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ORIGINAL PAPER

Chemometric Studies on zNose™ and Machine Vision Technologies for Discrimination of Commercial Extra Virgin Olive Oils

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Abstract The aim of this study was to classify Turkish commercial extra virgin olive oil (EVOO) samples according to geographical origins by using surface acoustic wave sensing electronic nose (zNose™) and machine vision system (MVS) analyses in combination with chemometric approaches. EVOO samples obtained from north and south Aegean region were used in the study. The data analyses were performed with principal component analysis class models, partial least squares-discriminant analysis (PLS-DA) and hierarchical cluster analysis (HCA). Based on the zNose™ analysis, it was found that EVOO aroma profiles could be discriminated successfully according to geographical origin of the samples with the aid of the PLS-DA method. Color analysis was conducted as an additional sensory quality parameter that is preferred by the consumers. The results of HCA and PLS-DA methods demonstrated that color measurement alone was not an effective discriminative factor for classification of EVOO. However, PLS-DA and HCA methods provided clear differentiation among the EVOO samples in terms of electronic nose and color measurements. This study is significant from the point of evaluating the potential of zNose™ in combination with MVS as a rapid method for the classification of geographically different EVOO produced in industry.

Keywords Extra virgin olive oil · Electronic nose · Machine vision system · Chemometrics

Introduction

The fragrant and unique aroma of olive oils with nutritional and health beneficial properties are the main reasons for the increased popularity of olive oil in the world [1]. The organoleptic quality and stability of olive oil are mainly attributed to the unsaponifiable fraction of olive oil. This fraction includes minor constituents consisting of volatile compounds that vary with vegetal species, climatic conditions, extraction and refining procedures, and storage conditions [2]. The volatile compounds identified in olive oils include aldehydes, alcohols, esters, hydrocarbons, ketones, furans and other compounds. The high quality olive oils are the most desired and expensive grades. The control of these olive oils is significant for producers and consumers [3, 4]. These controls are associated with authenticity and quality issues and certifications are done according to geographical origins by protected designation of origin (PDO), protected geographic indication (PGI) and typical geographic indication (IGT) [5]. Turkey makes a great contribution to the olive oil economy in the world with respect to the appropriate geographical conditions of the country [6]. Especially, the main cultivars Ayvalık and Memecik are harvested from the north and south Aegean of Turkey, respectively [7]. Color is a significant property for food products associated with other chemical and physical properties and its great influence in consumers' preferences. Olive oil color is also an important attribute for consumers related with quality [8].

Electronic nose technology is based on detection of the volatile compounds present in the headspace of a food sample

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[9]. The instrument enables classification of the food product by obtaining its aroma fingerprint [10]. Its advantages include a relatively small amount of sample preparation and the speed of analysis [11]. There have been some successful applications of electronic nose technology for the differentiation of olive oils regarding their geographical origins [12–14]. Chemometric methods have been widely carried out for discrimination among cultivars and geographical origins of olive oil in recent years [15–17]. Nevertheless, according to our knowledge, there is no detailed study in the literature that has proposed to investigate the effect of geographical origin on the aroma profiles and color of Turkish commercial olive oils by using easy, fast and non-destructive methods such as zNose™ and MVS in combination with different chemometric methods.

The major aim of this study was to evaluate the effect of aroma fingerprints and color of Turkish commercial EVOO samples obtained with zNose™ and MVS analyzed with chemometric methods on the classification of olive oils according to the geographical origins and crop years of olive oils. For this purpose, some of the basic chemometric methods such as principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA) and hierarchical cluster analysis (HCA) were applied to electronic nose and color data.

