.61 ± 0.02	0.59 ± 0.02	0.57 ± 0.17	0.69 ± 0.01	0.72 ± 0.04	0.72 ± 0.02
C20:0	0.38 ± 0.11	0.38 ± 0.10	0.44 ± 0.04	0.44 ± 0.05	0.45 ± 0.03	0.39 ± 0.20	0.52 ± 0.01	0.51 ± 0.03	0.50 ± 0.04
C20:1	0.22 ± 0.12	0.26 ± 0.09	0.27 ± 0.05	0.27 ± 0.05	0.29 ± 0.03	0.20 ± 0.17	0.30 ± 0.02	0.31 ± 0.04	0.32 ± 0.03
C22:0	0.07 ± 0.10	0.16 ± 0.03	0.07 ± 0.10	0.07 ± 0.10	0.09 ± 0.07	0.16 ± 0.02	0.13 ± 0.02	0.08 ± 0.11	0.11 ± 0.06
SFA	16.36 ± 0.72	16.56 ± 0.70	17.81 ± 1.01	15.93 ± 0.62	16.28 ± 0.22	15.15 ± 1.47	16.33 ± 0.20	15.86 ± 0.29	15.59 ± 0.80
MUFA	73.97 ± 1.18	74.08 ± 0.49	73.27 ± 0.73	73.08 ± 0.67	73.59 ± 0.17	73.74 ± 1.19	74.49 ± 2.24	73.16 ± 0.37	73.48 ± 0.73
PUFA	9.69 ± 0.44	9.39 ± 0.21	8.95 ± 0.26	11.02 ± 1.32	10.15 ± 0.02	11.16 ± 0.30	11.09 ± 0.21	10.99 ± 0.08	10.95 ± 0.06
C18:1/C18:2	8.02 ± 0.52	8.30 ± 0.19	8.61 ± 0.20	6.96 ± 0.92	7.57 ± 0.01	6.88 ± 0.16	7.05 ± 0.07	7.01 ± 0.07	7.07 ± 0.02
MUFA/PUFA	7.64 ± 0.47	7.89 ± 0.12	8.18 ± 0.16	6.68 ± 0.86	7.25 ± 0.00	6.61 ± 0.07	6.71 ± 0.07	6.66 ± 0.08	6.71 ± 0.03

SD: Standard deviation of two replicates, Hyt: Hydroxytyrosol, Tyr: Tyrosol, 4Hpa: 4-hydroxyphenyl acetic acid, 3Hpa: 3-hydroxyphenyl acetic acid, Caf: caffeic acid, Pin: Pinoresinol, Dba: 2,3dihydroxylbenzoic acid, Vnl; vanillin, pCu: p-Coumaric acid, Fer: Ferulic acid, Lut; Luteolin, O-der: sum of tyrosol and hydroxytyrosol, TPA: Total phenolic acids, C16:0: Palmitic acid, C16:1: Palmitoleic acid, C17:0: Margaric acid, C17:1: Cis-10-heptadecanoic acid, C18:0: Stearic acid, C18:1: Oleic acid, C18:2: Linoleic acid, C18:3: Linolenic acid, C20:0: Arachidic acid, C20:1: Cis-11-Eicosenoic acid, C22:0: Behenic acid, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.

Table 3
Phenolic and fatty acid profiles (mean ± SD) of Memecik olive oils at three harvest times (early, mid and late) and malaxation temperatures (27 °C, 37 °C and 47 °C).

