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ORIGINAL ARTICLE

Grape seed oil volatiles and odour activity values: a comparison with Turkish and Italian cultivars and extraction methods

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Abstract Valorization of bioactive-rich wastes of food industry, such as grape seeds, is one of the most popular topic worldwide. The present study is designed to examine the volatiles of grape seed oils obtained by two Turkish (*cvs.* Okuzgozu and Emir) and two Italian (*cvs.* Sangiovese and Moscatello) cultivars by using two well-known oil extraction methods, cold percolation (CP) and soxhlet (SX). In order to evaluate their volatile composition, obtained oil extracts were subjected to purge and trap aroma extraction chamber combined with gas chromatography–mass spectrometry GC–MS. Revealed results showed that the oil yield, volatile compositions and odor activity values (OAVs) of grape seed oils altered depending on both variety and extraction method of the oil. According to results, a total of 60 and 67 volatile compound were detected in CP and SX aromatic extracts. High temperature applied during SX led to form new volatiles and increase in overall volatile composition due to oxidation reactions. Among all aroma groups, alcohols were the dominating aroma group followed by esters in each cultivar for both extraction methods. GSOs obtained by red grape varieties exhibited apparently higher ester concentration while white varieties were abundant in terpenes. Additionally, SX method caused to form some heat derived volatiles. Moreover, a total of 26 and 33 aroma compounds possessed OAVs greater than 1 and ethyl octanoate (sweet-apple odour), nonanal (fatty-citrus odour) and 1-octen-3-ol (mushroom, earthy odour) were found to be dominant volatiles with respect to their OAVs.

Keywords Grape seed oil · Aroma · Okuzgozu · Emir · Sangiovese · Moscatello

Abbreviations

GSO	Grape seed oil
SX	Soxhlet method
CP	Cold percolation method
LRI	Linear retention index
OAV	Odour activity value

Introduction

Valorization of bioactive-rich wastes in food industry is one of the most striking research topic worldwide due to the limited chance of supplying new, cheap and healthy food sources to the food chain. During winemaking, a substantial amount of solid waste generated and this waste contains about 25% of grape peels and seeds, namely the grape pomace, is known to be an outstanding source of bioactive materials that are responsible for the several health promising effects (Dwyer et al. 2014). In the last decades, a great attention has been increasingly drawn to grape seed oil (GSO) due to its rich polyphenolic content, spectacular health effects, pleasing and peculiar aroma.

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Therefore this valuable oil, has a particular popularity in food, pharmaceutic and cosmetic industries. As a by-product of wine industry, GSO, is known to be the remarkable source of natural antioxidants and anti-aging components and so it is considered as a potential economic gain (Gupta et al. 2020). According to data mentioned in the study of Yeler and Nas (2020), China (11.7 million tons), Italy (8.6 million tons), U.S.A. (6.9 million tons), Spain (6.9 million tons), France (6.2 million tons), and Turkey (3.9 million tons) are spearheading countries for the grape production worldwide. The global grape production reached up to 77.8 million tons and each year substantial amount of grape seeds, revealing from wine and food industry as a disposable waste (Yeler and Nas 2020). Due to the increasing demand for health promising foods, implementation of grape waste management has become an attractive opportunity especially in these countries.

A grape seed comprise a significant amount of extractable oil (7–20%), 20–30% extractable bioactive compounds, particularly phenolics, 10–15% proteins, 5–10% water, 30–40% dietary fibers, volatiles and some of minerals (Matthäus, 2008). One of the most important factors affecting the preference of edible oils is flavor which is formed as a result of the interaction between taste and smell properties. Volatile compounds play a significant role in the aroma properties, overall product quality and consumer preference of oil samples. Aroma substances which consist of various chemical groups and have low perception threshold values have direct effect on food quality and consumer preference. Aroma compounds found mostly in GSOs have been reported as short chain acids, alcohols, esters, some aldehydes and ketones (Bail et al. 2008).

