

Characterization of Potent Odorant Compounds in Turkish Olive Oils by GC-MS-Olfactometric Techniques

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Abstract

The purpose of this study was to determine the most powerful aroma-active compounds of olive oils obtained from Ayvalik (AYV), Gemlik (GEM) and Memecik (MEM) cultivars harvested in 2011, using gas chromatography-mass spectrometry-olfactometry (GC-MS-O). Simultaneous distillation and extraction (SDE) with dichloromethane was used for extraction of volatile components. The aroma-active compounds of olive oils were evaluated by aroma extract dilution analysis (AEDA). A total of 14, 12 and 12 aroma-active compounds within the range of ≥ 64 –1024 flavor dilution (FD) factors were detected in aromatic extracts of olive oils obtained from AYV, GEM and MEM cvs., respectively. The compounds having the highest FD factor (1024) were (*Z*)-3-hexenol (cut grass, herbal) and β -sesquiphellandrene (floral) for AYV oil and (*Z*)-3-hexenyl acetate (fruity) for MEM oil. Among these compounds, terpenes were the overwhelmingly largest aroma-active components followed by aldehydes.

Keywords: Olive oil; Aroma-active compounds; Olfactometry; Sensory analysis

1 Introduction

Olive oil is a main food for the people and an important issue for the economic activity of the Mediterranean region. In the specialty food arena, olive oil is a dominant species that continues to grow in popularity. According to olive oil quantity data released in 2012, Spain (1,383,000 tonnes), Italy (572,000 tonnes), Greece (350,200 tonnes), Turkey (206,300 tonnes), Syria (200,000 tonnes) and Tunisia (192,600 tonnes) are the leading countries (FAO, 2012). Olive oil is extracted from fresh healthy olives using gentle

physical procedures such as milling, malaxing and centrifugation. The composition of virgin olive oil is influenced by many factors such as variety, degree of fruit ripeness, climatic conditions, growing region and extraction process techniques (Rigane, Bouaziz, Sayadi, & Ben Salem, 2013). Virgin olive oil (VOO), when extracted from fresh and healthy olives (*Olea europaea* L.) and properly processed and stored, is characterized by a unique, highly appreciated combination of aroma and taste (Kiritsakis, 1998; Angerosa, Mostallino, Basti, & Vito, 2000). Fresh and good-quality VOO is appreciated by consumers

for its delicious taste and aroma. The formation of volatiles is the basic component of VOO quality that directly relates to the mechanical extraction process itself. The characteristic aroma of VOO, and in particular, the green and fruity attributes, depend on many volatile compounds derived from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase (LOX) pathway taking place during the oil extraction process. The sensory qualities of unsaturated C₆ aldehydes and alcohols are related to the so-called 'green odour' of this product. The presence of these compounds in good-quality virgin olive oils is critical to provide the typical fresh 'green notes' that they are known for (Preedy & Watson, 2010).

Only a small fraction of this large number of volatiles in olive oil actually contributes to the overall aroma. With the aid of an "olfactometric technique", aroma-active substances can be detected in the complex mixture of hundreds of aroma compounds (Acree, 1997; Jackson & Linskens, 2002). Olfactometry has been used almost since the introduction of gas chromatography, as the human nose is the most appropriate detector to monitor the presence of an odorant in the effluent of a gas chromatograph (Fuller, Tisserand, & Steltenk, 1964). A few researchers have studied the odour active compounds of olive oils: Reiners and Grosch (1998) studied the potent odorants of virgin olive oils from Italy, Spain and Morocco by aroma extract dilution analyzes (AEDA) and GC-O of headspace samples; Dierkes, Bongartz, Guth, and Hayen (2012) studied aroma and aroma-active compounds of 95 olive oil samples obtained from olive cultivars of different countries; Kesen, Kelebek, and Selli (2014) characterized the key aroma compounds in Turkish Nizip yaglik and Kilis yaglik olive oils from different geographic origins by application of aroma extract dilution analysis.

To date, only limited studies have been published in the literature on the aroma-active compounds of olive oils with a representativeness evaluation of its extract. Therefore, the aim of this study was first to assess the representativeness of oil aroma extract obtained by simultaneous steam distillation-extraction and second to characterize the potent aroma compounds in Turkish olive

oils using aroma extract dilution analysis with GS-MS-Olfactometry.