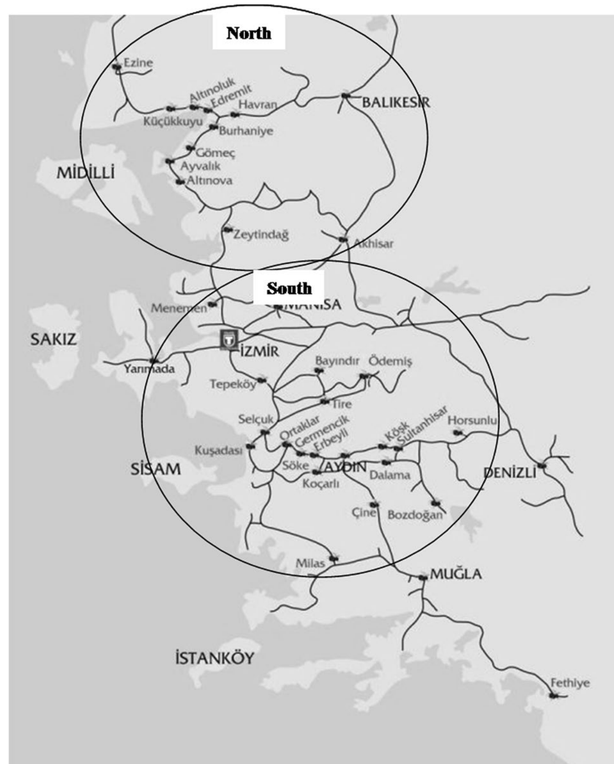

Fig. 1 Commercial EVOO samples obtained from north and south Aegean regions

Materials and Methods

Olive Oil Samples

Commercial extra virgin olive oils from different locations of the north and south Aegean regions of Turkey (Fig. 1) were obtained from Tariş Olive and Olive Oil Agricultural Sales Cooperatives Union and analyzed over two consecutive crop years (2005–2006 and 2006–2007). They were categorized into two groups that included 21 and 25 olive oil samples (2005/06 stated as the crop year 1 and 2006/07 stated as crop year 2). These commercial EVOO were obtained using the same process. Approximately 500–1000 ml was obtained for each oil sample and stored in dark brown bottles at 8 °C until they were analyzed. The names and codes of these oil samples are given in Table 1. The olive oil samples of crop year 1 were denoted by a sample code and the number 1, the olive oil samples of crop year 2 were denoted by the sample code and the number 2.

Electronic Nose Analysis

Analysis was performed by using an electronic nose (zNose™ 7100 vapor analysis system, Electronic Sensor Technology, CA, USA) to obtain the aroma fingerprints of commercial EVOO samples. The zNose™ consists of a 1-m DB-5 column and a surface acoustic wave (SAW) detector. The aroma fingerprint of the olive oil sample is composed of volatile compounds that were not identified by the electronic nose system.

The zNose™ used the Microsense software to interpret the results of the analysis. The frequency was read directly from the SAW detector that was shifted by the effect of the mass of each volatile compound. The frequency *versus* time plot was transformed to the first derivative of frequency *versus* time graph by the software. In the derivative plot, each peak corresponded to a specific volatile compound having a retention time. The area under the peak correlated with the concentration of the volatile compound and it was expressed in counts. Therefore, the zNose™ plot was constructed with the counts *versus* retention time. The counts of each peak were used in data analysis.

Ten milliliters of oil sample was transferred into a 40-ml septa-sealed vial and left overnight at room temperature prior to analysis. The vials were then placed into a water bath at 30 °C for 15 min. During this time, the oil samples were allowed to equilibrate with the headspace in the vial and then the sample's vapor was pumped into the zNose™ with a side-ported sampling needle through the septa. Before the sample analysis, the system was calibrated with n-alkane solution (C_6–C_{14}). After calibration, the samples were measured one at a time with the zNose™. For each oil sample, the average of six vials with four readings that corresponded to an average of 24 independent measurements was calculated to use in the data analysis.

