Analysis of olive oil for authentication and shelf life determination

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Abstract High-field proton (¹H) nuclear magnetic resonance (NMR) spectroscopy was applied for screening the fatty acyl contents of 33 olive oil samples from five different geographical regions of Turkey to do geographical discrimination analysis. The ¹H NMR data were subjected to principal component analysis (PCA) and analysis of variance (ANOVA) to discriminate the origin of olive oils. The combination of discriminative screening by ¹H NMR and ANOVA did not require further analysis of olive oil such as gas chromatography (GC). ANOVA was employed for samples from three regions separately: Marmara Region (MR), Aegean Region (AR), and Mediterranean Region (MeR). The best discriminative parameter was the ¹H NMR signal of linoleoyl acyl content. Applying both quantitative NMR and statistical analysis indicated that samples from different provinces within each geographical region (MR, AR, and MeR) could be discriminated. Comparing the linoleoyl and oleoyl acyl contents of Turkish olive oils with these of some Mediterranean olive oils showed the possibility of distinguishing the Turkish olive oils. ¹H NMR spectra of three olive oil samples with the production years of January 2013, January 2014, and January 2015 were acquired in 2016 for discussing shelf life of olive oil by quantifying fatty acyls content, and

determining minor constituents and possible by-products during storage. The effects of altitude and soil quality on fatty acyl contents of olive oil samples from a small olive growing village in Northeastern Turkey were also tested.

Keywords Olive oil authentication · Fatty acids · Minor constituents · Quantitative NMR · Chemometrics · Shelf life determination

Introduction

Olive oil is a genuine fruit juice with excellent nutritional, sensory and functional quality obtained from the fruit of *Olea europea* L. by mechanical or physical procedures. Olive oil contains more than 98% triglycerides responsible for its nutritional and physico-chemical properties and 1–2% of minors such as sterols, squalene, and α -tocopherol, the unsaponifiable fraction (Alonso-Salces et al. 2010). The chemical composition of olive oil may change due to climate, genetics of the olive tree, agronomic factors, and the practice of farmers (Gurdeniz et al. 2008). The consumption of olive oil increases in the daily human diet due to its positive health effects (Manai-Djebali et al. 2012; Reddy et al. 2015). Therefore, there are regulations that define olive oil genuineness and quality grade (Sacchi et al. 1998). One of the other important aspects of olive oil is its shelf life determination. The quality level of food samples is directly related to acceptability limit, and upon reaching this limit the product defined as unsuitable for consumption due to different factors such as nutritional or sensory criticisms. Shelf life is considered as the finite length of time after food production and packaging needed to reach this acceptability level under well-defined storage conditions (Nicoli 2012). Because fats and oils decompose into

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different oxidation by-products over time, there is an increasing interest in determining shelf life of olive oil and studying the occurrence of the chemical process of the oxidation into by-products. Most olive oil producers and researchers consider 1–1.5 years as the maximum commercial storage period from bottling to consumption, depending on the varietal composition of the olive oil (Krichene et al. 2015). However, there is still necessity for determining shelf life of olive oil due to its not only economic importance but also significance for human health.

A significant act of legislation, the Protected Designation of Origin (PDO) allows the identification of some Mediterranean extra virgin olive oils with the names of their origins [Council Regulation (EEC) No. 2081/92, 1992] (Council Regulation 1992). For this reason, in order to contribute to the efforts of authentication studies, there is a need for analyzing olive oil samples from different countries such as Turkey.

Due to such international requirements, there are several analytical protocols developed for the analysis of olive oil. Among various methods, spectroscopy has recently emerged as an important tool. One spectroscopic technique employed in olive oil analysis is high-resolution NMR (Alonso-Salces et al. 2010). A typical ^1H NMR spectrum of an olive oil sample discriminates only classes of fatty acyls with 0, 1, 2 or 3 double bonds (Sacchi et al. 1997). The main advantages of NMR are high precision, short analytical time, and easy sample preparation (Sacchi et al. 1996). The NMR technique, unlike traditional methods, permits analyzing olive oils without solvent extraction or chemical treatment. The fatty acyl composition at fatty acyl class level can be determined analyzing the integral or the intensity of properly selected resonances (Vigli et al. 2003). Quantitative NMR offers a unique and critical overview of the analyte, whether the analyte has biological, nutritional, pharmacological, or toxicological importance (Pauli et al. 2005).

The first goal inhere is fast compositional screening of thirty-three olive oils from five different olive growing geographical regions of Turkey by ^1H NMR spectroscopy. The olive growing regions in Turkey are Marmara (MR), Aegean (AR), Mediterranean (MeR), Southeastern (SeR), and Black Sea (BSR). Upon analyzing and integrating the peaks in the ^1H NMR spectra of the olive oil samples, the most discriminative NMR signals were determined by PCA and ANOVA analysis in order to differentiate and authenticate the origin of the samples. The second aim is chemical stability determination of three olive oil samples obtained from the region of İzmir–Kemalpaşa in three consecutive years 2013, 2014, and 2015 in order to focus on olive oil shelf life. Two important aspects of these three olive oils in contributing to the possible shelf life of olive oil were discussed: (1) quantification of fatty acyls, (2)

determination of minor constituents and possible by-products during storage. The effects of altitude and soil quality on contents of olive oil samples from the same village were also tested. Thus, we aim to show the continuation of our efforts in analyzing olive oils from Turkey with respect to previous harvest seasons (Gurdeniz et al. 2008; Ok 2014) and to compare our results with the fatty acyl contents of olive oil samples from different countries.