2 Materials and Methods

2.1 Reagents

The water used in the study was purified by a Millipore-Q system (Millipore Corp., Saint Quentin, France). Dichloromethane, sodium chloride, sodium sulphate, hexanal, (*E*)-2-hexenal and (*Z*)-3-hexenal were obtained from Fluka (Buchs, Switzerland). 1-Penten-3-ol, 3-penten-2-ol, hexanol, (*Z*)-3-hexenol, hexyl acetate, (*Z*)-3-hexenyl acetate, β -pinene, 2-ethyl-(*E*)-2 butenal, 2-ethenyl-2-butenal and (*Z*)-2-pentenol were purchased from Sigma-Aldrich (Steinheim, Germany), and α -copaene, zingiberene, (*E*, *Z*)- α -farnesene, α -farnesene, (*E,E*)- α -farnesene, (*E,E*)-2,4-hexadienal, β -sesquiphellandrene, hexanoic acid and γ -dodecalactone came from Merck (Darmstadt, Germany).

2.2 Materials

For each olive variety, an average sample of 50-70 kg olives was processed. Ayvalik, Gemlik and Memecik olives cultivated in Balikesir, Bursa and Mugla provinces (western part of Turkey), respectively, were harvested at optimum maturity stage in the 2011 season. The sampling was established in a randomized design with three replications and six trees were considered as a recurrence. The maturity index was 4.0, 4.0 and 3.9 in Ayvalik, Gemlik and Memecik olive samples, respectively. This method was based on the assessment of the colour of the olive skin and flesh (IOOC, 2011).

2.3 Methods

Olive oil extraction

A cold pressing process with dual phase centrifugation (Oliomio mini, Italy) was used for olive oil extraction. Fresh olive oils were put into glass bottles and were preserved in a dark and cool place until analysis. Cold press had

already been used to produce high quality olive oils (Kanavouras, Kiritsakis, & Hernandez, 2005; Ranalli, Contento, Schiavone, & Simone, 2001).

Standard chemical analysis

The moisture content (%) by heating at $105 \pm 1^\circ\text{C}$ in the oven and oil content (%) by soxhlet apparatus using n-hexane solvent were analyzed. At the same time, free fatty acids (AOCS, 2009), peroxide value (AOCS, 2003) and colour measurements using Hunter colorimeter (HunterLab, Color QuestXE-USA) were determined. Colour results for L^* , a^* and b^* colour system profile were recorded using CIE (Commission Internationale de l'Eclairage). Measurements were made at room temperature and in triplicate.

Extraction of the volatile compounds

Simultaneous distillation and extraction (SDE) in a Likens-Nickerson apparatus was used to extract the volatile compounds of olive oils. This method has already shown its reliability for the extraction of volatile components of olive oils (Caja, del Castillo, Alvarez, Herraiz, & Blanch, 2000; Vichi, Guadayol, Caixach, Lopez-Tamames, & Buxaderas, 2007). For the extraction, 40 mL of olive oil, 100 mL of pure water and 25 mL of 30% NaCl were put into a 500 mL distillation flask on one hand, and on the other 40 mL of dichloromethane solvent was pipetted into the other 100 mL distillation flask. Both of them were inserted into each heater, and then extraction was performed for approximately 3 hours (Kesen et al., 2014). After the dehydration by anhydrous sodium sulfate, the organic extracts were condensed to 5 mL in a Kuderna-Danish concentrator and then to 200 μL under a gentle stream of pure nitrogen. The entire process was repeated three times.

Representativeness test of the extract

A cardboard smelling strip (reference 7140 BPSI, Granger-Veyron, Lyas, France) was used to check the representativeness of the extract obtained SDE. Two solvents (dichloromethane and pentane + dichloromethane) were evaluated for representativeness using SDE. The representative-

ness of the extracts was determined according to Kesen, Kelebek, and Selli (2013). Results were analyzed by analysis of variance with Statgraphics Plus software (Manugistic, Inc. Rockville, MD, USA).