Table 1 Locations, geographical regions, and sample codes of commercial EVOO samples

Location	Geographical region	Samples codes of CY1	Sample codes of CY2
Ezine	N	E1	E2
Ezine Gülpınar Organik	N	EZ1	
Küçükkuyu	N	KK1	KK2
Altınoluk	N	AOL1	AOL2
Altınoluk-Sulubaskı	N	AS1	
Edremit	N	ED1	ED2
Havran	N	H1	H2
Burhaniye	N	B1	B2
Gömeç	N	G1	G2
Ayvalık	N	A1	A2
Altınova	N	AO1	AO2
Zeytindağ	N	Z1	Z2
Akhisar	S	AK1	
Menemen	S	M1	
Tepeköy	S	T1	T2
Bayındır	S	BA1	BA2
Selçuk	S	S1	S2
Aydın	S	AY1	AY2
Ortaklar	S	O1	O2
Koçarlı	S	K1	K2
Milas	S	ML1	ML2
Ödemiş	S		OD2
Tire	S		T2
Kuşadası	S		KA2
Germencik	S		GE2
Köşk	S		KS2
Dalaman	S		DA2
Erbeyli	S		ER2
Çine	S		C2

N north Aegean region, *S* south Aegean region, *CY1* 2005/2006 crop year, *CY2* 2006/2007 crop year

Machine Vision System Analysis

Twenty five milliliters of oil samples was transferred into glass Petri dishes (60 × 15 mm) and placed in the machine vision system (MVS) having two D65 fluorescent lamps at the top of the light box (ECS Inc., Gainesville, FL, USA) and a CCD digital camera (Sony DFK 21BF04, The Imaging Source Europe GmbH, Bremen, Germany). Two images of each oil sample were taken and five color features, lightness ($L*$), redness–greenness ($a*$), yellowness–blueness ($b*$), chroma, and hue, were extracted with the ColorExpert software (ECS Inc., Gainesville, FL, USA). $L*$ values vary from 0 (black) to 100 (white). Hue angle and chroma are derived from $a*$ and $b*$ values: the saturation index or chroma [$C* = (a*^2 + b*^2)^{0.5}$] is associated with brightness or vividness of a color, and the hue angle describes the sense of color [$h° = \tan^{-1}(b*/a*)$].

Multivariate Statistical Analysis

Discrimination of olive oil samples based on their aroma profiles was performed by using chemometric methods such as PCA class model, PLS-DA and HCA. PCA class model and PLS-DA were applied to classify oil samples according to their geographical origin by using Soft Independent Modeling of Class Analogy (SIMCA-P v.11.5) software (Umetrics, Umea, Sweden). Coomans' plot is used to show the orthogonal distances of the samples to two selected class models [18]. PLS-DA was carried out to make an estimation of the classification model for all olive oil samples by analyzing the zNose data and color data in two crop years [19]. In PCA class and PLS-DA models, the data matrix X consisted of 46 observations (olive oil samples) and 21 variables [zNose data (16 Peaks), color data (five color coordinates)]. The data matrix was used according to the variable that was taken into account.