Responses	Early harvest			Mid harvest			Late harvest		
	27 °C	37 °C	47 °C	27 °C	37 °C	47 °C	27 °C	37 °C	47 °C
Phenolic profile (mg/kg)									
Hyt	0.28 ± 0.01	0.18 ± 0.18	0.72 ± 0.40	0.77 ± 0.53	1.16 ± 1.01	2.65 ± 0.88	0.24 ± 0.13	0.38 ± 0.15	0.36 ± 0.12
Tyr	2.90 ± 0.18	10.17 ± 0.62	14.0 ± 1.73	12.4 ± 5.92	6.64 ± 2.60	18.0 ± 2.01	2.49 ± 1.24	3.61 ± 1.40	3.50 ± 0.85
4Hpa	4.88 ± 2.78	2.94 ± 1.44	6.24 ± 0.82	3.39 ± 1.54	6.32 ± 3.70	3.13 ± 0.64	2.89 ± 2.99	0.91 ± 0.05	1.01 ± 0.26
3Hpa	0.21 ± 0.00	0.21 ± 0.10	0.20 ± 0.04	0.15 ± 0.04	0.16 ± 0.02	0.14 ± 0.10	0.22 ± 0.03	0.17 ± 0.11	0.48 ± 0.54
Caf	0.33 ± 0.18	0.30 ± 0.08	0.40 ± 0.06	0.17 ± 0.06	0.26 ± 0.07	0.13 ± 0.03	0.13 ± 0.11	0.08 ± 0.03	0.09 ± 0.04
Pin	12.54 ± 1.48	10.89 ± 2.18	22.3 ± 2.45	4.74 ± 0.67	8.98 ± 2.11	7.75 ± 1.14	4.29 ± 1.24	4.74 ± 0.12	4.35 ± 0.67
Dba	0.12 ± 0.08	0.12 ± 0.00	0.31 ± 0.06	0.10 ± 0.01	0.16 ± 0.01	0.12 ± 0.04	0.08 ± 0.03	0.09 ± 0.01	0.07 ± 0.09
Vnl	0.06 ± 0.02	0.08 ± 0.01	0.12 ± 0.00	0.20 ± 0.18	0.16 ± 0.06	0.11 ± 0.01	0.04 ± 0.03	0.05 ± 0.01	0.08 ± 0.01
pCu	0.75 ± 0.25	2.05 ± 0.75	2.89 ± 1.17	0.42 ± 0.02	0.53 ± 0.16	0.87 ± 0.23	0.11 ± 0.13	0.10 ± 0.01	0.19 ± 0.06
Fer	0.17 ± 0.13	0.19 ± 0.10	0.27 ± 0.12	0.06 ± 0.01	0.10 ± 0.01	0.08 ± 0.03	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Lut	4.55 ± 0.87	6.64 ± 1.47	5.57 ± 0.49	0.72 ± 0.66	2.86 ± 0.16	2.88 ± 0.26	3.05 ± 1.44	3.08 ± 0.40	2.43 ± 0.25
O-der	3.18 ± 0.16	10.35 ± 0.43	14.72 ± 1.20	13.13 ± 5.52	7.79 ± 3.61	20.65 ± 1.12	2.73 ± 1.38	3.99 ± 1.25	3.86 ± 0.97
TPA	6.45 ± 3.42	5.81 ± 2.47	10.29 ± 2.28	4.28 ± 1.61	7.51 ± 3.61	4.47 ± 1.07	3.45 ± 3.24	1.35 ± 0.22	1.84 ± 0.49
Fatty acid profile (%)									
C16:0	12.96 ± 0.18	13.47 ± 1.10	13.32 ± 0.52	12.23 ± 0.37	11.50 ± 0.41	11.60 ± 0.08	11.80 ± 0.25	11.97 ± 0.04	11.79 ± 0.09