Materials and methods

Chemicals and olive oils

Deuterated chloroform ($\text{CHCl}_3\text{-d}$), 99.8% atom% D stabilized with 0.5 wt% silver foil, was purchased from Aldrich. All olives were harvested by hand during the period November 2011 through-February 2012 and processed via mechanical techniques without any chemical treatment to olive oil by local producers. The three samples of İzmir–Kemalpaşa were products of January 2013, January 2014, and January 2015. The fatty acyl content of the olive oil sample from İzmir–Kemalpaşa produced in January 2011 was published previously (Ok 2014). The olive oils were “virgin” oils because they were not refined or further processed upon production. Olive oils were obtained from local producers in several cities of Turkey (Table 1). The olive oils were kept in dark at 18 °C. Prior to and during analysis, all the samples were protected from light and maintained at a constant temperature. The NMR experiments were conducted as soon as the olive oils were received.

Analytical methods

NMR spectroscopy

Olive oils (150 μl) were placed into 5 mm NMR tubes and dissolved in chloroform- d (0.7 ml), followed by sealing the NMR tube. ^1H NMR data were acquired on a VARIAN 500 MHz INOVA instrument with a multi-nuclei 5 mm solution probe (ID-PFG). The ^1H NMR spectra were acquired with recycle delay time of 10 s, long enough for quantitative analysis, and with 32 repetitions. The proton spectra were internally referenced to the signal of the solvent at 7.25 ppm.

Data analysis

The quantitative analysis of the region between 0 and 4 ppm in ^1H NMR spectra of olive oil samples is done referring to the reports of Sacchi et al. (1996, 1997). The resonances at 0.98 ppm (E), 0.88 ppm (F), 2.01 ppm (C),

Table 1 Percentage fraction of Ln (18:3), L (18:2), O (18:1), and S in olive oils from different regions, towns and villages of Turkey

Region (S#)	City	Town	Village	% fraction of acids in olive oils			
				Ln (18:3)	L (18:2)	O (18:1)	S
MR (1)	Çanakkale	Bayramic	Kutluoba	1.4 ± 0.0	9.3 ± 0.1	78.9 ± 0.8	10.4 ± 0.9
MR (2)	Çanakkale	Bayramic	Saçaklıköyü	1.3 ± 0.1	7.9 ± 0.2	81.1 ± 0.4	9.8 ± 0.5
MR (3)	Çanakkale	Kemalli	–	1.4 ± 0.1	10.0 ± 0.2	76.9 ± 0.9	11.7 ± 1.0
MR (4)	Çanakkale	Gelibolu	–	1.8 ± 0.1	7.2 ± 0.2	72.3 ± 0.6	18.7 ± 0.7
MR (5)	Çanakkale	Ezine	Geyikli	1.9 ± 0.1	5.9 ± 0.3	74.8 ± 0.5	17.4 ± 0.6
MR (6)	Çanakkale	–	–	1.8 ± 0.1	7.2 ± 0.3	72.8 ± 1.4	18.3 ± 1.5
MR (7)	Çanakkale	Bozcaada	–	1.4 ± 0.1	8.8 ± 0.2	79.4 ± 0.5	10.4 ± 0.5
MR (8)	Balıkesir	Burhaniye	Ören	1.4 ± 0.1	6.6 ± 0.2	85.2 ± 0.8	6.9 ± 0.8
MR (9)	Balıkesir	Burhaniye	–	2.0 ± 0.1	4.8 ± 0.3	76.6 ± 0.9	19.5 ± 1.1
MR (10)	Bursa	Gemlik	–	1.7 ± 0.1	3.9 ± 0.3	78.9 ± 0.5	15.3 ± 0.8
MR (11)	Bursa	Mudanya	–	1.7 ± 0.1	4.3 ± 0.3	76.4 ± 1.2	17.7 ± 1.4
AR (12)	İzmir	Bağyurdu (1)	–	1.2 ± 0.0	6.4 ± 0.1	86.2 ± 0.4	6.2 ± 0.4
AR (13)	İzmir	Bağyurdu (2)	–	1.1 ± 0.1	8.0 ± 0.3	83.3 ± 0.5	7.7 ± 0.4
AR (14)	İzmir	Bergama	Mahmudiye	1.9 ± 0.1	7.7 ± 0.3	72.4 ± 0.4	18.1 ± 0.5
AR (15)	Manisa	Turgutlu	Derbent	1.9 ± 0.1	3.4 ± 0.2	79.2 ± 0.5	15.4 ± 0.6
AR (16)	Manisa	Soma	Avdan	1.9 ± 0.1	4.3 ± 0.4	76.8 ± 0.8	17.0 ± 1.0
AR (17)	Manisa	Kırkağaç	–	1.8 ± 0.1	4.3 ± 0.3	77.6 ± 0.4	16.3 ± 0.5
AR (18)	Aydın	Nazilli	–	2.1 ± 0.1	9.0 ± 0.3	72.4 ± 0.5	16.6 ± 0.7
MeR (19)	Antalya	Alanya	Çamlıca	2.0 ± 0.2	12.7 ± 0.3	65.4 ± 0.5	19.9 ± 0.7
MeR (20)	Antalya	Alanya	Yeşilöz	1.9 ± 0.0	13.0 ± 0.2	65.9 ± 0.7	19.2 ± 0.9
MeR (21)	Mersin	Tarsus	–	1.9 ± 0.1	6.8 ± 0.4	71.8 ± 0.6	19.6 ± 0.7
MeR (22)	Adana	Salbaş	–	1.9 ± 0.1	4.5 ± 0.3	74.1 ± 0.6	19.5 ± 0.6
MeR (23)	Osmaniye	Kadirli	–	1.5 ± 0.1	13.5 ± 0.2	73.2 ± 0.4	11.1 ± 0.5
MeR (24)	Hatay	Samandağı	Cevlek	1.9 ± 0.1	4.3 ± 0.4	77.6 ± 0.4	16.3 ± 0.5
SER (25)	Gaziantep	–	–	1.2 ± 0.0	6.2 ± 0.1	79.1 ± 0.4	13.5 ± 0.4
BSR (26)	Artvin	Yusufeli	–	1.7 ± 0.1	6.1 ± 0.3	77.1 ± 0.6	15.2 ± 0.6

