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Classification of Turkish olive oils with respect to cultivar, geographic origin and harvest year, using fatty acid profile and mid-IR spectroscopy

Gozde Gurdeniz · Banu Ozen · Figen Tokatli

Abstract Fatty acid composition and mid-infrared spectra of olive oils in combination with chemometric techniques were used in the classification of Turkish olive oils with respect to their varieties, growing location and harvest year. In particular, olive oil samples belonging to five different cultivars were obtained from the same orchard in the middle part of Aegean region and two of these varieties were also received from another orchard in northern part of the same region of Turkey in two consecutive harvest years. Evaluation of nine different fatty acid compositions with principal component analysis revealed clear differentiation with respect to variety, geographical origin and harvest year. On the other hand, mid-infrared spectra also achieved varietal and seasonal discrimination to some extent, but differentiation is not as clear as that obtained using fatty acid compositions.

Keywords Olive oil · FTIR · Fatty acid composition · Classification · Chemometrics

Introduction

Olive oil has been receiving special attention from consumers, because of its sensorial quality and health beneficial effects. As a result, special types of olive oils generally produced from olives of certain varieties cultivated in selected regions (monovarietal) have been appearing in the market. The chemical composition of olive oils might differ due to the influence of geographical, agronomic and technological factors. Differences in composition depending on geographic origin are the basis of the legislations such as protected denomination of origin (PDO) and protected geographical indication (PGI). PDO and PGI certifications allow labeling of food products with growing areas and provide extra economical benefits for producers of designated areas. Determination of the variability in the composition of olive oils obtained from olives grown at different locations is necessary for the classification of oils as well as for the determination of adulteration of monovarietal oils. Various major and/or minor compositional data such as triglycerides, fatty acids and sterols in combination with chemometrics were employed for classification of oils according to variety [1–5], origin [6], extraction method [7] and harvest year [8]. Among the constituents of olive oil, fatty acids are extremely useful in characterization. Lanteri et al. [9] demonstrated usefulness of fatty acids to distinguish the monovarietal olive oils belonging to some particular Calabrian cultivars. Result of another study proved that fatty acid compositional data have a significant discriminating power even within close geographical areas [10].

Over the last few years, there is an increasing demand by food processors, consumers and regulatory bodies for rapid methods for confirming authenticity or detecting adulteration of food products. To solve this problem, infrared (IR) spectroscopy has recently been applied to detect adulteration of extra virgin olive oil with refined oils and different types of vegetable oils [11–13]. Although Fourier-transform infrared (FTIR) spectroscopy data evaluated with chemometric techniques have been found to have sufficient ability to quantify adulteration, there are only a few studies involving its use for classification purposes. Near-infrared reflectance spectroscopy was evaluated for its ability to classify olive oils from eastern Mediterranean, and promising

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results were obtained [14]. Tapp et al. [15] also claimed that mid-infrared spectroscopy combined with multivariate analysis has the potential to verify the country of origin of extra virgin olive oils.

There are several examples of olive oil classification studies performed in major olive oil-producing countries, Italy, Spain and Greece [3, 16–18]; however, number of studies about the characterization of Turkish olive oils is limited. Turkey produces 80,000–200,000 tons of olive oil annually, and this corresponds to about 5% of the world production [19]. Aim of this study is to characterize and classify Turkish extra virgin olive oils using fatty acid and mid-infrared spectral profile in combination with chemometric techniques with respect to olive variety, growing location and harvest year.

Materials and methods

Oil samples

Ayvalik (A), Gemlik (G), Memecik (M), Erkence (E) and Nizip (N) varieties were obtained from Olive Research Institute (Izmir, Turkey), and Ayvalik (AE) and Gemlik (GE) cultivars were provided by Olive Nursery (Edremit, Turkey) for two consecutive harvest years (2005/06 denoted by 1 and 2006/07 denoted by 2). Olives from each cultivar were picked from trees at the same maturity stage and then milled with a maximum 5 kg capacity laboratory scale olive oil mill (TEM Spremoliva, Italy). Two to four oil pressings were done for each cultivar. The samples were stored in dark brown bottles at a temperature of 8 °C. All chemical analyses were performed at least twice for each sample, and all analyses were completed within 2 months after pressing of the oils.

Free fatty acid value

European official methods of analysis [20] were used for the determination of free fatty acid value. Results were expressed as % oleic acid.