The concentration and the quality of aroma and bioactive ingredients of GSO, highly depends on the variety, canopy management, ripening stage, climatic conditions and extraction procedure of the oil (Bombai et al. 2017). In seed oil industry, considering the usage area, extraction methodologies split in half whether they aimed to obtain the highest amount or the highest quality of oil from seeds. In this sense, the GSO used in the food industry is generally extracted by a solvent-free cold press extraction to protect its high nutritional quality and edibility (Kornsteiner et al. 2006). On the other hand, cosmetic industry is much more interested in the higher extraction yields due to their economical profit dependence. Solvent assisted extractions, supercritical liquid extraction, microwave, ultrasound and enzyme treatments are the most used commercial extraction procedures to obtain GSO (Sevindik and Selli, 2017). Sabir et al. (2012) investigated 21 grape varieties by means of their oil yields of soxhlet extraction and researchers declared that grape seeds possessed different amount of oil in a range of 7.3–22.3%. In another study, Lachman et al. (2015) examined GSO from 23 different grape varieties and found that the oil yield ranged between 3.9–17.3%. Apart from the quantity, the quality of this particular oil affects significantly with respect to oil extraction methodology due to the fragile structure of bioactive compounds. Therefore, extraction technique and its conditions such as temperature, pressure, solvent flow, particle size and extraction time play a crucial role to maintain the high quality grape seed oil. Among these parameters, high temperature applied during the seed drying or oil extraction influence aroma profile deeply (Bail et al. 2008).

Although a number of researches has been focused on the grape seed oil extraction, oil yield and characterization of bioactive compounds, there is a limited number of study investigating both variety effect and different extraction methods on the GSO volatile profile and oil yield. According to present knowledge, this is the first study investigating the volatile profiles of two Turkish (*cvs.* Okuzgozu and Emir) and two Italian (*cvs.* Sangiovese and Moscatello) grape seed oils, focusing on the effect of different extraction methods coded as CP (cold percolation) and SX (soxhlet extraction). Furthermore, the OAVs were calculated with the aim of estimating the contribution of aroma compounds to the overall scent of GSOs. Additionally, oil yields of four different grape seeds were determined in dry basis.

Materials and methods

Chemicals

Standard aroma compounds such as 3-penten-2-ol, 2-hexanol, isoamyl alcohol, 2-heptanol, 1-hexanol 1-octen-3-ol, 2-dodecanol 1,2-ethanediol, benzyl alcohol, phenyl ethyl alcohol, ethyl hexanoate, hexyl acetate, ethyl octanoate, ethyl nonanoate, ethyl benzoate, ethyl decanoate, diethyl succinate, benzyl acetate, phenyl ethyl acetate, buthyl butanoate, ethyl palmitate, ethyl laureate, ethyl linoleate, hexenal, octanal, (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-heptadienal, 2-nonenal, benzene acetaldehyde, (*E*,*E*)-2,4-decadienal, vanillin, (*E*)-2-heptenal, nonanal, (*E*,*E*)-2,4-nonadienal, α-copaene, linalool, α-caryophyllene, citronellol, δ-cadinene purchased from Sigma-Aldrich (Steinheim, Germany), and 4-nonanol, n-hexane and dichloromethane were obtained from Merck (Gernsheim, Germany).

Grape seeds

The grape cultivars used in the present study were selected upon their colour and local importance. Turkish varieties, *cvs.* Okuzgozu and Emir, are the most produced red and

white grape cultivars respectively, as same as the Italian red and white cultivars, *cvs.* Sangiovese and Moscatello. Turkish grape clusters were collected from the vineyards located in Nevsehir, Turkey (38°36′43.2"N, 34°50′04.0"E), while Italian varieties were harvested from the experimental vineyards of University of Bologna (44°17′7''N, 11°52′59''E) in Faenza, Italy. All grapes were harvested when they reached up about 25° brix value. Once the seeds were seperated manually from the flesh, they were dried in a laboratory scale oven (Memmert UNB 400, Germany) at 50 °C for 24 h and vacuum packaged, sealed (DZ-300/2SA, China) and stored in dark conditions at room temperature until analysis. Shortly before the analysis, dried grape seeds were grinded with a grinding machine Vibratory disc mill (RS 200, Retsch, Germany) and grinded grape seed powders were classified with a laboratory scale sifter up to 0,250—0,425 mm mesh size. Once the grape seeds became in powder form, divided into two lots for the oil extraction step. Two methods were used to extract oil from the grinded grape seeds; the cold percolation (CP) and the soxhlet (SX). Both of extractions were carried out in triplicates for each grape variety and oil yields were calculated in dry basis.