MR the Marmara Region (northwestern Turkey), AR the Aegean Region (western Turkey), MeR the Mediterranean Region (southern Turkey), SeR the Southeastern Region, BSR Black Sea Region (north Turkey)

and 2.77 ppm (A) arise from the following functional groups having different number of protons, respectively: $-\text{CH}_3$ (terminal group) ((Ln (18:3)); E: 3H); $-\text{CH}_2$ (terminal group) ((L (18:2)) (F: 3H) + (O (18:1)) (3H) + saturated fatty acyl (S) (3H); 9H); $-\text{CH}_2-\text{CH}=\text{CH}$ (allylic group) ((L (18:2)) (4H) + (Ln (18:3)) (4H) + (O (18:1)) (4H); C: 12H); $\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}$ (diallylic group) ((L (18:2)) (2H) + (Ln (18:3)) (4H); A: 6H). The $-\text{CH}_3$ (E) chemical shift of Ln (18:3) is ~ 0.10 ppm deshielded with respect to the analogous terminal groups of the other major fatty acyls. This is attributed to deshielding of $-\text{CH}_3$ (E) by the nearby double bond electrons. The same deshielding effect also explains the approximate 0.76 ppm difference between the signals of allylic (C) and diallylic (A) $-\text{CH}_2$ groups (Alonso-Salces et al. 2010; Sacchi et al. 1996). The integral value of each signal is proportional to the number of nuclei contributing to that signal. Taking the multiplicity

of proton atoms per functional group into account, the relative fraction of each functional group is directly derived. The integral of the lowest intensity peak arising from the terminal $-\text{CH}_3$ of (Ln (18:3)) (E) is taken as 1. The integrals of the remaining peaks are determined with respect to the integral value of E. The integral values of the peaks (E, A, C, F) are equalized to the number of corresponding protons. The content of Ln (18:3) is determined by dividing the integral value of E at 0.98 ppm with 3 due to three hydrogen of the terminal group of Ln (18:3).

$$\begin{aligned}\text{CH}_3(\text{Ln}) &= \text{E} \\ \text{CH}(\text{Ln}) &= \text{E}/3\end{aligned}\quad (1)$$

Then taking the multiplicity of diallylic protons into account, the integral value of A at 2.77 ppm yields the content of L (18:2).

$$\begin{aligned}
 \text{CH}_2(\text{L}) + 2 * \text{CH}_2(\text{Ln}) &= \text{A} \\
 2 * \text{CH}(\text{L}) + 4 * \text{CH}(\text{Ln}) &= \text{A} \\
 \text{CH}(\text{L}) &= [\text{A} - 4 * \text{CH}(\text{Ln})]/2
 \end{aligned}
 \quad (2)$$

The relative amount of O (18:1) is calculated referring to the allylic protons, so the integral value of C at 2.01 ppm.

$$\begin{aligned}
 2 * \text{CH}_2(\text{L}) + 2 * \text{CH}_2(\text{Ln}) + 2 * \text{CH}_2(\text{O}) &= \text{C} \\
 4 * \text{CH}(\text{L}) + 4 * \text{CH}(\text{Ln}) + 4 * \text{CH}(\text{O}) &= \text{C} \\
 \text{CH}(\text{O}) &= [\text{C} - 4 * \text{CH}(\text{L}) - 4 * \text{CH}(\text{Ln})]/4
 \end{aligned}
 \quad (3)$$

Similarly, the content of S is measured from the integral value of F arising from the terminal CH_3 protons of free acids at 0.88 ppm.

$$\begin{aligned}
 \text{CH}_3(\text{L}) + \text{CH}_3(\text{O}) + \text{CH}_3(\text{S}) &= \text{F} \\
 3 * \text{CH}(\text{L}) + 3 * \text{CH}(\text{O}) + 3 * \text{CH}(\text{S}) &= \text{F} \\
 \text{CH}(\text{S}) &= [\text{F} - 3 * \text{CH}(\text{L}) - 3 * \text{CH}(\text{O})]/3
 \end{aligned}
 \quad (4)$$

Each fatty acyl value obtained from the equations is summed, followed dividing the value of each fatty acyl with the sum and multiplying the result by 100. Integration of each peak in the ^1H NMR spectra is repeated 6 times by using the iNMR 4.3.0 software to get average and standard deviation (see Table 1 showing the geographical origins of olive oil samples, and the relative contents of each fatty acyl in olive oil samples). This approach was already used for the characterization of various olive oils from different countries (Sacchi et al. 1996, 1997) but used for Turkish olive oils only recently (Ok 2014).

Statistical analysis

^1H NMR data were analyzed by analysis of variance (ANOVA) using Tukey's test at a p level < 0.05 (5% significance level) with Origin 8.5. The ANOVA analysis was performed on the percentage fraction of the free acyl contents obtained integrating the ^1H NMR resonances. Principal component analysis (PCA) was performed with statistical package SPSS version 22.0.