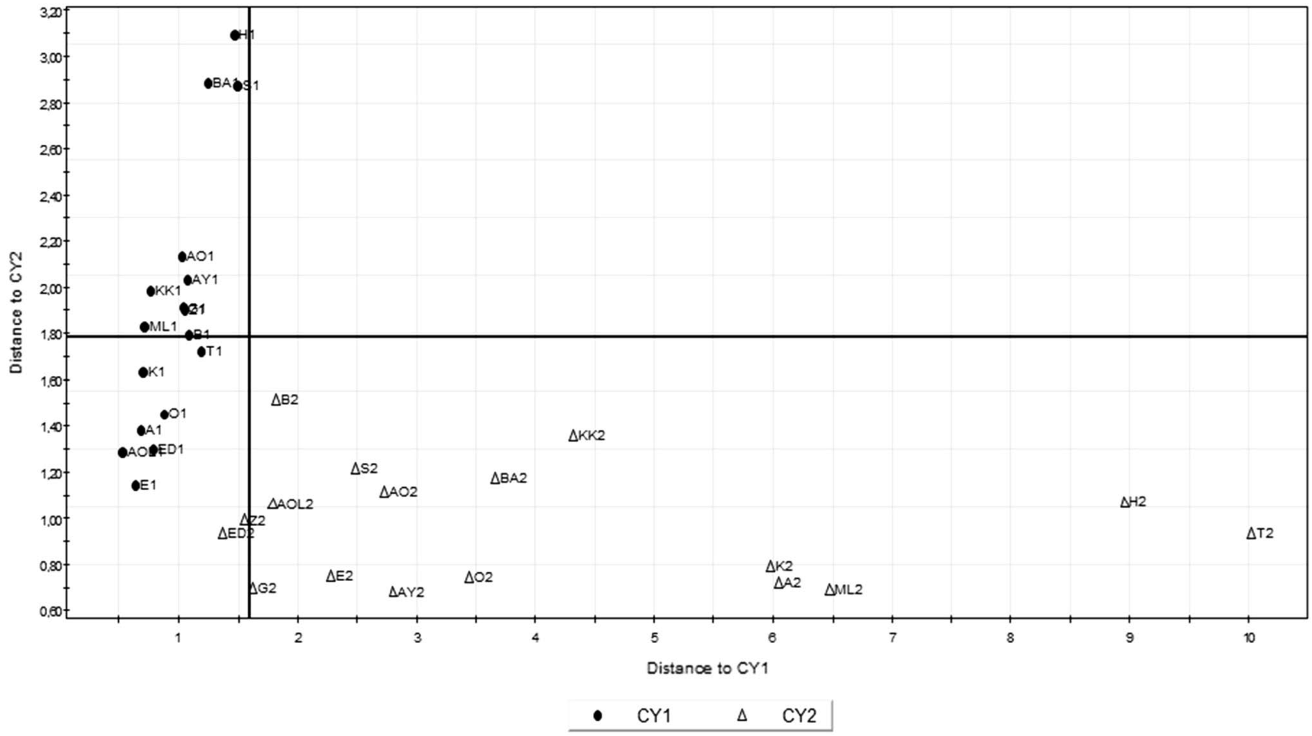

Fig. 2 Coomans' plot for the classification of olive oils according to crop years using electronic nose data

The pretreatment was not applied on data. The leave-one-out cross validation was used for validation of models by SIMCA software.

HCA was used as an unsupervised pattern recognition technique to find the natural tendency of the olive oil samples to classify them according to different geographical origins using Statistica software (StatSoft Inc., Tulsa, OK, USA). The similarity matrix is calculated by using the distance between the two oils with the Manhattan distance [20]. The complete linkage method is used to cluster the olive oil samples and figured by a vertical hierarchical tree plot.

Results and Discussion

This study presents the potential of zNose™ and MVS for the classification of olive oil samples with respect to the crop year and geographical origin. The commercial olive oil samples used in this study are economically important olive oil samples extracted with the same system. Therefore, the effect of crop year and geographical region on the aroma profiles and color of olive oils was investigated in a comparable way. The aroma fingerprints of olive oil samples and the color measurements were utilized to build classification models. All data were analyzed using PCA class model, PLS-DA and HCA methods and illustrated graphically.

In the PCA class model, the discrimination of olive oil samples was performed by analyzing the electronic nose data set with respect to crop years. The Coomans' plot showing the clustering of CY1 olive oils against CY2 olive oils is shown in Fig. 2. This plot was constructed by plotting the Mahalanobis distance between olive oils of different crop years. The olive oils of CY2 were grouped correctly in their own region showing variable aroma fingerprints among the samples. However, some olive oil samples that belong to CY1 class model fell in the common plot area closer to the CY2 olive oils. This can be related to the similar aroma profiles of these olive oil samples analyzed with the electronic nose. The impact of crop years on the discrimination of different varieties of olive oil samples was shown by using mid-IR spectra and fatty acid profiles of olive oil samples and PCA method, previously [21].