GSO extraction

Cold percolation (CP)

CP methodology was designed according to an earlier study performed by Shiozaki and Murakami (2016). Briefly, a 40 g of seed powder was put into a flask and 200 ml of n-hexane was added. The flask was covered with aluminum foil and corked. The flask was placed into a closed-system incubated shaker and was held at 25 °C for 24 h. As soon as the extraction was completed, solid residues were subjected to a centrifugation at 15 °C and 6000 rpm for 10 min to separate from the liquid phase. The liquid phase was then filtered by a filtration paper (11 µm) with the help of separating funnel. Afterwards n-hexane was removed from the oily fraction with the help of rotary vacuum evaporator (Buchi, Rotavapor, Switzerland) at 40 °C and under a low pressure (2,5 kPa). Eventually, the obtained oil was weighed in a precision balance and preserved in a capped amber glasses and stored at 10 °C °C until analysis.

Soxhlet extraction (SX)

The SX extraction performed in the present study was slightly modified from the method mentioned by Malićanin et al. (2014) and Sevindik et al. (2020). In short, a 40 g of dried and grinded seeds were equally divided into four lots and separately placed into cellulosic porous cartridges and outer side of extraction chambers were filled with 200 ml n-hexane. In order to achieve a complete extraction of oil, the soxhlet system (Soxtherm SOX404, Gerhardt, Germany) was set to a high temperature extraction at 80 °C for 5 h and then 45 min for rinsing and finally 30 min of solvent removal. Once this step was completed, residual solvent was removed by a rotary vacuum evaporator (Buchi, Rotavapor, Switzerland) at 40 °C and under a low pressure (2,5 kPa). Finally, the obtained oil was weighed in a precision balance and preserved in a capped amber glasses and stored at 10 °C until analysis.

Extraction and analysis of volatiles

The extraction procedure carried out in the present study was designed with respect to our earlier paper (Guclu et al. 2016). A 3 ml of oil samples were subjected to a purge and trap system consist of a flow regulator in order to control and split nitrogen flow which enables to purge four sample at the same time. Volatiles were purged with a constant flow and trapped by a specific adsorbent resins, namely Lichrolut EN (200 mg, Merck). This adsorbent was already stated in an earlier study as an appropriate material regarding the retention of volatiles (Sonmezdag et al., 2018). Previous to the gas flow, vials containing the oil samples were pre-incubated at 60 °C for 10 min. Afterwards, volatile compounds were trapped at 60 °C for 90 min under a 500 ml minute^{-1} nitrogen flow. Trapped volatiles were confined into 6 ml of dichloromethane and the aromatic extract was subjected to anhydrous sodium sulphate to remove existed water. Afterwards, the extract was concentrated to 1 mL by a Kuderna Danish concentrator embed with a Snyder column at 40 °C. The amounts of the volatile compounds were computed by the internal standard method with 4-nonanol (43.3 µg/kg). The ratio of peak area was corrected with response factors of each compound, and response factors were calculated from the intensity ratio of each compound to 4-nonanol.

GC–MS analysis

The GC–MS conditions were set with slight differences of the work cited by Topi (2020). The Agilent 6890 chromatograph interfaced with a flame ionization detector (FID) and Agilent 5973-Network-mass selective detector (MSD) (Wilmington, DE, USA) constituted the gas chromatography (GC) system. DB-Wax column (30 m length × 0.25 mm i.d. × 0.5 µm thickness, J&W Scientific, Folsom, CA, USA) was used to separate the volatile compounds of the GSO samples. An amount of 3 µl of extract was injected in pulsed splitless mode (40 psi; 0.5 min). 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 conditions of the oven program was 50 °C to 250 °C with 4 °C/min, 10 min hold. The identical oven program was also used for the mass-selective detector. The MS (electronic impact ionization) conditions were as follows: ionization energy of 70 eV, mass range m/z of 30–300 a.m.u., scan rate of 2.0 scan/s, interface temperature of 250 °C and source temperature of 180 °C. The volatiles were analyzed from their retention index and their mass spectra based on a commercial spectra database (Wiley 9, NIST 11, NBS 75 K). Subsequently, the mean values of the triplicate GC analyses were calculated. The retention indices of the volatiles were also computed by using n-alkane (C_8–C_{32}) series and comparing the Kovats Retention Index.

Odor activity value (OAV)

In order to examine the influence of each volatile compound of total GSO aroma, the OAVs of volatiles were calculated by a formula based on dividing the concentration with the odour threshold value that is cited in the extant literature (Selli and Kelebek, 2011). Regarding this calculation, only the compounds with an OAV greater than 1 were evaluated as a potential contributor of the overall GSO aroma.