Results and discussion

Fatty acyl contents determination and discrimination of olive oils based on ANOVA and seasonal comparison of fatty acyl contents

Figure 1 shows a representative ^1H NMR spectrum of Mersin/Tarsus with both letter and number labels on the peaks of interest that arise from the major components of olive oil. There was no evidence of free fatty acids, which appeared in rancid oil. Fatty acyls are extremely useful in

Fig. 1 ^1H NMR spectrum of olive oil from Mersin/Tarsus. Signals labelled with the numbers 1, 2, 3, and 8 refer to $-\text{CH}=\text{CH}-$ (triglycerides at 5.33 ppm), $-\text{CH}-$ glycerol (triglycerides at 5.25 ppm), $-\text{CH}_2-$ of glycerol (triglycerides at 4.11 ppm and 4.26 ppm) and $-\text{CH}_2-$ of (1,2 Diglycerides at 4.15 ppm and 4.30 ppm), respectively, while the signals with both capital letter and number labelling A-4, B-5, C-6, D-7, E-9, F-10 arise from $-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$ (linoleoyl acyl and linolenoyl acyl at 2.76 ppm), $-\text{OCOCH}_2-$ (linoleoyl acyl, oleoyl acyl, saturated fatty acyls, and linolenoyl acyl at 2.76 ppm), $-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-$ (linoleoyl acyl, oleoyl acyl, and linolenoyl acyl at 2.29 ppm), $-\text{OCOCH}_2-\text{CH}_2-$ (linoleoyl acyl, oleoyl acyl, saturated fatty acyls, and linolenoyl acyl at 1.59 ppm), $-\text{CH}_3$ (linolenoyl acyl at 0.96 ppm), $-\text{CH}_3$ (linoleoyl acyl, oleoyl acyl, and saturated fatty acyls at 0.87 ppm), respectively (Sacchi et al. (1997))

the characterization of olive oils (Ok 2014). Table 1 includes the geographical origin of each olive oil sample, the relative contents of linolenoyl (Ln, 18:3), linoleoyl (L, 18:2), oleoyl (O, 18:1), and saturated (S) fatty acyls in each sample.

After quantitative analysis of NMR data and determining fraction of L, Ln, O, and S, ANOVA was used to assess significance differences among the obtained treatment means. This was based on whether their variance is bigger than expected due to random variation (Krzywinski and Altman 2014). The aim of the statistical analysis was to determine the most important frequency band with the highest discrimination power so that geographical origin of the olive oil samples could be distinguished (Sacchi et al. 1996). ANOVA was performed in order to find a possible clustering of olive oil samples from different regions of the world (D'Imperio et al. 2007). Principle component analysis (PCA) permits obtaining linear combinations of the chosen variables from the original variables with a large number (Giansante et al. 2003) PCA was employed (using four fatty acyl fractions) to the 25 oil samples. Grouping of samples based on their geographical origins was not possible to observe with PCA, and only the values of saturated fatty acyls and linolenoyl acyl are highly correlated. For this reason, we preferred using linoleoyl acyl content in performing ANOVA that shows the discrimination of olive oils. Figure 2 indicates the ANOVA results for the

Fig. 2 The ANOVA analysis based on the origin of a city within each geographical region of MR, AR, MeR. For ANOVA subset for $\alpha = 0.05$. The numbers on the x-axis refer to the same numbers in Table 1. **a** Marmara Region, **b** Aegean Region, and **c** Mediterranean Region

linoleoyl acyl (L, 18:2) content of the cities or towns within the three geographical regions MR, AR, MeR separately. Among the four resonances considered, only the linoleoyl

acyl content resulted in significant discrimination between oils of different origin. The numbers in the x-axis of the plots in Fig. 2 refer to the city or town names in Table 1.

Table 1 shows that the three samples of MeR (southern Turkey) have the highest linoleoyl acyl content. The two samples of Antalya/Alanya contain less than 70% oleoyl acyl content. Such data discriminate MeR samples easily. Some of the samples of MR (northwestern Turkey) such as the ones from Çanakkale/Bayramic/Saçaklıköyü [MR (2)] and Balıkesir/Burhaniye/Ören [MR (8)] have higher than 80% oleoyl acyl and less than 10% saturated acyl fraction. A similar result was observed in the case of İzmir/Bağyurdu samples [AR (12) and AR (13)] located in Aegean region. The contents of olive oil samples as a function of season indicated that the fractions of linolenoyl acyl and linoleoyl acyl are within the same range. Saturated acyl percentage in İzmir, Manisa and Gaziantep samples of 2012 exhibit ~ 60, ~ 25, and ~ 30% difference with respect to the samples of 2011 (Ok 2014). The results of Antalya/Alanya samples (MeR (19) and MeR (20)) are independent of harvest season. These harvest season independent and consistent results make it easy to discriminate Antalya/Alanya olive oil samples from the other samples in the same geographical region and the rest of the samples in Turkey.

In the MR region, the olive oil samples, excluding [MR (4)] and [MR (6)], were easily discriminated from each other. The fatty acyl contents of the samples from Çanakkale/Gelibolu [MR (4)] and Çanakkale [MR (6)] have closer values, and they cannot be distinguished (Table 1). In the case of AR, olive oil samples from Manisa/Soma/Avdan [AR (16)] and Manisa/Kırkağaç [AR (17)] are not discriminated based on the ANOVA result. The two samples are from the same city, and again the contents of the other three fatty acyls of the two olive oil samples show closer values. Further, the two olive oil samples from İzmir/Bağyurdu (2) [AR (13)] and İzmir/Bergama/Mahmudiye [AR (14)] have closer linoleoyl acyl content. These two samples of [AR (13)] and [AR (14)] yielded significantly different oleoyl and saturated acyl contents. Considering the ANOVA results of MeR, we can conclude that the fatty acyl contents of the two villages Çamlıca [MeR (19)] and Yeşilöz [MeR (20)] in the same town Alanya of province Antalya are closer to each other. In addition, the single sample of Black Sea region (BSR) from Artvin/Yusufeli [BSR (26)] has closer oleoyl and saturated fatty acyl percentage values with respect to the data of season 2011 (Ok 2014).