The aroma profiles of olive oil samples belonging to two crop years were analyzed with PLS-DA to demonstrate the effect of the different geographical origins on the electronic nose analysis. Figure 3 demonstrates that the olive oil samples that belong to the north and south regions were classified correctly. However, AS1, H1, and H2 olive oil samples that were obtained from the north region could not be differentiated from the olive oils obtained from south

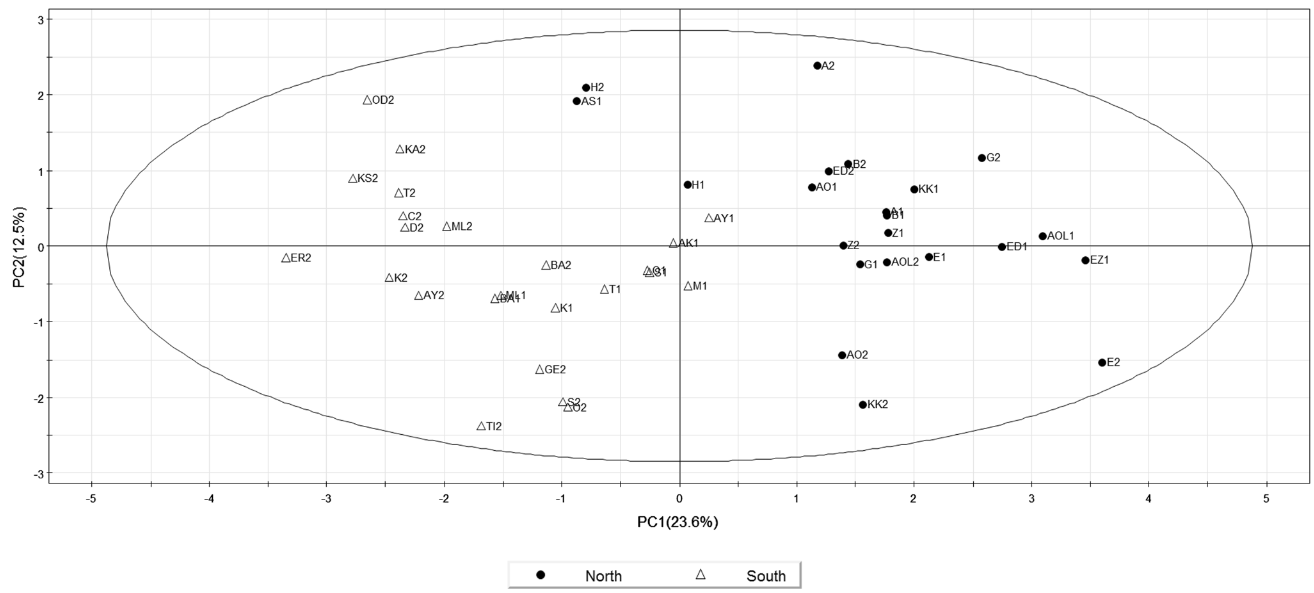

Fig. 3 PLS-DA score plot for the classification of olive oils according to geographical origin using electronic nose data