Statistical analysis

In the present study, the data was collected in triplicates and the revealed results of volatile analysis were given in mean with their standard deviations. An analysis of variance test (ANOVA) was performed and statistical analysis followed by the level of significance using SPSS 18.0 (SPSS Inc., Chicago, Illinois, USA). In order to display the effects of variety and extraction procedure on volatiles, Duncan's multiple-comparison test and a PCA (principal component analysis) were carried out. P values of < 0.05 were adopted as the criterion for significant correlation in statistical analysis.

Results and discussion

Oil extraction yields

The oil yields (g oil 100 g/dry weight) in CP samples were found to be 14.1, 9.6, 14.9 and 16.6 while in SX samples 12.1, 11.6, 15.1 and 15.5 in *cvs.* Okuzgozu, Emir, Sangiovese and Moscatello, respectively. Significant differences in oil yield results were observed between Turkish and Italian GSO samples in both CP and SX extracts ($p < 0.05$). In a general aspect, both of Italian grape seeds possessed a higher amount of oil with respect to Turkish cultivars. Among samples, Moscatello seeds exhibited the highest oil amount having 16.6 g oil/100 g and 15.5 g oil 100 g/seed in CP and SX extracts, respectively. Whilst, Turkish samples contained relatively lower amount of oil. The Emir grape contained the lowest oil quantity having 9.6 g oil 100 g/seed and 11.6 g oil 100 g/seed. These data were in accordance with the published yields in the literature (Sabir et al. 2012; Lachman et al. 2015; Wen et al. 2016). The variation across the varieties and extraction methods were found to be significant by statistical analysis ($p < 0.05$). These kind of variations in oil content not only caused by varietal distinctions and oil extraction procedure but also the maturity, seasonal differences, seed drying conditions (Bombai et al., 2017).

Volatile composition and odor activity values (OAVs) of grape seed oils

Volatile compositions of the Turkish and Italian GSO samples extracted with two different methods are given in Table 1 and 2. When extraction methods were compared, it was found that soxhlet extraction (SX) significantly increased the amount of volatiles due to heat effect during extraction compared to cold percolation method (CP) in both Turkish and Italian GSO samples. A total of 60 aroma compounds were identified and quantified in CP samples including thirteen alcohols, sixteen esters, four aldehydes, nine terpenes, four ketones, eight carboxylic acids, three volatile phenols, one lactone and one furane compound. On the other hand, 67 aroma compounds detected in SX samples were twelve alcohols, fourteen esters, eleven aldehydes, eight terpenes, four ketones, eight carboxylic acids, three lactones, three volatile phenols, two furans, and two pyranones. The dominant aroma groups in Turkish and Italian GSO samples were alcohols, esters, aldehydes and carboxylic acids with the abundance of 2-hexanol, 3-hexanol, phenylethyl alcohol, ethyl octanoate, hexanal and hexanoic acid (Table 1 and 2). Some of the aroma compounds detected in the present work have already been mentioned in the extant literature related to GSO (Bail et al. 2008). In both extraction methods, Okuzgozu seed oils exhibited the highest total aroma concentrations (59,207.6 and 87,463.5 µg/kg, respectively) while Emir seed oils were found to be the lowest (24,953.8 and 50,571.2 µg/kg, respectively). Expectedly, some of well-known heat derived aroma compounds, such as 2-pentyl furan and maltol, were found only in SX extracts due to applied high temperature.

On the other hand, to observe the impact of the volatile compounds of overall GSO aroma, the OAVs were calculated as given in Table 3 and 4. Among all aroma compounds of CP and SX samples, 26 and 33 compound possessed an OAV greater than 1, in other words showed