Comparison with respect to other seasons of Turkish olive oils and olive oils of different countries

Earlier Andjelkovic et al. (2009) analyzed only ten commercial olive oils from North Aegean of Turkey by gas chromatography (GC) (Andjelkovic et al. 2009) while Ilyasoglu et al. (2010) applied liquid chromatography–mass spectrometry (LC–MS) for analyzing only thirteen samples of the same region. In the present study, the number of samples was higher than that of the studies in the literature. It was mentioned that saturated fatty chains elevate the amount of cholesterol in human blood. For this reason, saturated fatty chains are not considered as “healthy” constituents from a nutritional point of view. Monounsaturated fatty acyls contribute to limit cardiovascular risks by decreasing the level of cholesterol in blood (Mannina et al. 2001). Considering this factor, we can say that olive oil samples from Antalya region have less “healthy” characteristics due to low amount of oleoyl fatty acyl content. In this regard, samples with oleoyl acyl fraction higher than eighty percentage from Çanakkale/Bayramiç/Saçaklıköyü [MR (2)], Balıkesir/Burhaniye/Ören [MR (8)], İzmir/Bağyurdu (1) [AR (12)] and (2) [AR (13)] have more “healthy” characteristics from the nutritional point of view. The influence of olive growing area and harvest date on organic acid composition of olive fruits was studied by Arslan and Ozcan (2011). Clustering obtained by PCA showed that olive fruits from closer locations had closer organic acid compositions. For instance, Alanya–Antalya–Karaman locations formed a group together. Similar to that study by Arslan and Ozcan (2011), closer fatty acyl contents of the two villages Çamlıca [MeR (19)] and Yeşilöz [MeR (20)] in the same town Alanya is attributed to closer geographical locations. The linoleoyl acyl fraction of samples Yeşilöz [MeR (20)] and Osmaniye/Kadirli [MeR (23)] cannot discriminate the two samples based on the ANOVA results. Oleoyl and saturated fatty acyl fractions of samples coded 20 and 23 in Table 1 are easily distinguished.

Gurdeniz et al. (2008) applied FT-MIR for analyzing fatty acyl contents of olive oils obtained from various Turkish olive varieties such as Ayvalik and Gemlik as a function of harvest seasons of 2005/2006 and 2006/2007. Olive oils analyzed in the study by Gurdeniz et al. (2008) were Aegean region varieties and Nizip variety, the only one from northeastern Turkey. Most of the samples from Aegean region of the current study have higher oleoyl acyl and lower linoleoyl acyl fraction than the ones in the report by Gurdeniz et al. (2008). AR (14) and AR (18) samples of İzmir–Bergama and Aydınlı–Nazilli, respectively, have nearly same linoleoyl and oleoyl contents as in the case of Gemlik–Edremit variety. Gemlik is widespread in the Aegean and Marmara regions of Turkey (Isik et al. 2011).

The fatty acyl composition of different Turkish olive varieties has been also studied (Tanilgan et al. 2007). Tanilgan et al. (2007) showed that excluding the content of olive oil of Uslu type variety, both the saturated and unsaturated fatty acyl fractions of Gemlik, Kilis, Tirilye and Ayvalık varieties were closer. Since total saturated acyl fraction also matches the amount of Gemlik–Edremit, we can conclude that AR (14) and AR (18) samples should have been obtained from Gemlik–Edremit variety. Gurdeniz et al. (2010) studied the contents of olive oils from Marmara and Aegean regions. Overall, the olive oil samples of the current study have higher oleoyl acyl and lower linoleoyl acyl fractions than the ones in the report by Gurdeniz et al. (2010). The data of Aydınlı–Nazilli sample is closer to the data of Aydınlı olive oil in the harvest seasons of 2005/06 and 2006/07. Diraman and Dibeklioglu (2014) studied fatty acyl compositions of different cultivars obtained from various geographical regions of Turkey such as south and north Aegean. Among these samples only Kemalpaşa-1 (İzmir) and Selimiye (Milas-Muğla) samples have oleoyl acyl content values closer to the ones in the current report. The average of MR and AR oleoyl acyl contents in the current report are higher than the one reported by Diraman and Dibeklioglu (2014). The monounsaturated fatty acyls in particular oleoyl acyl are of great importance since oleoyl acyl contributes to health benefits of oxidative stability of olive oils (Gurdeniz et al. 2010; Diraman and Dibeklioglu 2014).

Diraman et al. (2011) classified three Turkish olive cultivars from Aegean region for the crop years of 2001/2002 and 2002/2003, when we compared our data belonging to Aegean region to the data of Diraman and co-workers (2011), oleoyl acyl contents of the olive oils studied in this study were overall higher than the ones from 2001/2002 to 2002/2003 seasons. The samples analyzed in the current report have also lower linoleoyl acyl fractions, and higher saturated acyl percentages than the ones reported by Diraman et al. (2011). Yorulmaz et al. (2014) analyzed contents of olive oils obtained from different Turkish varieties. The fatty acyl with the highest percentage in these samples was oleoyl acyl ranging from 60.15% (Domat) to 80.46% (Girit). linoleoyl acyl fraction was varying between 5.34% (Girit) and 18.29% (Antalya yağlık). We did not have oleoyl acyl content as low as 60.15%, while the maximum oleoyl acyl content belonged to İzmir–Bağyurdu sample with 86.2%. Oleoyl and linoleoyl contents of Antalya samples of the present report are closer to the values reported in the work by Yorulmaz et al. (2014). Arslan et al. (2013) reported a similar result for the oleoyl and linoleoyl acyl fractions of Sariulak Turkish variety grown in Antalya region. This also confirms the easy discrimination of Antalya olive oils.