GC-MS-O analysis

The GC system consisted of an Agilent 6890 chromatograph equipped with a flame ionization detector (FID) (DE, USA), an Agilent 5973-Network-mass selective detector (MSD) (DE, USA), and a Gerstel ODP-2 (MD, USA) sniffing port using deactivated capillary column (30 cm x 0.3 mm) heated at 240°C and supplied with humidified air at 40°C . GC effluent was split 1:1:1 among the FID, MSD, and sniffing mode. Aroma compounds were separated on a DB-Wax (30 m length x 0.25 mm i.d. x $0.5\mu\text{m}$ thickness, J&W Scientific Folsom, CA, USA) column. A total of 3 μL of extract was injected in pulsed splitless (40 psi; 0.5 min) mode. Injector and FID detectors were set at 270°C and 280°C , respectively. The flow rate of carrier gas (helium) was 1.5 mL/min. The oven temperature of the DB-Wax column was first increased from 50°C to 200°C at a rate of $5^\circ\text{C}/\text{min}$ and then to 260°C at $8^\circ\text{C}/\text{min}$ with a final hold at 260°C for 5 min. The same oven temperature programs were used for the MSD. The mass detector was operated in the electron impact mode at 70 eV. The GC-MS interface and ionization source temperature was set at 250°C and 180°C , respectively.

Aroma extract dilution analysis (AEDA)

The original aroma extracts were analyzed by GC-MS-O using three experienced sniffers. For AEDA, the concentrated aromatic extracts (200 μL) of olive oils were diluted 1:1 stepwise using dichloromethane as the solvent to obtain dilutions of 1:1, 1:2, 1:4, 1:8, 1:16 and so on up to 1:1024 of the original extracts (Schieberle & Grosch, 1987; Steinhaus & Schieberle, 2000). Sniffing of dilutions was continued until no odorant could be detected by GC-MS-O. Each odorant was thus assigned a flavour dilution factor (FD factor) representing the last dilution in which the odorant was still detectable. FD factor

of aroma compounds increases, also the degree of flavour activity increases (Fickert & Schieberle, 1998; Tairu, Hofmann, & Schieberle, 2000). This method, as well as the determination of olive oil aroma-active compounds are used in many studies, including samples of orange juices (Averbeck & Schieberle, 2009) and fish (Cayhan & Selli, 2011).

Sensory assessment of olive oils

Sensory analysis of olive oil samples were evaluated by a group of 10 trained panelists according to the published methods of International Olive Oil Council determining the terms of organoleptic assessment (IOOC, 2013a). Olive oil samples (15 mL) were placed in a tasting glass coded with different four digits numbers. The temperature of samples was kept at $28 \pm 2^\circ\text{C}$. Each panelist smelled, then tasted the oil in the tasting glass and scaled intensity of the positive and negative attributes in the profile sheet.

Statistical analysis

The findings of this study were subjected to analysis of variance using the SPSS 17 software package (SPSS Inc, USA), and Duncan's multiple-comparison test was used to find significant differences at the $p < 0.05$ level.

3 Results and Discussion

3.1 Chemical composition of olive oils

Chemical composition of olive fruits and general quality parameters of olive oils are shown in Table 1. As can be seen, oil and moisture content of olives were found between 22.10 and 24.40% and 42.75 and 54.73%, respectively. Free fatty acids, peroxide values and colour values are usually used to evaluate the initial quality of edible oils (Martin-Polvillo, Marquez-Ruiz, & Dobar-ganes, 2004). Free fatty acid values of the three olive oils did not exceed the limit of 0.8% established for the extra virgin olive oil (IOOC, 2013b) and calculated 0.53, 0.54 and 0.62% for AYV, GEM and MEM, respectively. Peroxide values

were also below the limit of 20 meq oxygen/kg of oil, which is the accepted limit for extra quality of virgin olive oil. Peroxide values of AYV, GEM and MEM were, respectively, 7.82, 5.71 and 7.60 meq oxygen/kg of oil. Colour values of oil samples were statistically different ($p < 0.05$).

3.2 Representativeness results

Based on representativeness results, the dichloromethane solvent was selected for the extraction of olive oil aroma compounds. As previously stated, it is of great importance to assess the representativeness of the aromatic extracts in a matrix with characteristics similar to those of the original product (Selli et al., 2008; Serot, Prost, Visan, & Burcea, 2001). The similarity score of the aromatic extract obtained by SDE was found to be 75.7 mm and intensity score of aromatic extract was found to be 63.5 mm on a 100-mm unstructured scale. These scores were high and acceptable (Kesen et al., 2013). Briefly, the similarity and intensity results indicated that the representative aromatic extract was achieved with the SDE method in order to determine the aroma-active compounds of the olive oils.