C16:1	0.76 ± 0.05	0.84 ± 0.06	0.88 ± 0.00	0.81 ± 0.00	0.54 ± 0.29	0.74 ± 0.02	0.85 ± 0.05	0.88 ± 0.01	0.87 ± 0.04
C17:0	0.10 ± 0.00	0.09 ± 0.01	0.09 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
C17:1	0.15 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.09 ± 0.03	0.11 ± 0.05	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.00	0.06 ± 0.00
C18:0	2.35 ± 0.10	2.28 ± 0.02	2.50 ± 0.07	2.69 ± 0.06	2.40 ± 0.11	2.36 ± 0.04	2.45 ± 0.10	2.41 ± 0.04	2.38 ± 0.02
C18:1	71.63 ± 0.36	70.97 ± 0.18	71.55 ± 0.00	74.37 ± 0.11	72.73 ± 3.53	75.16 ± 0.38	72.89 ± 0.38	73.13 ± 0.13	73.88 ± 0.31
C18:2	10.51 ± 0.92	10.47 ± 1.43	9.72 ± 0.57	8.16 ± 0.46	11.26 ± 4.38	8.57 ± 0.56	10.17 ± 0.09	9.78 ± 0.03	9.26 ± 0.03
C18:3	0.76 ± 0.17	0.91 ± 0.04	0.94 ± 0.01	0.75 ± 0.02	0.78 ± 0.06	0.72 ± 0.02	0.87 ± 0.04	0.90 ± 0.02	0.89 ± 0.06
C20:0	0.42 ± 0.03	0.43 ± 0.02	0.48 ± 0.01	0.44 ± 0.00	0.36 ± 0.06	0.39 ± 0.01	0.44 ± 0.02	0.44 ± 0.01	0.43 ± 0.02
C20:1	0.29 ± 0.02	0.31 ± 0.00	0.31 ± 0.01	0.29 ± 0.00	0.26 ± 0.03	0.28 ± 0.02	0.33 ± 0.02	0.34 ± 0.02	0.33 ± 0.03
C22:0	0.11 ± 0.01	0.12 ± 0.00	0.12 ± 0.01	0.11 ± 0.00	0.05 ± 0.07	0.09 ± 0.01	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.00
SFA	15.94 ± 0.32	16.39 ± 1.15	16.51 ± 0.59	15.54 ± 0.32	14.38 ± 0.62	14.49 ± 0.13	14.86 ± 0.37	14.97 ± 0.11	14.76 ± 0.09
MUFA	72.84 ± 0.41	72.25 ± 0.22	72.86 ± 0.00	75.56 ± 0.14	73.65 ± 3.80	76.25 ± 0.41	74.15 ± 0.32	74.41 ± 0.09	75.15 ± 0.24
PUFA	11.27 ± 0.75	11.38 ± 1.38	10.66 ± 0.55	8.90 ± 0.44	12.03 ± 4.44	9.30 ± 0.54	11.04 ± 0.05	10.68 ± 0.01	10.15 ± 0.11
C18:1/C18:2	6.84 ± 0.63	6.84 ± 0.95	7.37 ± 0.43	9.13 ± 0.53	7.06 ± 3.06	8.79 ± 0.62	7.17 ± 0.03	7.48 ± 0.01	7.98 ± 0.08
MUFA/PUFA	6.48 ± 0.47	6.40 ± 0.79	6.84 ± 0.36	8.50 ± 0.43	6.63 ± 2.76	8.22 ± 0.52	6.72 ± 0.00	6.96 ± 0.00	7.41 ± 0.11