Gas chromatographic analysis

Fatty acid methyl esters (FAMEs) containing C8–C24 (2–11% relative concentration) were used as reference standard (Supelco # 18918). The olive oil samples were dissolved in hexane and saponified to their methyl esters with the addition of methanolic KOH solution as described in Official Journal EU (1991). Gas chromatographic analyses were performed on an Agilent 6890 GC with a flame ionization detector (FID) and a split/splitless (1/50) injector equipped with HP 88 capillary column (100 m × 0.25 mm

ID × 0.2 μm). The carrier gas was helium with constant flow of 2 mL/min. Injection and detector temperatures were 250 and 280 °C, respectively. The oven temperature program was run at 120 °C for 1 min, varied at 3 °C/min to 220 °C and held at this temperature for 5 min. Peak quantification is expressed as percentage of FAMEs, using FAME standards and samples chromatograms.

FT-MIR analysis

All infrared spectra (4,000–650 cm^{-1}) were acquired with a Perkin Elmer Spectrum 100 FTIR spectrometer (Perkin Elmer Inc., Wellesley, MA). This instrument was equipped with a horizontal attenuated total reflectance (ATR) sampling accessory (ZnSe crystal) and a deuterated tri-glycine sulphate (DTGS) detector.

ATR accessory was used to collect the spectral data of oil. The resolution was set at 2 cm^{-1} and the number of scans collected for each spectrum was 128. ZnSe crystal was cleaned with hexane in between sample runs.

Statistical analysis

Data analysis was performed using multivariate statistical methods with SIMCA software (Umetrics, Sweden). For the analysis, 3,620–2,520 and 1,875.5–675 cm^{-1} regions of FTIR spectra were used. These regions were selected because absorption peaks were observed only in these areas (Fig. 1). Selected region corresponds to 4,603 wavenumbers. Wavelet compression was used as spectral filtering method. Daubechies was chosen as wavelet function with wavelet order of 10 [21]. The discrimination of olive oil samples was achieved with principal component analysis (PCA). PCA is a multivariate analysis technique that is used frequently for classification problems. It is commonly used to represent a multivariate data table as a low-dimensional plane, usually consisting of two to five dimensions, such that an overview of the data is obtained. SIMCA uses leave one out class validation technique in PCA models.

It is important to accurately determine the number of components that should be included in the model, since it is linked to the difference between the degree of fit and the predictive ability. Degree of fit increases as the number of components increases, but predictive ability does not increase after a certain model complexity. So, it is important to reach an optimal balance between fit and predictive ability. Significant principal components of each category build the class model, which is computed after a separate scaling for each category. Using PCA class model, Coomans' plot is constructed for the classification of each cultivar. In class models, number of principal components based on model's fit and prediction abilities varies between 1 and

Fig. 1 Mid-IR spectra of A and
N olive oils
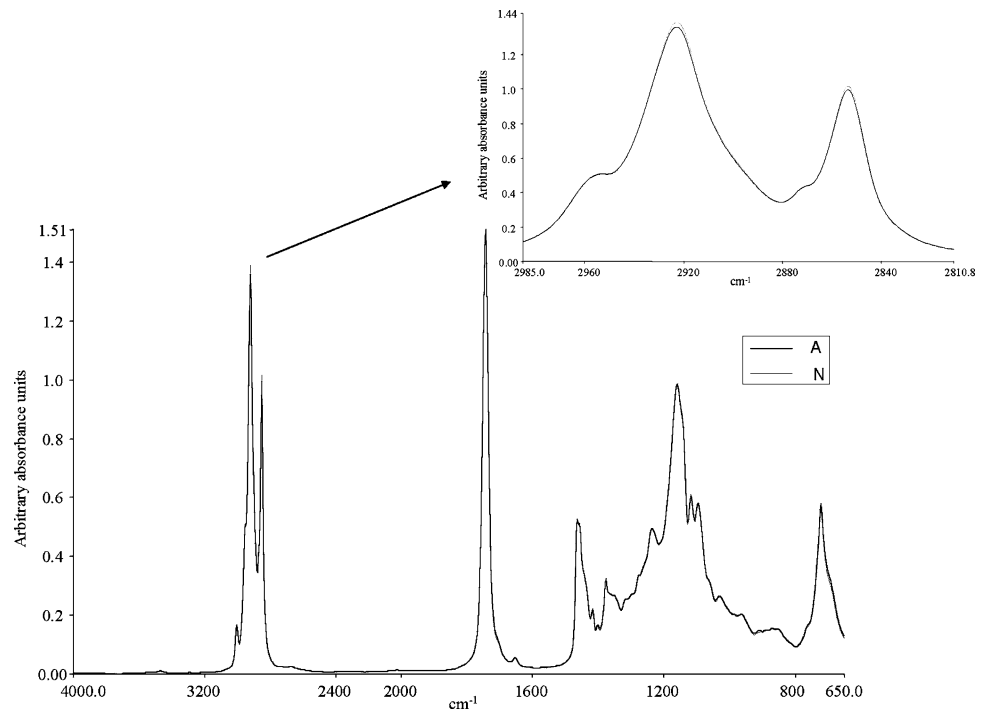
4 and between 2 and 5 for PCA of fatty acid composition and spectral data, respectively.