Table 1 Aroma profiles of GSOs obtained by CP method

No	LRI	Compounds	Concentration (μg/kg)				*p*	Identification
			Okuzgozu	Sangiovese	Emir	Moscatello		
Alcohols								
1	1170	3-Penten-2-ol	$290.6^{c} \pm 0.5$	$378^{b} \pm 0.4$	$327.1^{bc} \pm 2.2$	$475.2^{a} \pm 1$	*	LRI, MS, Std
2	1211	3-Hexanol	$8627.9^{a} \pm 7.6$	$9919.6^{a} \pm 3.6$	$5547.5^{b} \pm 11.4$	$8899.3^{a} \pm 2.2$	*	LRI, MS, Tent
3	1217	2-Hexanol	$10{,}230.1^{a} \pm 1.6$	$11{,}229.2^{a} \pm 5.6$	$6680^{b} \pm 13.2$	$9175.1^{a} \pm 1.7$	*	LRI, MS, Std
4	1218	Isoamyl alcohol	$236.5^{b} \pm 11$	$211.4^{b} \pm 3.5$	nd	$392.1^{a} \pm 0.8$	*	LRI, MS, Std
5	1273	2-Heptanol	nd	$120.6^{a} \pm 2.2$	$89.8^{ab} \pm 5.2$	$43.3^{b} \pm 2.1$	*	LRI, MS, Std
6	1342	3-Methyl cyclopentanol	$411.9^{a} \pm 9.1$	$376.6^{a} \pm 3.5$	$267.3^{b} \pm 10.9$	$349.7^{ab} \pm 2.3$	*	LRI, MS, Tent
7	1359	1-Hexanol	$989.1^{a} \pm 3.3$	$964.6^{a} \pm 8.1$	$365.8^{c} \pm 15.3$	$572.8^{b} \pm 8.6$	*	LRI, MS, Std
8	1430	1-Octen-3-ol	$165^{b} \pm 2.3$	$280.9^{a} \pm 1.2$	$140.1^{b} \pm 18.2$	$85.1^{c} \pm 8.7$	*	LRI, MS, Std
9	1413	2-Dodecanol	nd	nd	78.2 ± 14.9	nd		LRI, MS, Std
10	1635	1,2-Ethanediole	nd	119.5 ± 10.0	nd	nd		LRI, MS, Std
11	1786	Butoxyethoxy ethanol	nd	48.5 ± 7.1	nd	nd		LRI, MS, Tent
12	1861	Benzyl alcohol	$55.8^{c} \pm 4.9$	$143^{b} \pm 9.7$	$128^{b} \pm 7.2$	$472.24^{a} \pm 2.7$	*	LRI, MS, Std
13	1923	Phenylethyl alcohol	$2195.3^{a} \pm 3.0$	$1694^{b} \pm 0.7$	$357.4^{c} \pm 15$	$318.6^{c} \pm 1.5$	*	LRI, MS, Std
		Total	$23{,}786.5 \pm 46.3$	$25{,}485.6 \pm 58.2$	$14{,}281.2 \pm 111$	$20{,}504.9 \pm 31.6$		
Esters								
14	1126	Isoamyl acetate	$905.2^{a} \pm 6.1$	$527.3^{b} \pm 1.8$	$261.7^{c} \pm 12$	$410.8^{b} \pm 5.2$	*	LRI, MS, Tent
15	1240	Ethyl hexanoate	$1335.7^{a} \pm 0.2$	$871.9^{b} \pm 2$	$756.3^{b} \pm 11$	$768.2^{b} \pm 2.7$	*	LRI, MS, Std
16	1255	Isoamyl butanoate	126.9 ± 4.3	nd	nd	nd		LRI, MS, Std
17	1276	Hexyl acetate	$503.4^{a} \pm 0.9$	$132.1^{c} \pm 3.3$	$200.1^{b} \pm 15.4$	$179.5^{bc} \pm 5.8$	*	LRI, MS, Std
18	1430	Ethyl octanoate	$7216.9^{a} \pm 4.2$	$566.3^{b} \pm 7.1$	$1160.8^{bc} \pm 16.9$	$1797.6^{b} \pm 3.8$	*	LRI, MS, Std
19	1526	Ethyl nonanoate	$358.9^{a} \pm 15.5$	$116.7^{b} \pm 6.7$	$96.7^{b} \pm 27.9$	nd	*	LRI, MS, Std
20	1650	Ethyl benzoate	nd	nd	678.3 ± 10.1	416.3 ± 10.4		LRI, MS, Std
21	1652	Ethyl decanoate	$8596.6^{a} \pm 10.6$	$325.2^{b} \pm 1.62$	$826.1^{b} \pm 9.6$	$666.1^{b} \pm 7$	*	LRI, MS, Std
22	1686	Diethyl succinate	196 ± 2.3	nd	nd	nd		LRI, MS, Std
23	1754	Benzyl acetate	nd	nd	45.2 ± 13.7	nd		LRI, MS, Std
24	1786	Phenyl ethyl acetate	$893.2^{a} \pm 9.7$	$185.4^{b} \pm 6.7$	$125.4^{b} \pm 7.8$	$142.8^{b} \pm 10.3$	*	LRI, MS, Std
25	1835	Ethyl dodecanoate	842.5 ± 13.9	nd	nd	nd		LRI, MS, Tent
26	1861	Buthyl butanoate	44.8 ± 11.2	273.2 ± 5.6	nd	324.2 ± 6.3	*	LRI, MS, Std
27	2130	Phenoxy ethyl acetate	$37.4^{c} \pm 7.4$	$136.2^{bc} \pm 14.8$	$1100.4^{a} \pm 12.1$	$390^{b} \pm 7.8$	*	LRI, MS, Tent
28	2259	Ethyl palmitate	$498.8^{a} \pm 11.0$	$185^{b} \pm 2.8$	nd	$100.5^{b} \pm 5.1$	*	LRI, MS, Std
29	2449	Ethyl laureate	nd	120.2 ± 2.7 7	102.6 ± 16.4	nd		LRI, MS, Std
30	2511	Ethyl linoleate	441.5 ± 9.4	nd	nd	nd		LRI, MS, Std
		Total	$21{,}997.6 \pm 108.5$	3439.3 ± 55.2	5256.8 ± 124.7	5196.1 ± 61.5		
Aldehydes								
31	1078	Hexanal	$1763.1^{ab} \pm 5.7$	1436.8b ± 5.9	$1310.8^{b} \pm 7.9$	$2089.7^{a} \pm 8.8$	*	LRI, MS, Std
32	1334	(*E*)-2-Heptenal	114.3 ± 2.2	43.9 ± 5.6	nd	nd		LRI, MS, Std
33	1395	Nonanal	$651.4^{a} \pm 7.7$	$556.6^{a} \pm 2.4$	$258.8^{b} \pm 20.6$	535.9 ± 0.6^{b}	*	LRI, MS, Std
34	1702	(*E*,*E*)-2,4-Nonadienal	144.2 ± 9.6	nd	nd	nd		LRI, MS, Std
		Total	2952.9 ± 31.3	2037.3 ± 13.9	1569.6 ± 14.8	2625.7 ± 9.4		
Terpenes								
35	1459	α-Cubebene	nd	nd	136.8 ± 14.1	nd		LRI, MS, Tent
36	1493	α-Copaene	nd	nd	149.3 ± 13.9	nd		LRI, MS, Std
37	1537	Linalool	nd	nd	nd	486 ± 9.3		LRI, MS, Std
38	1612	(*E*)-Caryophyllene	nd	nd	121.6 ± 19	nd		LRI, MS, Std