SD: Standard deviation of two replicates, Hyt: Hydroxytyrosol, Tyr: Tyrosol, 4Hpa: 4-hydroxyphenyl acetic acid, 3Hpa: 3-hydroxyphenyl acetic acid, Caf: caffeic acid, Pin: Pinoresinol, Dba: 2,3dihydroxylbenzoic acid, Vnl; vanillin, pCu: p-Coumaric acid, Fer: Ferulic acid, Lut; Luteolin, O-der: sum of tyrosol and hydroxytyrosol, TPA: Total phenolic acids, C16:0: Palmitic acid, C16:1: Palmitoleic acid, C17:0: Margaric acid, C17:1: Cis-10-heptadecanoic acid, C18:0: Stearic acid, C18:1: Oleic acid, C18:2: Linoleic acid, C18:3: Linolenic acid, C20:0: Arachidic acid, C20:1: Cis-11-Eicosenoic acid, C22:0: Behenic acid, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.

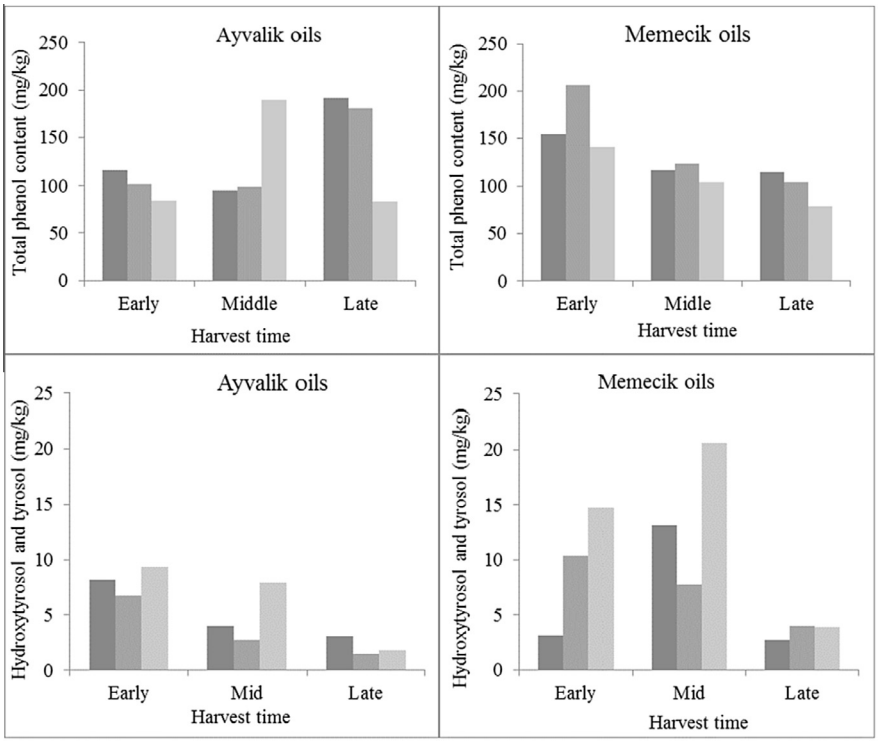

Fig. 1. Changes in the total phenol content (TPC) and the sum of hydroxytyrosol and tyrosol (O-der) of Ayvalik and Memecik olive oils with respect to malaxation temperatures (27 ■, 37 ■, 47 ■ °C).