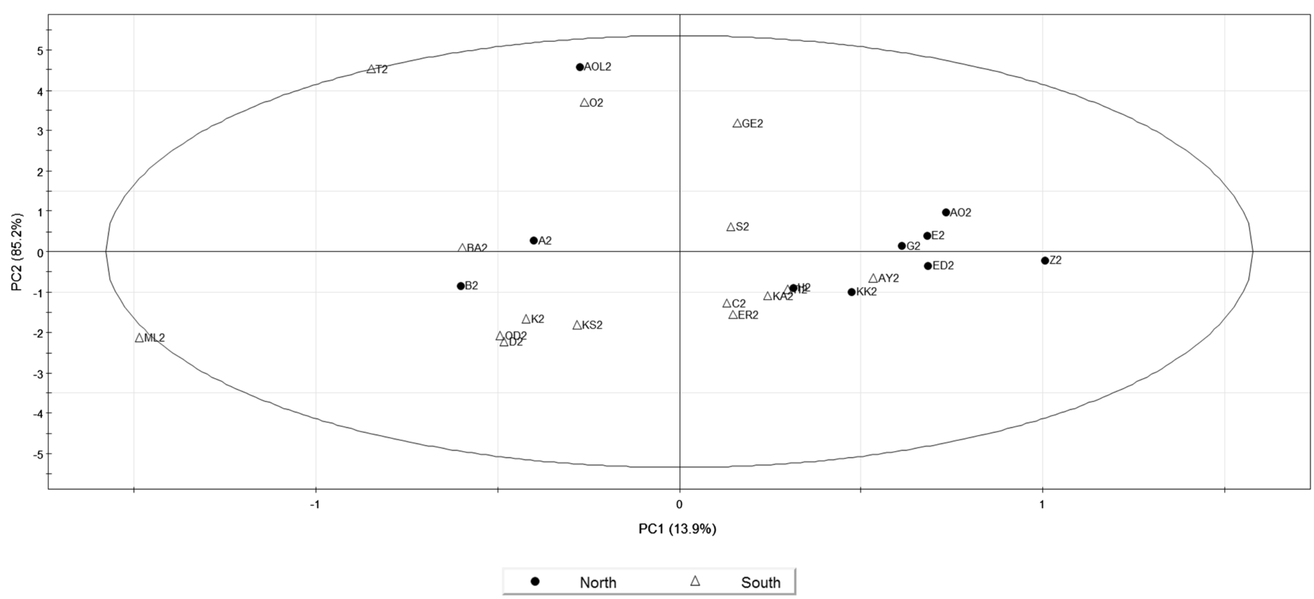

Fig. 4 PLS-DA score plot for the classification of olive oils using color data

region which could depend on the similar aroma profiles of these olive oil samples. In a previous study, the effect of geographical origin, ripening degree and irrigation regime on the volatile profiles of olive oils obtained from Tunisian and Sicilian cultivars was investigated [22]. Similarly to our findings, the results demonstrated that the volatile compounds obtained by using SPME-GC/MS and GC/FID methods were significantly affected by agronomic conditions.

The effect of color on the discrimination of olive oil samples of different regions was analyzed by constructing the score plot for the first two dimensions of the PLS-DA model with CY2 olive oils. The plot shows that north and south classes of olive oils display a broad spread and could not be separated clearly based on their color values as illustrated in Fig. 4. Therefore, the electronic nose and color data were combined to visualize the effect of these two quality parameters on differentiation of olive oils. It