Results and discussion

Olive varieties used in this study are commercially important cultivars in olive oil production. All varieties except Nizip are mainly cultivated in western part of Turkey called Aegean region. Nizip, on the other hand, originates from southeastern part of Turkey. Five varieties (A, G, M, E and N) used in this study were obtained from the same orchard in Izmir, which is in the middle part of Aegean region and two of these varieties (A and G) were also supplied from another orchard in northern part of the same region (Edremit) for two consecutive harvest years. Same extraction system was used in obtaining oils. Therefore, analysis of oils from varieties grown in Izmir allowed us to investigate the variety effect only. On the other hand, comparison of two varieties (A and G) from two different areas (Izmir and Edremit) provided information about the effect of growing location. Free fatty acid values as the basic quality indices of olive oil are presented in Table 1. All olive oil samples have free fatty acid values lower than 1%, which is the maximum limit of EU (1991) regulations [20] for extra virgin olive oil.

Fatty acid compositions were determined for seven different olive oil samples belonging to two consecutive harvest years (Table 2). The oleic acid content was at the lowest concentration (63.57%) for E variety of second

Table 1 Free fatty acid values of olive oils expressed as % oleic acid

Variety	Harvest year	
	2005–2006	2006–2007
Ayvalik (A)	0.24 ± 0.02^b	0.31 ± 0.1^{ab}
Gemlik (G)	0.16 ± 0.03^a	0.16 ± 0.02^a
Memecik (M)	0.13 ± 0.03^a	0.29 ± 0.1^a
Erkence (E)	0.38 ± 0.07^b	0.14 ± 0.01^a
Nizip (N)	0.94 ± 0.18^c	0.45 ± 0.3^b
Ayvalik–Edremit (AE)	0.17 ± 0.02^a	0.80 ± 0.04^c
Gemlik–Edremit (GE)	0.32 ± 0.02^b	0.25 ± 0.06^{ab}

In each column, values with same superscript letter(s) show that there is no statistical difference between free fatty acid values at $P > 0.05$

harvest year and at the highest concentration (72.95 %) for GE of second harvest year. The content of linoleic acid varied between 16.89% for E and 7.41% for GE varieties, both belonging to second harvest year. Also, the amount of individual fatty acids of each variety changed with respect to year of harvesting.

On the other hand, mid-IR spectra of olive oil samples are very similar. The entire spectra of A and N varieties having distinct fatty acid profiles is presented in Fig. 1. However, it is not possible to discriminate spectra belonging to different varieties visually. The slight differences in the intensities of peaks around 2,960 and 2,840 cm^{-1} are shown in the inset of Fig. 1. Multivariate statistical analysis was used to visualize these slight intensity differences in the spectral profile of olive oil samples and to discriminate the