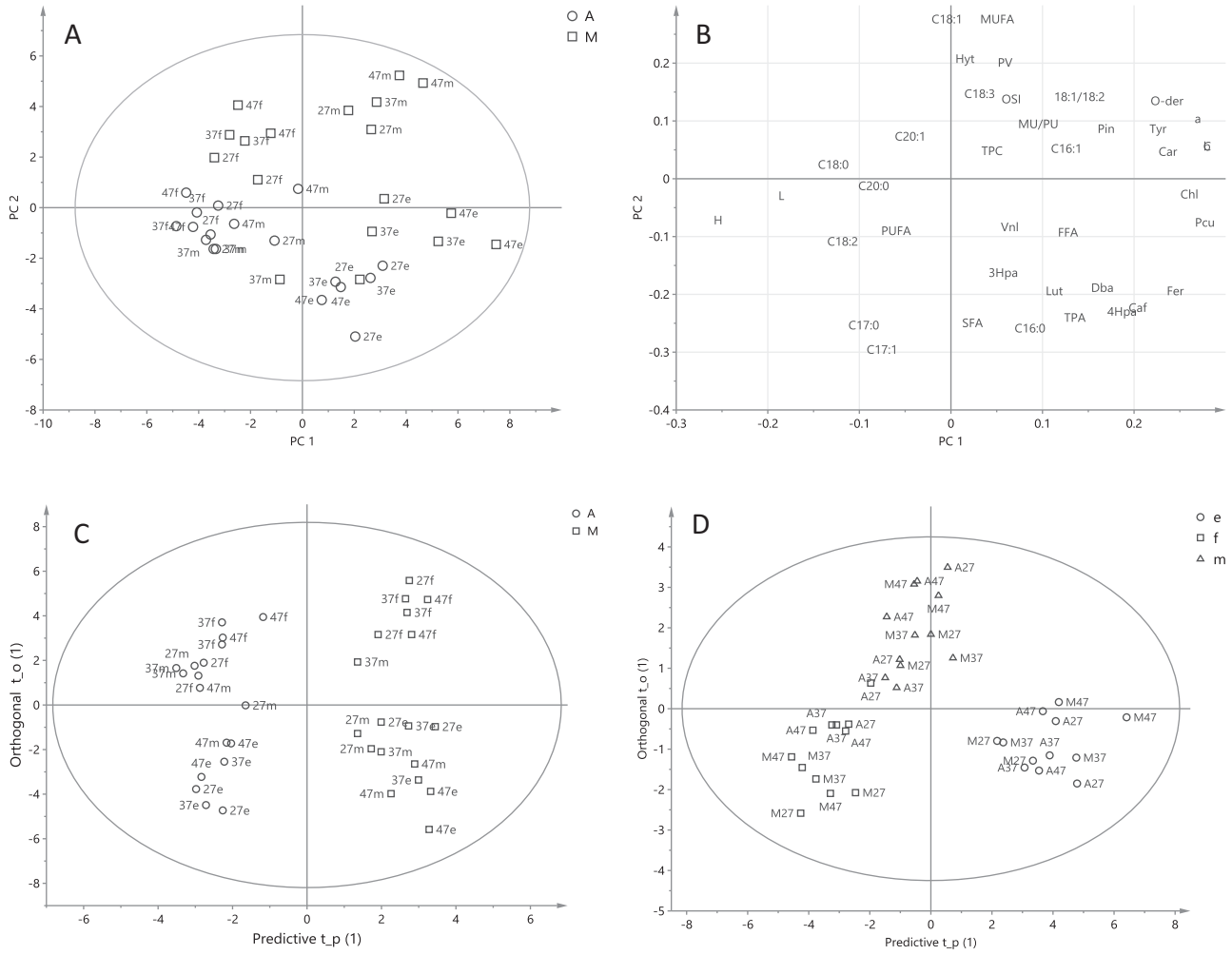

Fig. 2. Results of multivariate models: (A) Score plot of PCA model, (B) Loading plot of PCA model; (C) Score plot of OPLS-DA model of varietal classification, Memecik (□) and Ayvalik (○); (D) Score plot of OPLS-DA model of harvest time classification, early (○), mid (△) and late (□).

(EFSA, 2012). Higher amounts of oleuropein derivatives (sum of hydroxytyrosol and tyrosol) in oils were found in early and mid-harvest olives of both varieties (Tables 2 and 3). Tyrosol appeared to be more abundant than hydroxytyrosol in the olive oil samples. Tyrosol of Ayvalik oils linearly decreased with harvest time with the late harvest having less than 20% of the initial value. Memecik oils of early and mid-harvest had higher tyrosol contents in comparison to the oils of late harvest, with its value ranging between 2.49 and 18.0 mg/kg, almost twice that of Ayvalik (1.19–9.26 mg/kg). This finding implies that Ayvalik and Memecik olives obtained within early to mid-harvest, and processed at 47 °C are expected to contain higher amounts of tyrosol. Pinoresinol is an important phenolic compound belonging to the group called lignans. According to Rigane, Ayadi, Boukhris, Sayadi, and Bouaziz (2013), lignans and its derivatives are the main phenolics of olive seed. They might be present in the oil due to the breaking of pits during crushing (Cecchi et al., 2013). Pinoresinol is the most abundant phenolic compound in Memecik olive oil samples (Table 3). There was a decline in the amount of pinoresinol in Memecik oils from early harvest to late, whereas no significant change was observed in the Ayvalik oils. Pinoresinol was significantly affected by the main factors and their interactions. In the study of Taticchi et al. (2013), olive oils of different varieties were different in their pinoresinol contents, however, no significant difference was detected with respect to malaxation temperature. Studies show that olive drupes are rich in luteolin, which may exist in the form of luteolin and luteolin-7-glucosides. The presence of luteolin in the hydrophobic oil phase may be explained by the amphiphilic nature of its glucoside derivatives (Bendini et al., 2007). Luteolin was significantly affected by all factors and their interactions. The highest values of luteolin were observed at mid-harvest for Ayvalik and early harvest for Memecik oils. Luteolin content of oil of early harvest Memecik was more than twice that of mid and late harvest. Unlike another report about the relative stability of flavones and lignans with the malaxation temperature (Boselli et al., 2009), Ayvalik oils obtained at 27 °C was significantly higher in luteolin compared to oils obtained at malaxation temperatures of 37 and 47 °C. Memecik oils obtained at 37 °C had better luteolin content in all the harvest seasons. Total phenolic acids (TPA) of oil samples was significantly affected by variety, harvest time and combined effect of both. Ayvalik oils had higher TPA (1.86–19.13 mg/kg) than Memecik oils (1.43–10.38 mg/kg).