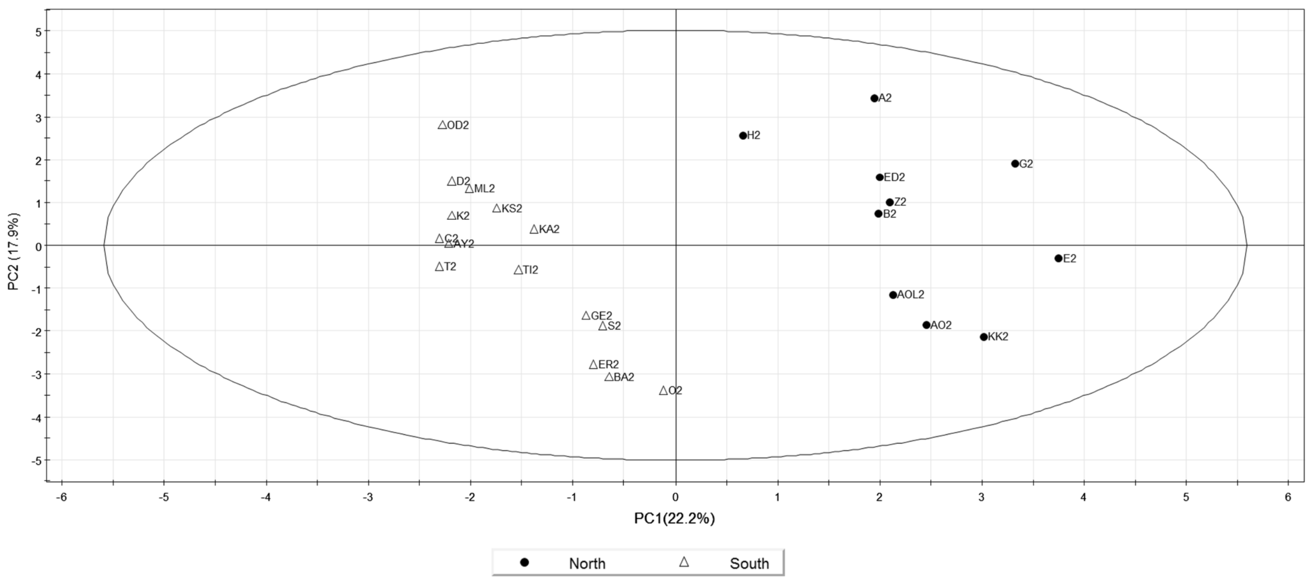

Fig. 5 Score plot of PLS-DA for the classification of olive oils using color and electronic nose data

was observed that color is not a strong discriminative factor on the classification of commercial olive oils based on their geographical origins. Otherwise, it can be used as an additional factor with the electronic nose data because the olive oil samples could be classified successfully by applying PLS-DA to both electronic nose and color data as given in Fig. 5 according to their geographical regions. In previous studies, the relationship between the volatile composition of olive oils and different factors such as fruit genotype, ripening, processing equipment, climate, soil type and geographical origin was reported [23, 24]. In one of these studies, the effect of cultivar, processing methods, anthracnose attack and stone removal on the volatile profiles of the olive oil samples of different regions in Italy was demonstrated by using the SPME-GC/MS method with PCA analyses [25]. In another study, the influence of fruit variety and ripeness stage of the olive oil samples on the volatile composition of the oils were studied by using GC–MS and sensory analyses [26].

The electronic nose analysis of olive oils in two crop years were evaluated together by HCA and is shown in Fig. 6. The dendrogram based on HCA results could be separated into two groups as Erbeyli and all other oil samples, based on their aroma profiles. The second group consists of one block corresponding to the north region and three blocks corresponding to the south region of the Aegean area. North region olive oil samples, Altınoluk-sulubaskı and Havran, were found to be categorized with the south region olive oil samples. The dendrogram confirmed the results found with PLS-DA. PCA and HCA analyses were performed previously to classify olive oils based on some quality parameters such as acidity and peroxide values.

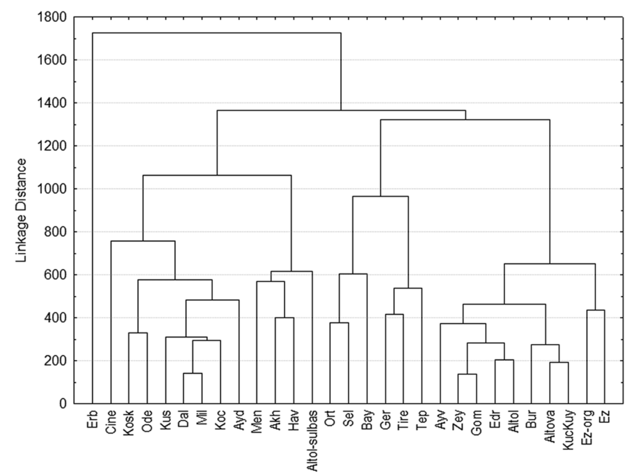

Fig. 6 Dendrogram showing the clustering of olive oil samples of crop years 1 and 2 based on electronic nose data

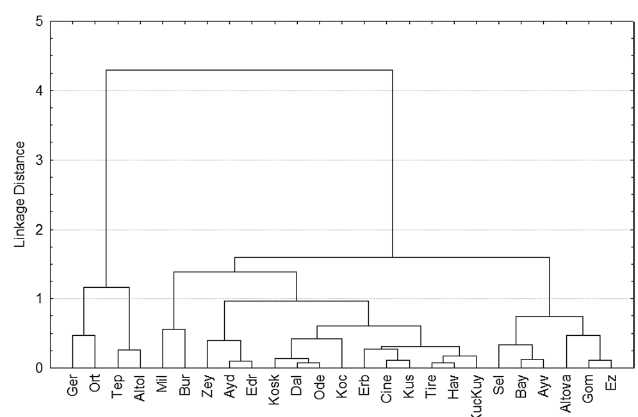

Fig. 7 Dendrogram showing the clustering of olive oil samples of crop year 2 based on color data