Table 2 Fatty acid compositions of olive oils

Variety	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0
2005/06									
A	0.03 ± 0.00	14.24 ± 0.37	0.84 ± 0.01	2.77 ± 0.09	69.58 ± 0.44	11.22 ± 0.11	0.45 ± 0.02	0.75 ± 0.01	0.13 ± 0.02
G	0.01 ± 0.00	14.01 ± 0.75	0.93 ± 0.07	2.92 ± 0.06	70.67 ± 1.21	10.13 ± 0.39	0.39 ± 0.03	0.78 ± 0.02	0.15 ± 0.06
M	0.02 ± 0.00	14.95 ± 0.73	0.89 ± 0.05	2 ± 0.08	66.32 ± 0.96	14.5 ± 0.72	0.35 ± 0.04	0.86 ± 0.01	0.11 ± 0.03
E	0.01 ± 0.00	14.09 ± 0.88	0.75 ± 0.07	2.39 ± 0.09	66.44 ± 1.59	14.95 ± 0.85	0.38 ± 0.02	0.86 ± 0.03	0.12 ± 0.02
N	0.01 ± 0.01	15.19 ± 0.52	0.81 ± 0.47	4.57 ± 0.37	68.35 ± 0.97	9.95 ± 0.22	0.49 ± 0.02	0.53 ± 0.05	0.09 ± 0.02
AE	0.02 ± 0.00	14.16 ± 0.25	1.03 ± 0.02	2.29 ± 0.02	69.03 ± 0.23	11.33 ± 0.09	0.42 ± 0.04	0.67 ± 0.02	0.13 ± 0.03
GE	0.01 ± 0.00	13.72 ± 0.31	1.06 ± 0.02	3.19 ± 0.17	72.05 ± 0.31	8.88 ± 0.21	0.37 ± 0.04	0.59 ± 0.01	0.14 ± 0.06
2006/07									
A	0.02 ± 0.00	16.51 ± 0.37	2.65 ± 0.03	2.08 ± 0.03	65 ± 0.26	12.69 ± 0.16	0.49 ± 0.02	0.42 ± 0.01	0.13 ± 0.02
G	0.01 ± 0.00	14.45 ± 0.45	2.07 ± 0.21	3.37 ± 0.39	71.2 ± 1.65	7.82 ± 0.66	0.49 ± 0.02	0.48 ± 0.02	0.12 ± 0.01
M	0.02 ± 0.00	12.71 ± 0.62	1.29 ± 0.03	2.13 ± 0.10	72.88 ± 0.61	10.01 ± 0.16	0.46 ± 0.01	0.4 ± 0.02	0.12 ± 0.01
E	0.02 ± 0.00	14.62 ± 0.31	1.6 ± 0.04	2.28 ± 0.07	63.57 ± 0.46	16.89 ± 0.43	0.52 ± 0.01	0.4 ± 0.01	0.12 ± 0.01
N	0.01 ± 0.00	14.98 ± 0.65	1.54 ± 0.02	5.1 ± 0.19	67.21 ± 0.66	10.14 ± 0.14	0.32 ± 0.01	0.56 ± 0.02	0.13 ± 0.02
AE	0.02 ± 0.00	15.03 ± 0.64	1.87 ± 0.06	2.11 ± 0.09	66.29 ± 0.45	13.77 ± 0.33	0.39 ± 0.01	0.4 ± 0.02	0.13 ± 0.02
GE	0.01 ± 0.00	13.48 ± 0.40	2.08 ± 0.09	3.13 ± 0.12	72.95 ± 0.53	7.41 ± 0.12	0.39 ± 0.01	0.43 ± 0.02	0.11 ± 0.01

All values indicate the mean ± standard deviation of at least two batches of oils

olive oils. Similar conclusion was also stated by Dabson et al. [22] for the spectra of most pure fats and oils.

Fatty acid profile and mid-IR spectra were used in the classification of olive oils, using multivariate analysis techniques. PCA was employed as the multivariate analysis tool to visualize principal groupings. After the construction of PC model, loading plot supplies the necessary information to determine which variables are responsible for the patterns seen among the observations. Loading plot obtained from PCA of fatty acid data is represented in Fig. 2. Variables C16:1, C18:1, C18:2 and C18:3 are placed far from the origin, meaning that they have an important effect on the classification of oil samples with respect to cultivar, geographical origin and harvest year. The high discriminant power of C16:0, C18:1 and C18:2 was also stated in the classification of Sicilian cultivars with respect to cultivar and geographical effects [17].