3.2. Multivariate analysis

Statistical models built with the data of quality parameters, phenolic compounds and fatty acids were used to highlight the differences/similarities among oil samples with respect to the significant factors. According to a PCA model with 5 PCs, R^2 of 0.81 and R^2_{CV} of 0.53, the distribution patterns of oils is clearly with respect to

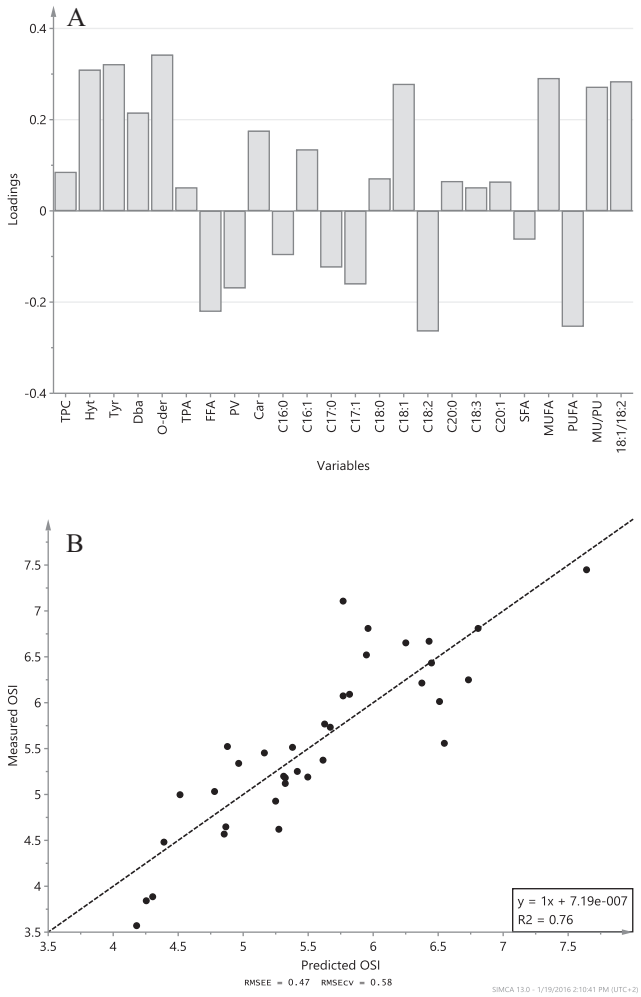

Fig. 3. Prediction results of OSI with OPLS model. The loading weights of chemical variables of the regression model (A) and scatter plot of predicted to measured values (B). RMSEE: root mean square error of estimation; $RMSE_{CV}$: root mean square of the cross validation.

harvest time and olive variety rather than malaxation temperature (Fig. 2A). The most recognizable pattern in this unsupervised model is that the early harvest Ayvalik oils are different than mid and late harvest oils. Early harvest Ayvalik oils are mainly defined by their comparatively higher amount of phenolic acids and saturated fatty acids according to the loading plot (Fig. 2B). In case of Memecik oils, the early harvest oils show similar characteristics as Ayvalik oils of the same harvest, however mid harvest Memecik oils have differences from other olive oils as they are clustered in the upper right quarter in the score plot (Fig. 2A). The presence of vanillin and phenolic acids such as 4Hpa, 3Hpa, caffeic, ferulic, and Dba can contribute to stability and nutritional quality of Ayvalik oils. Even though phenolic acids can be relatively less potent to oxidative stability compared to oleuropein derivatives (hydroxytyrosol, tyrosol etc.) and secoiridoids compounds, they can form a protective action against to oxidation (Servili et al., 2013). The lower linolenic acid content of early and mid-harvest Ayvalik oils can be considered as another factor for the stability. The oxidative stability parameters and health implicative variables such as phenolic compounds, pigments, oxidative stability index, and also degree of saturation are all localized at the right part of the loading plot corresponding to the characteristics of early or mid-harvest of both olive varieties. The late harvest oil samples located in the upper part of the control ellipse can be characterized with certain fatty acids such as linoleic, stearic and arachidic acids and higher PV.