3.3 GC-MS-O results

Olfactometric analysis results are shown in Table 2. Application of the AEDA on the olive oil extracts revealed 14, 12 and 12 aroma-active compounds in the AYV, GEM and MEM, respectively. The differences in the number of aroma-active compounds of the oils are mainly caused by concentration differences of these compounds. Among these compounds, terpenes (7 different terpenes) were overwhelmingly the largest aroma-active components, followed by aldehydes. The flavour dilution (FD) factors of the compounds were found to fall within the range of ≥ 64 -1024 (Table 2). The compounds having the highest FD factor (1024) were (*Z*)-3-hexenol and β -sesquiphellandrene for AYV oil and (*Z*)-3-hexenyl acetate for GEM oil. A total of six aldehydes and five alcohols were identified in all olive oils as aroma-active compounds (Table 2). Aldehydes are generally characterised by an in-

Table 1: Olive fruits and olive oils: general properties

Olive properties	AYV	GEM	MEM
Maturity index	4.0	4.0	3.9
Oil content (%)	24.40±0.15 ^a	22.80±0.08 ^b	22.10±0.06 ^b
Moisture content (%)	42.75±0.32 ^c	50.23±0.24 ^b	54.73±0.12 ^a
Olive oil properties			
Free fatty acids (oleic acid %)	0.53±0.01 ^b	0.54±0.01 ^b	0.62±0.10 ^a
Peroxide value (meq O ₂ /kg oil)	7.82±0.13 ^a	5.71±0.12 ^b	7.60±0.06 ^a
<i>L</i> [*]	61.59±0.05 ^a	44.24±0.01 ^c	60.57±0.07 ^b
<i>a</i> [*]	-4.69±0.01 ^b	-0.65±0.02 ^a	-5.04±0.01 ^c
<i>b</i> [*]	49.25±0.03 ^b	50.26±0.04 ^b	51.06±0.10 ^a

±: standard deviation. AYV: Ayvalik; GEM: Gemlik; MEM: Memecik.

Table 2: Aroma-active compounds of olive oil samples (FD ≥ 64)

No	LRI ^a	Aroma compounds	Odour description ^b	FD factor ^c		
				AYV	GEM	MEM
1	1074	Hexanal	Cut grass	-	-	64
2	1108	β-Pinene	Green plant, floral	64	-	-
3	1135	(<i>Z</i>)-3-Hexenal	Vegetables, grassy, herbal	256	64	256
4	1171	2-Ethyl-(<i>E</i>)-2-butenal	Grassy, floral	-	512	-
5	1176	1-Penten-3-ol	Grassy, green plant	512	128	128
6	1178	3-Penten-2-ol	Green, grassy	128	64	64
7	1190	(<i>E</i>)-2-Hexenal	Cut grass, green	-	-	512
8	1254	2-Ethenyl-2-butenal	Fruity, green	128	-	64
9	1285	Hexyl acetate	Fruity	64	128	64
10	1300	(<i>Z</i>)-3-Hexenyl acetate	Fruity	64	1024	-
11	1326	(<i>Z</i>)-2-Pentenol	Fatty	64	-	-
12	1350	Hexanol	Floral, grass	256	64	64
13	1378	(<i>Z</i>)-3-Hexenol	Cut grass, herbal	1024	512	-
14	1521	α-Copaene	Sweet, fruity	-	64	128
15	1702	Zingiberene	Floral	64	-	-
16	1715	(<i>E,Z</i>)-α-Farnesene	Floral	512	-	-
17	1745	α-Farnesene	Floral, green plant	-	256	64
18	1749	(<i>E,E</i>)-α-Farnesene	Floral, herb	64	-	-
19	1759	(<i>E,E</i>)-2,4-Hexadienal	Fatty, solvent	-	128	-
20	1784	β-Sesquiphellandrene	Floral	1024	64	-
21	1810	Hexanoic acid	Oily	-	-	64
22	2384	γ-dodecalactone	Fruity	-	-	512

^a LRI: Linear retention index calculated on DB-WAX capillary column.

^b Odour description: Odour description as perceived by panelists during olfactometry.

^c FD factor is the highest dilution of the extract at which an odourant is determined by aroma extract dilution analysis.