Coomans' plot is an extremely useful tool to visualize principal groupings, in which the two axes represent the distance of individual model. In Coomans' plot, PCA model is applied to each class separately and two class models are plotted against each other with the critical levels as straight lines displaying the boundaries. Any sample having a distance to the corresponding centroid greater than the critical distance is considered as being outside the class model and, as a consequence, rejected as an outlier for the specific category. Coomans' plots, using fatty acid compositions (Fig. 3a) and mid-IR spectra (Fig. 3b), were constructed for discrimination of olive oil samples. PCs of 14 class models belonging to two harvest years were calculated independently, and overall statistics of PC class models
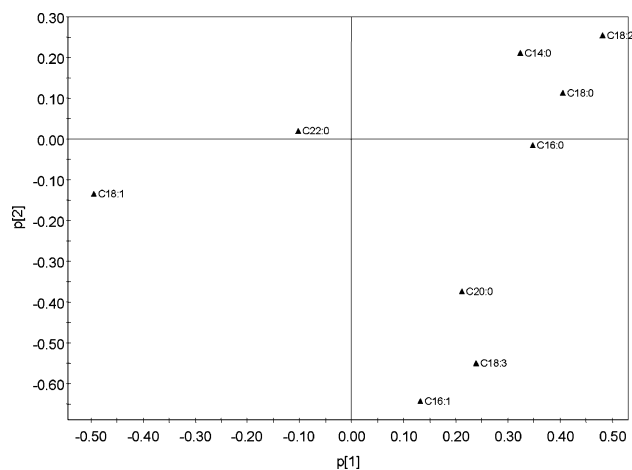
Fig. 2 PCA loading plot of the fatty acid profile data

for each multivariate data set was provided in Table 3. Among class models, A2 and N2 were preferred to be plotted against each other due to their different fatty acid compositions. Inspection of Fig. 3a reveals that each sample of A2 and N2 was correctly plotted in its critical limits apart from the origin, which indicates quite good discrimination of two models. Also, most of the other models were identified as separate classes and placed in the outer space of A2 and N2 models. Only N1 is plotted in the GE2 class model. Also M2 and AE1, having similar fatty acid compositions, cannot be differentiated from each other. These observations reveal that harvest year and cultivar significantly influence the fatty acid composition of olive oil. Usefulness of fatty acid composition in differentiation of Calabrian [9]

Table 3 PCA class models and general statistics for fatty acid profile and mid-IR spectra

PCA class models	Fatty acid profile		Mid-IR spectra	
	Number of PCs	R²X(cum) (%)	Number of PCs	R²X(cum) (%)
A1	3	94.9	3	91.8
A2	4	97.4	5	97
G1	2	88.9	2	74.5
G2	3	92.7	4	93.3
M1	3	89.7	3	94.1
M2	3	92.8	2	72.8
E1	4	98.3	4	96.9
E2	3	78.6	5	95.5
N1	1	75.8	3	93.1
N2	2	96.8	2	96.8
AE1	2	84.2	2	88.6
AE2	4	94.4	5	91.8
GE1	3	94.7	2	84.8
GE2	2	61.5	3	85.6

R²X(cum): cumulative sum of squares of the entire Xs explained by all extracted components

and Sicilian [17] cultivars was also demonstrated previously.

On the other hand, Fig. 3b shows that mid-IR spectra have lower discrimination ability than fatty acid profile. Actually, each sample of A2 and N2 were placed on its own model region. However, in the outer space of A2 and N2 models, the only classification is observed for AE2 and M2, and other samples were completely spread, not showing any groupings. Also, to demonstrate the discrimination ability of harvest year, each variety belonging to 2005/06 and 2006/07 harvest years were plotted against each other on the same graph, and results reveal that all cultivars could be differentiated according to harvest year. Further application of PCA on spectral data of olive varieties for each harvest year separately reveals better differentiation of cultivars using Coomans' plot (graph is not shown). Boggia et al. [23] stated that variation of climatic conditions from one year to the next affects the olive oil quality. A further study on Cornicabra virgin olive oil revealed significant statistical differences in quality indices, major fatty acids and sterol compositions with respect to the year of production, with the exception of total phenols [7].