An OPLS-DA model with 1 + 2 PCs, R^2 of 0.945 and R^2_{CV} of 0.855 was built to show the classification of oils with respect to cultivar. As can be seen in the score plot of the model, Ayvalik and Memecik oil samples are clustered in different parts of the control ellipse (Fig. 2C). In case of Memecik oils, the early and mid-harvest oils separated themselves in the lower right quarter of the ellipse with their typical properties such as higher contents of tyrosol, pinoresinol, p-coumaric acid, color pigments and different color characteristics. The oxidative stability of Memecik oils, despite their significant high peroxide values, free fatty acids, and linolenic acids content, can be explained by the defensive effect of phenols against lipid oxidation. The classification of olive oils with respect to harvest time is shown in the score plot of OPLS-DA model with 2 + 2 PCs, R^2 of 0.82 and R^2_{CV} of 0.62 (Fig. 2D). Early harvest olive oils were distantly located from other oils. And yet, the mid and late harvest olive oils formed clusters of their own. There is no differentiation of malaxation temperatures in the clusters of two models (Fig. 2C and D). In terms of high phenolic content, pigment concentration and stability (high OSI, low PV and FFA), early harvest oils of both varieties and Memecik oils from mid-harvest olives showed similar characteristics irrespective of malaxation temperature between 27 and 47 °C. The comments on the high malaxation temperature of 47 °C are limited to this statement, since sensory analysis was not performed on the olive oils.

In order to explain the effects of chemical and quality parameters on oxidative stability, a regression model for OSI was built with OPLS technique. According to VIP values of the model, chlorophyll content, pinoresinol, vanillin, 3Hpa, 4Hpa and C18:0 were not effective in modelling of OSI and removed from the data set (VIP less than 0.3). The OPLS model was rebuilt with the remaining variables. The loadings of predictive component of the model with 1 + 2 PCs, R^2 of 0.76 and R^2_{CV} of 0.6 are given in Fig. 3 (loadings bar plot and regression plot for OSI). With respect to their weights in the regression model, total concentration of hydroxytyrosol and tyrosol (O-der), the ratio of oleic/linoleic acids and oleic acid have positive effects on OSI of olive oils. The high concentrations of free fatty acid, peroxide value, linoleic acid, and total polyunsaturated fatty acids have adverse effects on the stability. It was also observed that high total phenol content did not necessarily cause stability, but rather carotenoids and individual phenolic substances such as hydroxytyrosol and tyrosol did. The significant effects of hydroxytyrosol and total hydroxytyrosol and tyrosol concentrations on the oxidative stability were reported by other researchers (Allalout et al., 2009; Uncu & Ozen, 2015).

4. Conclusion

The degree of maturation as early, mid and late harvest olives was the most significant factor affecting chemical and quality profiles of olive oils from Ayvalik and Memecik varieties. The malaxation temperatures between 27 and 47 °C were found significant on the changes of hydroxytyrosol, tyrosol, pinoresinol, p-coumaric acid contents and peroxide values. It was also observed that the interaction between olive variety and harvest time was significant, that is the oils of different types had different characteristics with respect to harvest time. As a result of multivariate classification models, it was concluded that early and mid-harvest Memecik oils showed similar properties, and early harvest Ayvalik oils had significantly different chemical qualities than its mid and late harvest oils. The olive oils with high oxidative stability and nutritional quality could be produced from early harvest olives even at high temperatures up to 47 °C.

Oxidative stabilities of oil samples were investigated with a multivariate regression model (OPLS) to determine the effect of chemical compositions. The sum of hydroxytyrosol and tyrosol concentrations, carotenoids and oleic acid/linoleic acid ratio had positive effects on the stability; high free fatty acid, peroxide value and linoleic acid contents might have caused a decrease on the stability parameter. Total phenol and chlorophyll contents were not found effective on the oxidative stability index.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data (the results of ANOVA as p-values of factors) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.05.134.

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