AYV: Ayvalik; GEM: Gemlik; MEM: Memecik

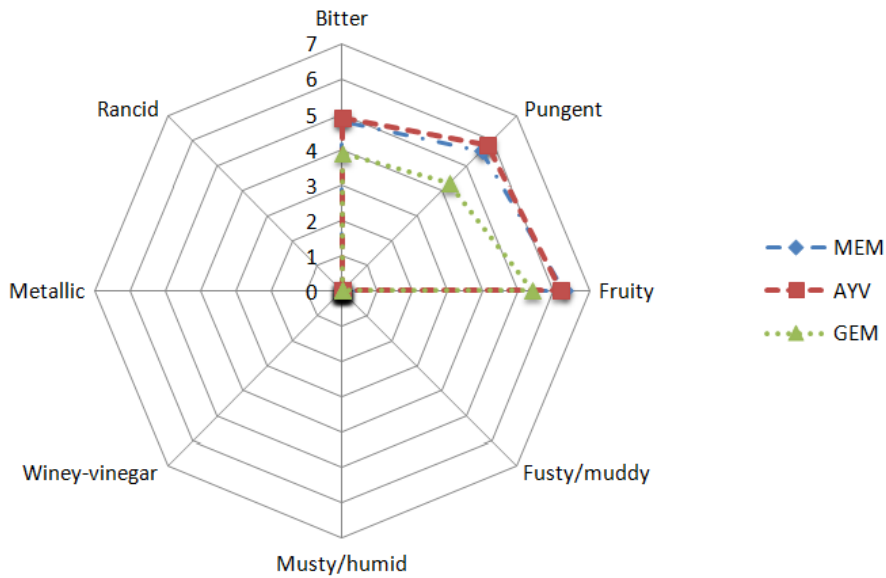


Figure 1: Sensory properties of AYV, GEM and MEM olive oils. (AYV: Ayvalik; GEM: Gemlik; MEM: Memecik)

tense sensory description by the panelists, associated with green, cut grass, green plant, citrusy and sweet notes (Kesen et al., 2014). The total number of aroma-active terpenes were 5, 3 and 2; aldehydes were 2, 3 and 4; and alcohols were 5, 4, 3 in AYV, GEM and MEM oils, respectively. Terpenes were the most dominant odour-active compounds in oil samples. β -Pinene (green plant, floral), α -copaene (sweet, fruity), zingiberene (floral), (*E,Z*)- α -farnesene (floral), α -farnesene (floral, green plant), (*E,E*)- α -farnesene (floral, herb) and β -sesquiphellandrene (floral) were detected as aroma-active terpene compounds in olive oil extracts. Terpene compounds generally provide a floral odour to olive oils. In a previous study, Kesen et al. (2014) also determined the terpenes as the key aroma compounds in Turkish olive oils. Based on GC-MS-O, the most potent aroma-active terpene was β -sesquiphellandrene (FD: 1024) followed by (*E,Z*)- α -farnesene (FD: 512) in AYV oil. β -sesquiphellandrene was also detected in GEM (FD: 64), but not in MEM oil. α -Farnesene in GEM (FD: 256) and α -copaene in MEM oils (FD: 128) were the most powerful aroma-active

compounds to contribute to the aroma profile. Among the terpenes detected by GC-O, copaene and α -farnesene were identified as being the most potent odorants in Moroccan green olives Iraqi, Vermeulen, Benzekri, Bouseta, and Collin (2005).

Aldehydes were the second largest class of odour-active compounds in oil samples. Among the aroma-active aldehydes detected by GC-MS-O, 2-ethyl-(*E*)-2-butenal (FD:512) for GEM oil and (*E*)-2-hexenal (FD:512) for MEM oil were the most powerful aroma-active compounds to contribute to the aroma profile. The first has grassy, floral and the second has cut grass, green notes. These compounds were not detected in other oil samples. It is interesting to note that 2-ethyl-(*E*)-2-butenal was detected in Nizip yaglik variety with a FD factor of 256 and (*E*)-2-hexenal was detected in Nizip yaglik and Kilis yaglik varieties with FD factors of 256 by Kesen et al. (2014). Brkić Bubola, Koprivnjak, Sladonja, and Lukić (2012) suggested that monovarietal virgin olive oils could be distinguished by the presence of (*E*)-2-hexenal. The next most important aroma-active aldehyde in terms of FD factors

was (*Z*)-3-hexenal (vegetables, grassy, herbal). The FD factor values of this compound were 256, 64 and 256 for AYV, GEM and MEM, respectively. Within the aroma-active aldehydes, (*Z*)-3-hexenal was common to all varieties. As previously stated, hexanal (green), (*E*)-2-hexenal (green, apple-like), nonanal (citrus-like), (*E*)-2-nonanal (paper-like, fatty) and (*E,E*)-2,4-decadienal were detected in olive oils by Dierkes et al. (2012) as aroma-active aldehyde compounds. It is worth noting that other important aroma-active aldehydes were 2-ethenyl-2-butenal (fruity, green) in AYV (FD: 128) and (*E,E*)-2,4-hexadienal (fatty solvent) in GEM (FD: 128).