To investigate the effect of growing location on fatty acid profile and mid-IR spectra, PCA was performed separately on varieties A and G from two different areas (Izmir and Edremit). Overall statistics of PCA class models was shown in Table 4. Fig. 4a displays Coomans' plot in which A2 and AE2 are plotted against each other using fatty acid profiles. Samples of 2006/07 harvest year, A2
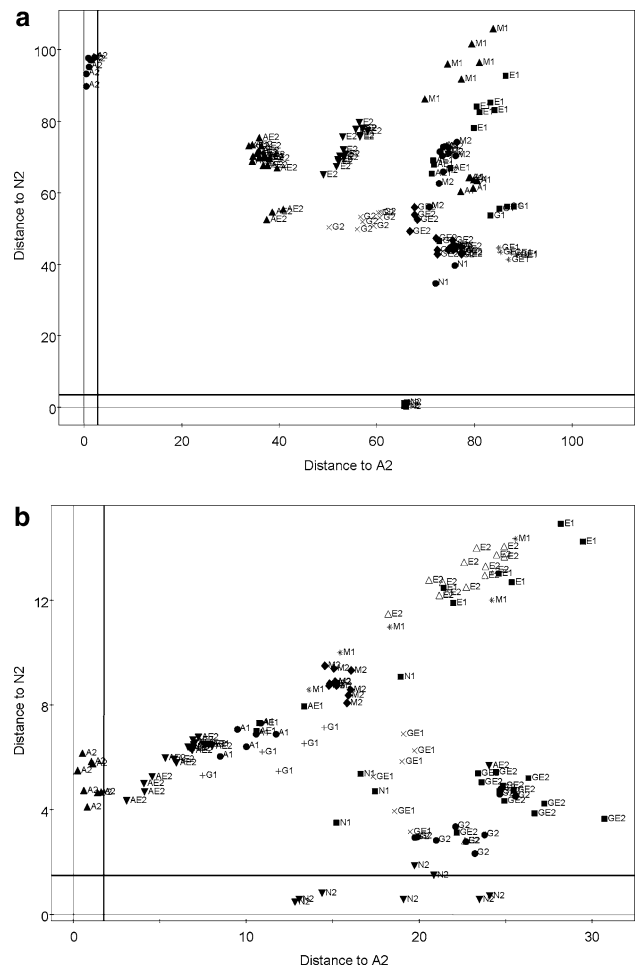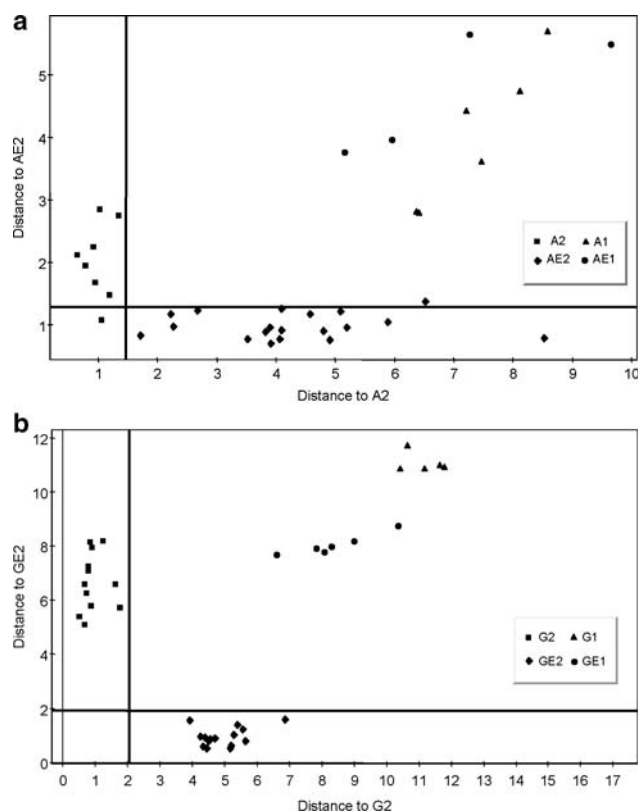

Fig. 3 Coomans' plot for the classification of olive oil samples with respect to variety and harvest year (2005/06 denoted by 1 and 2006/07 denoted by 2), using (**a**) fatty acid profile and (**b**) mid-IR spectra