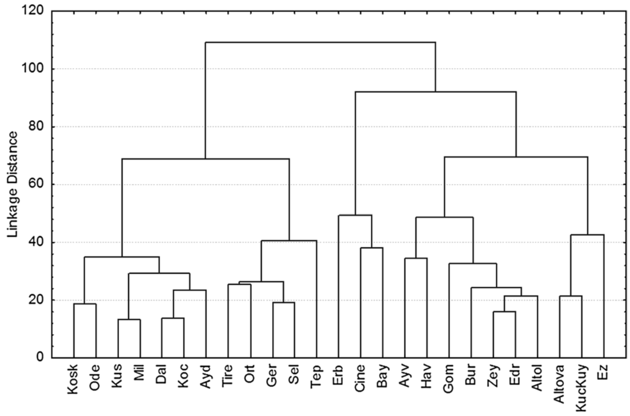

Fig. 8 Dendrogram showing the clustering of olive oil samples of crop year 2 based on electronic nose and color data

Acidity is also the only difference between the edible and lampante olive oils. The discrimination between these olive oils was established successfully by measuring total luminescence and synchronous fluorescence spectra of olive oils combined with PCA and HCA methods obtained previously [27]. In our study, the dendrograms constructed with different olive oils exhibited good classification with olive oils of different origins.

The dendrogram given in Fig. 7 shows the clusters formed in consequence of HCA of color data of the olive oil samples obtained in crop year 2. Cluster analysis using Manhattan distance produced two main groups with one block of Kosk, Dal, Ode, Koç and another block of Erb, Çine, Kus that separate from other olive oil samples. Clear classification could not be obtained by this method as demonstrated in PLS-DA analysis for using only color data. The responses obtained by electronic nose and the combination of electronic nose and color data processing were quite similar, this being the reason why only the case of dendrogram of electronic nose data was not presented. The dendrogram based on HCA of electronic nose and color data obtained for crop year 2 are shown in Fig. 8. This figure indicates that EVOO samples could be separated into two groups based on their aroma and color profiles. Olive oils in the first group consist of the samples obtained from olives cultivated from south region of Western Turkey. However, the second group mostly includes the olive oils from the north region olive oils except Erb, Çine and Bay; those essentially belong to the south Aegean region.

The differentiation of olive oils according to taste and aroma is significant for the consumer preferences as well as conserving genetic diversity. There have been a lot of successful applications of electronic nose technology for the differentiation of olive oils on the basis of geographical origin. An electronic nose based on MOS sensors and an electronic tongue were used for the discrimination of olive oils from different geographical regions in Morocco in a study [28]. It was stated that PCA, cluster analysis (CA) and support vector machine (SVM) data analysis methods were applied to discriminate the olive oil samples. The results showed that a low level of abstraction data fusion developed in combination with PCA, CA and SVM enhanced the discrimination capability of olive oils of different geographical origins. An electronic nose with chemometric analysis has also been used to verify the geographical origin of EVOO by Casale *et al*. [29] and successful results were obtained in the classification of 46 oil samples from three different areas of Liguria by the application of linear discriminant analysis (LDA). Similar to our study, all these previous studies demonstrated the usefulness of an electronic noses based on different sensors combined with chemometric methods for the classification of olive oils of different origins.

Conclusion

The potential of zNose™ and MVS for the classification of olive oils from different geographical regions and crop years was evaluated in this study. The results demonstrated that Turkish commercial EVOO samples could be discriminated well based on the aroma profiles and color measurements. The statistical analysis of the effect of geographical region on color revealed that the olive oils were not clearly differentiated based on their color individually. PCA class model, PLS-DA and HCA methods yielded successful classification patterns taking into consideration the differences in aroma and color profiles of olive oil samples. However, a more detailed study is needed to build classification models with a great number of data sets that would increase reproducibility and repeatability.

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