Alcohols were the third largest class of odour-active compounds in oil samples. A total of five aroma-active alcohol compounds were found in the samples. These were 1-penten-3-ol (grassy, green plant), 3-penten-2-ol (green, grassy), (*Z*)-2-pentenol (fatty), hexanol (floral, grass) and (*Z*)-3-hexenol (cut grass, herbal). 1-Penten-3-ol, 3-penten-2-ol and hexanol were detected in all the samples as aroma-active alcohol compounds. The FD factors of these compounds were varied, depending on cultivars. Within the alcohols, (*Z*)-3-hexenol for AYV oil was the most powerful aroma-active compound to contribute to the aroma profile of the olive oils. The FD factor of this compound in AYV was 1024 and in GEM was 512. The other alcohol having the high FD factor (FD: 512) was 1-penten-3-ol in AYV oil. The FD factor of this compound was 128 in GEM and MEM oils. Similar potent alcohols found in our samples have already been identified in different olive oils (Luna, Morales, & Aparicio, 2006; Dierkes et al., 2012) and green olives (Iraqi et al., 2005).

Hexyl acetate (fruity) and (*Z*)-3-hexenyl acetate (fruity) were detected in oil extracts as aroma-active esters. Esters are responsible for the fruity notes (Selli & Kelebek, 2011). The presence of these compounds can be explained by the activity of alcohol acetyl transferase (AAT) enzyme. Increasing the AAT activity can enhance the production of volatile in olive oils (Aparicio & Morales, 1998). Among the esters, (*Z*)-3-hexenyl acetate had the highest FD factor (1024) value followed by hexyl acetate with FD factor of 128 detected by GC-O in GEM oil. These esters were also observed in olive oils by Luna et al. (2006)

and Kalua et al. (2007), as green, fruity and green notes.

The other detected aroma-active compounds were lactones and carboxylic acids. Lactones contribute to the characteristics fruity odours of the olive oil samples (Kesen et al., 2014). Only one γ -dodecalactone (fruity) compound was detected in MEM oil. The FD factor of this compound was 512. γ -Dodecalactone has also identified in black olives by Collin, Nizet, Muls, Iraqi, and Bouseta (2008). Hexanoic acid was only detected in MEM oil as aroma-active carboxylic acid. The FD factor of this volatile acid was 64.

3.4 Sensory analysis of olive oils

The evaluation of the sensory quality of olive oils involves perception of both positive and negative sensory attributes. The results of the sensory analysis of olive oils are given in Figure 1. Olive oils are classified as extra virgin olive oils if the median of defects is equal to zero and the median of fruity is more than zero (IOOC, 2013a). According to sensory analysis, the studied oils showed no defects, so they were considered extra virgin olive oils. The highest fruity median was found for MEM oil (6.30). This value was 6.20 and 5.40 for AYV and GEM oils, respectively. The values of olive oil bitterness was detected between 3.90 and 4.90. According to median value of bitterness, GEM oil had the lowest value and AYV oil had the highest value. Pungent property was detected at highest value (5.80) in AYV oil with lowest value (4.30) in GEM oil. The median of this positive attribute was observed as 5.60 in MEM oil. According to Regulation EEC/640/2008 (EEC, 2008) the term “medium” may be used where the median of the positive attribute concerned is between 3 and 6 and the term “intense” is used if higher than 6. In this study all olive oil samples were characterized as medium by bitter and pungent properties but intense in terms of fruity properties. Sensory analysis of olive oils were evaluated in previous studies by using the medians of defects and positive attributes (Anastasopoulos et al., 2012; Dierkes et al., 2012).

4 Conclusions

This study revealed potent aroma components that are responsible for the overall aroma of the Turkish olive oils obtained from AYV, GEM and MEM cultivars. A total of 14, 12 and 12 aroma-active compounds in the FD factor range of 64 to 1024 were observed in the AYV, GEM and MEM, respectively. Results show that the complex combination of several aroma-active compounds contributes to the overall aroma profile of the olive oils. The majority of aroma-active compounds were terpenes and aldehydes, followed by alcohols. The most powerful aroma-active compounds were (*Z*)-3-hexenol, β -sesquiphellandrene and (*Z*)-3-hexenyl acetate. Due to sensory analysis, the olive oils studied in the present work were defined as extra virgin. The highest fruity median was found in MEM oil followed by AYV and GEM, respectively.

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