and AE2, were accepted by their own class models. On the other hand, samples of 2005/06 harvest year, A1 and AE1, were plotted among the critical limits of A2 and AE2 model. Similarly, quite successful differentiation of G2 and GE2 is observed in Fig. 4b. According to these observations, geographical origin and harvest year have significant influence on fatty acid profile of the olive oil. A previous study on Sicilian olive oils suggested that although the effect of the cultivar is predominant in the olive oil classification based on the fatty acid composition, a minor but well-defined geographic effect is also present [17]. In another study, Stefanoudaki et al. [10] was able to discriminate olive oil samples with respect to growing location based on altitude due to differences in environmental factors such as relative humidity and rainfall. Figure 4 represents Coomans' plot constructed using mid-IR spectra to differentiate A from AE (Fig. 4a) and G from GE (Fig. 4b). Although, most samples of the A2 and AE2 are classified correctly (Fig. 5a), some samples of G2
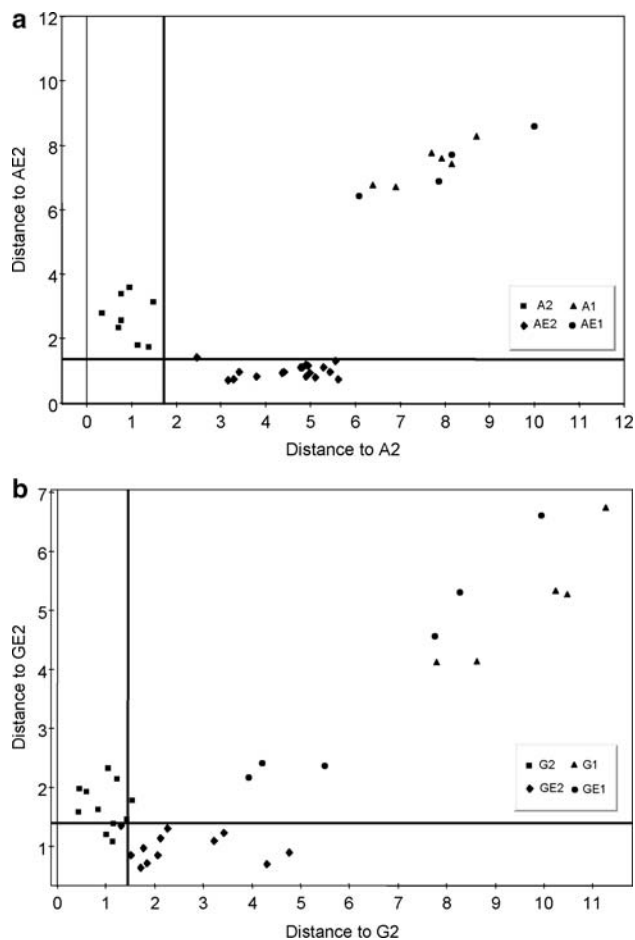
Table 4 PCA class models and general statistics of each A–AE and G–GE class models for fatty acid and Mid-IR spectral data

Class models	Fatty acid profile		Mid-IR spectra	
	Number of PCs	R^2X(cum) (%)	Number of PCs	R^2X(cum) (%)
PCA class models of A–AE				
A1	3	94.6	3	89.5
A2	4	97.5	5	94.6
AE1	2	84	2	86.3
AE2	4	92.7	6	86.2
PCA class models of G–GE				
G1	3	96.3	2	75.9
G2	3	93.4	5	95.3
GE1	4	87	2	83.3
GE2	2	84.2	3	84.7

R^2X(cum): cumulative sum of squares of the entire Xs explained by all extracted components

Fig. 4 Coomans' plot for classification with respect to geographical origin and harvest year (2005/06 denoted by 1 and 2006/07 denoted by 2) for (**a**) Ayvalık and (**b**) Gemlik olive oil varieties, using fatty acid composition

Fig. 5 Coomans' plot for classification with respect to geographical origin and harvest year (2005/06 denoted by 1 and 2006/07 denoted by 2) for (**a**) Ayvalık and (**b**) Gemlik olive oil varieties, using mid-IR spectra

countries, which probably have more variable chemical properties.

In this paper, the efficiency of fatty acid composition and mid-IR spectra in combination with PCA for the differentiation of 14 olive oil class models was demonstrated and compared. Results showed that application of PCA to fatty acid composition is quite successful for the classification of olive oil samples with respect to variety, geographical origin and harvest year. On the other hand, mid-IR spectra cannot supply distinct varietal, geographical or seasonal grouping as much as fatty acid composition does. Nevertheless, with the advantage of rapid and easy analysis ability, FTIR can be used for the more general authentication issues with respect to harvest year and variety.

are plotted beyond its critical limits (Fig. 5b). More distinct geographical differentiation compared to our study was achieved using FTIR in another study [15], because olive oil samples used in this study were from different

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