We also compare the contents of Turkish olive oils to some of the Mediterranean ones. Fatty acyl composition of virgin olive oils obtained from Cornicabra olive cultivar grown in the Provinces of Ciudad Real and Toledo (Castilla-La Mancha, Spain) from 1995/1996 to 1999/2000 crops indicated 76.5 and 82.5% as the lowest and the highest oleoyl acyl content, while the fraction of saturated fatty acyl ranged from 11.40 to 15.13% (Aranda et al. 2004). Casas et al. (2009) studied olive oils from three representative regions of Extremadura (Spain): Sierra Norte of Cáceres, Serena-Siberia, and Tierra de Barros for the purpose of geographical discrimination of olive oils based on fatty acyl, triglyceride, and sterol contents. The mean oleoyl acyl content was between 70.27 and 77.57%, while the mean linoleoyl acyl fraction ranged from 4.45 to 10.77% (Casas et al. 2009). Ollivier and co-workers (2003) analyzed 564 virgin olive oil samples of France for different seasons from such as 1997 (81 samples) to 1999 (128 samples), and 2000 (267 samples). The olive oil from Salonenque variety found in PDO “Vallée des Baux” had the lowest oleoyl content with the mean value 64.76% and the highest linoleoyl acyl fraction of 12.59% among the 372 olive oils from few main French cultivars (Ollivier et al. 2003). Del Coco and co-workers (2012) found by ^1H NMR spectral analysis that Italian Ogliarola and Coratina varieties have approximately 80, 8, and 12% oleoyl acyl, linoleoyl acyl, and saturated fatty acyl compositions. Among Turkish olive oils analyzed in the current study, the ones obtained from Çanakkale–Bayramiç–Saçaklıköyü, Ballıkesir–Burhaniye–Ören, and İzmir–Bağyurdu had oleoyl acyl content higher than 80%. In particular, Ballıkesir–Burhaniye–Ören and İzmir–Bağyurdu (1) samples have oleoyl acyl fraction higher than 85%. These four olive oils are easily distinguishable from majority of the European olive oils. In addition, approximately 40% of Turkish olive oils analyzed in the current report have higher saturated fatty acyl fractions than the Spanish (Aranda et al. 2004; Casas et al. 2009), and Italian (Del Coco et al. 2012) olive oils referred above. Besides the reports on European olive oil, in a recent work on discrimination of the geographical origin of monovarietal Tunisian olive oil produced from local Chemlali and Sahli cultivars, Souayah et al. (2017) showed the possible use of potentiometric fingerprints. The effect of olive cultivar, quality grade or geographical location on the sensory attributes of Tunisian olive oil was evaluated through one-way ANOVA. The similarity in the report by Souayah et al. (2017) with respect to the current study is combining experimental and statistical approaches, and showing that olive oil authentication is a challenging task in particular when agricultural factors such as soil quality influence the chemical content of olive oil. However, the major difference is that we applied ^1H NMR spectroscopy enabling the

detection of both minor and major constituents of olive oil samples without sample preparation or extraction of the constituents, while Souayah et al. (2017) conducted electronic-tongue potentiometry analyzing polar compounds found in olive oil. The comparisons of the current results with respect to the literature show that mean fatty acyl fractions of Turkish olive oils help with distinguishing olive oils from non-Turkish olive oils as well as compatibility of different experimental approaches with chemometrics methods in analyzing the chemical contents of olive oils.

Shelf life determination by quantifying fatty acyl content and detection of minor constituents

In the present study, we focused on three olive oil samples obtained from the same region as a function of three consecutive harvest seasons: late 2012, late 2013, and late 2014, and with the production dates of early 2013, early 2014, and early 2015. Analyzing ^1H NMR spectra of the olive oils from 2012, 2013, and 2014 reveal high number of resonances arising from minor constituents of olive oil samples in particular between 6.00 and 10.50 ppm (Table 2) (Guillen and Uriarte 2012; Dugo et al. 2015; Alonso-Salces et al. (2015)). In the case of 2012 sample, the number of sterol and alcohol peaks is relatively less than the samples of 2013, and 2014. For example, the signals at 3.61 and 3.45 ppm and attributed to the sterol fraction were not observed in the ^1H NMR spectra of 2012, and 2013 samples. There is an increase in the number of phenolic compounds of 2012 sample, whose signals are at 7.02, 7.00, and 6.74 ppm. Resonances of alcohol fraction at 3.98 and 3.78 ppm were not observed in the ^1H NMR spectrum of the 2012 sample. This indicated that the compounds of sterol and alcohol derivatives evaporated during shelf life time, and thus their signals were not observed in the ^1H NMR spectrum. The existence of signals attributed to the phenolic compounds in the ^1H NMR spectrum of 2012 sample is attributed to possible by-product formation during storage of the olive oil (Alonso-Salces et al. 2010). Cyclic minor compounds such as cyclopropene and cycloartenol are stable for the period of shelf life time. Among the aldehyde derivatives, butanal and hexanal whose CH_3 and COH peaks are observed at 0.95 and 9.74 ppm, respectively, are stable, while the other two aldehydes whose resonances appear between 8.00 and 10.00 ppm in the ^1H NMR spectra of the samples do not show systematic pattern in terms of shelf life stability. For this reason, we think that the stability of aldehyde compounds is independent of shelf life but related to production of olive oil itself. In other words, if the olive oil sample contains aldehyde compound(s) initially, it is possible to

Table 2 The list of ^1H NMR signals of the three Izmir–Kemalpasa olive oils from three consecutive harvest seasons of 2012, 2013, and 2014

δ ^1H NMR (ppm)	Assignment	Proton	Harvest season		
			2012	2013	2014
10.41	New signal		–	+	–
9.74	Hexanal	COH	+	+	+
9.56	Alka-2,4-dienals	–CHO	–	+	–
8.13	Hyrdperoxides	–OOH (hydroperoxide group)	–	+	–
8.10			+	–	–
8.03	Aldehyde		–	–	+
7.98	New signal		+	+	–
7.95	New signal		–	–	+
7.02	Phenolic compounds		+	–	–
7.00	Phenolic compounds		+	–	–
6.74	Phenolic compounds	–Ph–H (phenolic ring)	+	+	+
6.54	Hyrdperoxides	–CH = CH–CH = CH–(cis, trans conjugated diene system)	+	+	+
6.46	Hyrdperoxides	–CH = CH–CH = CH–(cis, trans conjugated diene system)	+	+	–
6.34	Tocopherol fraction		–	–	+
6.24			+	+	–
5.97	Hyrdperoxides	–CH = CH–CH = CH–(cis, trans conjugated diene system)	+	+	+
5.78			–	+	–
5.76			–	+	–
5.72			+	+	+
5.65	Tocopherol fraction		+	+	–
5.62	Tocopherol fraction		+	–	+
5.60	Tocopherol fraction		–	+	–
5.55	Tocopherol fraction		+	+	+
5.44	Sterol fraction		+	+	+
5.31	Triglycerides	CH–beta	+	+	+
5.24	Triglycerides	>CHOCOR (glyceryl group)	+	+	+
5.19	Squalene		+	+	+
5.12	sn-1,2 diglyceride	CHOCOR	+	+	–
5.07	1,2 diglycerides	ROCH ₂ –CH(OR')–CH ₂ OH	+	+	–
5.02	Cycloartenol		+	+	+
4.97	Terpene		+	+	+
4.91	Sterol fraction		+	+	+
4.86	Sterol fraction		+	+	+
4.69	Terpene	CH(s)	+	+	+
4.64	Alcohol fraction		+	+	+
4.57	Terpene	CH(d)	+	+	+
4.40	Triglycerides		+	–	+
4.27	Triglycerides		+	+	+
4.13	Triglycerides	CH ₂ –alpha	+	+	+
4.05	Hydrocarbon fraction		+	+	+
4.01	Triglycerides	CH ₂ –O–CO–R	+	+	+
3.90	Saturated alcohol		–	+	+
3.78	Alcohol fraction		–	–	+
3.70	Alcohol fraction		+	+	+
3.64	sn-1 in monoglyceride	CH ₂ OH	–	+	+
3.61	β -Sitosterol, stigmasterol, campesterol		–	–	+
3.58	β -Sitosterol, stigmasterol, campesterol		+	+	+

Table 2 continued

δ ^1H NMR (ppm)	Assignment	Proton	Harvest season		
			2012	2013	2014
3.49	β -Sitosterol, stigmasterol, campesterol		+	+	+
3.45	β -Sitosterol, stigmasterol, campesterol		–	–	+
3.26	Erythrodiol		–	–	+
3.01			+	+	+
2.92			–	+	–
2.87			+	+	+
2.78	Polyunsaturated fatty acids	$\text{CH} = \text{CH}-\text{CH}_2-\text{CH} = \text{CH}-\text{CH}_2-\text{CH} = \text{CH}$	+	+	+
2.75	Linoleic acid	$\text{CH} = \text{CH}-\text{CH}_2-\text{CH} = \text{CH}-$	+	+	+
2.69			+	+	+
2.64	^{13}C satellite	Linoleic chains	+	+	+
2.58	α -tocopherol	$-\text{CH}_3$ in the oxane ring	–	–	+
2.52			+	+	+
2.39	Aldehyde	CH_2	+	+	+
2.28	Fatty acid	$\text{OCO}-\text{CH}_2-$	+	+	+
2.18			+	+	+
1.99	Fatty acid	$\text{CH}_2(\text{trans})-\text{CH} = \text{CH}$	+	+	+
1.89	Oleoyl chains		+	+	+
1.78	Erythrodiol		+	–	–
1.65	Squalene	CH_3	+	+	+
1.59	1-Eicosanol	$-\text{CH}_2-\text{CH}_2-\text{OH}$	+	+	+
1.48	Hexenal	CH_2	+	+	+
1.25	Fatty acid	$(\text{CH}_2)_n$	+	+	+
1.01	Erythrodiol		+	+	+
0.99	Fatty acids	$\text{CH} = \text{CH}-\text{CH}_2-\text{CH}_3$	+	+	+
0.95	Butanal	CH_3	+	+	+
0.86	Fatty acids	CH_3	+	+	+
0.75	Erythrodiol		+	+	+
0.69			–	–	+
0.66			+	+	+
0.54	Alcohol fraction		–	+	+
0.52	Cyclopropene	CH_2	+	+	+
0.31	Cycloartenol	CH_2	+	+	+

detect the aldehyde compound proton NMR resonance even after 1 or 2 years later.

The percentages of fatty acyl contents of İzmir–Kemalpaşa samples were also determined using the equations mentioned in the experimental section (Table 3). The fatty acyl percentages of the olive oil samples from three consecutive harvest seasons from İzmir–Kemalpaşa are closer to each other as well as to the values published previously (Ok 2014). This shows that fatty acyls are stable during the shelf life time. The chemical contents vary in terms of minor components of the olive oils. There are also

unassigned ^1H NMR signals such as the one at 10.41 ppm belonging to the 2013 sample. The existences of new and unassigned signals show that during long period of storage time there could be formation of undefined by-products in olive oil.

Fatty acyl contents of a northwestern Turkish village

Kurşunlu is a coastline village with the coordinates of $40^\circ 22' 5''\text{N}$ and $29^\circ 2' 55''\text{E}$ within the border of Gemlik

Table 3 Percentage fraction of Ln (18:3), L (18:2), O (18:1), and S in İzmir–Kemalpasa olive oils from three consecutive harvest seasons of 2012, 2013, and 2014

Sample	Ln (18:3)	L (18:2)	O (18:1)	S
İzmir–Kemalpasa (2012)	1.0 ± 0.1	5.2 ± 0.4	72.9 ± 4.1	20.9 ± 4.5
İzmir–Kemalpasa (2013)	1.0 ± 0.1	5.3 ± 0.4	71.5 ± 3.9	22.2 ± 4.3
İzmir–Kemalpasa (2014)	1.1 ± 0.1	6.1 ± 0.8	69.3 ± 3.2	23.5 ± 3.7

Table 4 Percentage values of Ln (18:3), L (18:2), O (18:1), and S in olive oil samples from Kurşunlu village of Bursa/Gemlik

Geography location	Soil quality	% fraction of acids in olive oils			
		Ln (18:3)	L (18:2)	O (18:1)	S
Hill	Black soil	1.6 ± 0.1	12.4 ± 0.3	73.1 ± 0.3	12.9 ± 0.4
Hill	Wet black soil	1.3 ± 0.1	3.7 ± 0.2	81.2 ± 0.6	13.8 ± 0.6
Hill	Soil with stone	1.8 ± 0.1	3.3 ± 0.1	78.6 ± 0.3	16.3 ± 0.3
Hill	Wet soil with stone	1.7 ± 0.1	3.6 ± 0.1	80.4 ± 1.3	14.3 ± 1.4
Flat	Black soil with stone	1.8 ± 0.1	9.7 ± 0.2	74.4 ± 1.0	14.1 ± 1.2
Flat	Soil with stone	1.8 ± 0.1	5.7 ± 0.2	78.1 ± 0.5	14.5 ± 0.6
Flat	Black soil	1.7 ± 0.1	12.4 ± 0.3	73.4 ± 0.9	12.6 ± 1.1

district of Bursa Province of Turkey. The region lies between hills of approximately 300 m and the Gemlik Gulf in the Marmara Sea. In the current study, four of the Kurşunlu olive oil samples are obtained from the trees in the hills region with nearly height of 300 m, while three of them were obtained from the trees grown at the sea level. The average of linoleoyl acyl fraction in the hillside samples is lower than that of the sea level (Table 4). The average of oleoyl acyl percentage in the hillside samples is higher than that of the sea level. Similar results due to altitude difference were observed in our previous report (Ok 2014). The content of olive oil produced in that village was analyzed as a function of extension in the harvest season and altitude. As the harvest season was extended in the hillside region of the village, there was a significant increase in the oleoyl acyl fraction. The data of olive oil from sea level showed higher linoleoyl acyl and lower oleoyl acyl fractions compared to the samples of the hillside level. This was explained by the altitude difference. D'Imperio et al. (2007) analyzed olive oils both from olive trees grown on hills with the altitude higher than 400 m and from lower altitudes. The statistical result indicated that olive oils have been well grouped as a function of altitude. Stefanoudaki et al. (1999) studied fatty acyl content of olive oil produced from two major Cretan cultivars (Koroneiki and Mastoides) as a function of high- and low-altitude location for one harvesting period. Within the low-altitude group of samples, the oils of Chrysopigi and Falasarna form two separate groups with higher fraction of saturated fatty acyls. This was attributed to different environmental factors such as relative humidity. High-

altitude olive oils established one single group. By that study, it was proved that analysis of fatty acyl content of olive oil samples help with discriminating the olive oils from each other even within closer geographical regions. Arslan and Ozcan (2011) reported contents of different olive oils from various olive growing areas of Turkey, and showed that oxalic, succinic, and malic acids were higher in Karaman and Osmaniye samples, that result was partially related to higher altitude of the two locations. This also indicates that altitude may affect olive oil discrimination of a small olive growing village.

The best discriminant factor is the oleoyl acyl content in analyzing the effect of soil quality. Both of the olive oil samples from “black soil” olive oil have approximately same fatty acyl values, while samples obtained from olives of “soil with stone” closer oleoyl acyl contents. The samples from olives grown in “wet soil” have closer fatty acyl fractions. The sample from “black soil with stone” is easily discriminated with the linoleoyl acyl and oleoyl acyl percentages from the other samples of Kurşunlu village. The current results indicate that the fast screening of olive oil by ^1H NMR aids in distinguishing the contents of olive oils as a function of soil quality.

Conclusion

Several olive oil samples from Turkey were analyzed by the combination of non-destructive ^1H NMR spectroscopy and the chemo-metrics analysis. The results of Antalya/Alanya samples of MeR were independent of harvest

season. Data analysis permitted discrimination of the olive oils based on the sub-regions of the samples within three geographical regions (MR, AR, and MeR). It was also possible to distinguish Turkish and European olive oils partially by comparing oleoyl and linoleoyl acyl fractions. The highest fraction of oleoyl acyl found in samples from Northwestern providences of Turkey such as Balıkesir and Çanakkale, showing more “healthy” character. Considering the shelf life time of olive oil based on the chemical content analysis of İzmir–Kemalpasa samples from the harvest seasons of 2012, 2013, and 2014, we suggest that the existences of new and unassigned signals in the ^1H NMR spectra indicate formation of by-products in olive oil during storage. The sterol and alcohol derivatives are not stable during shelf life time, as a result their signals are not observed in the ^1H NMR spectrum of the 2012 olive oil sample of İzmir–Kemalpasa. Cyclic compounds such as cyclopropene and cycloartenol do not evaporate during the period of shelf life time. Among the aldehyde derivatives, butanal and hexanal are stable, while the other two aldehydes do not show systematic pattern in terms of shelf life stability. Therefore, the stability of aldehyde compounds is independent of shelf life but related to production of olive oil itself. The results on fatty acyl contents are reproducible showing that fatty acyls are stable during the shelf life time. The results also indicated that olive oil content depend on the quality of soil and the altitude of the olive trees.

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