Table 1 continued

No	LRI	Compounds	Concentration (μg/kg)				*p*	Identification
			Okuzgozu	Sangiovese	Emir	Moscatello		
39	1702	α-Caryophyllene	nd	nd	720.5 ± 6.1	nd		LRI, MS, Std
40	1715	Germacrene	nd	nd	nd	502.7 ± 2.5		LRI, MS, Tent
41	1771	Citronellol	116.8 ± 6.7	56.2 ± 4.7	nd	nd		LRI, MS, Std
42	1805	Δ-Cadinene	nd	nd	520.5 ± 10.2	181.1 ± 10.5		LRI, MS, Std
43	1835	(*E*)-Calamenene	nd	nd	232.9 ± 16.5	nd		LRI, MS, Tent
		Total	116.8 ± 6.6	56.2 ± 4.7	1881.6 ± 79.8	1169.8 ± 22.3		
Ketones								
44	1245	Acetoin	123.1 ± 3.4	135.8 ± 1.6	nd	nd		LRI, MS, Std
45	1285	2-Octanone	nd	161.4 ± 0.6	nd	nd	*	LRI, MS, Std
46	1415	2-Nonanone	301.9^{b} ± 10.2	1152.8^{a} ± 10.4	285.8^{b} ± 0.6	1117.9^{a} ± 3.1	*	LRI, MS, Std
47	1645	Acetophenone	nd	141.3 ± 7.5	nd	186.7 ± 7.2	*	LRI, MS, Std
		Total	425 ± 13.6	1591.2 ± 20.2	285.8 ± 0.6	1304.6 ± 10.3	*	
Carboxylic acids								
48	1686	Isovaleric acid	267.2^{a} ± 1.4	196.5^{b} ± 2.1	143.1^{c} ± 18.2	59.2^{d} ± 5.8	*	LRI, MS, Tent
49	1730	Pentanoic acid	nd	81.4 ± 1.4	34.4 ± 11.7	nd		LRI, MS, Std
50	1855	Hexanoic acid	1457.8^{a} ± 5.2	1186.4^{a} ± 10.1	668.8^{b} ± 7.9	454.6^{b} ± 6.9		LRI, MS, Std
51	1960	Heptanoic acid	46.0 ± 9.2	nd	nd	nd		LRI, MS, Std
52	1990	Octanoic acid	484.7^{a} ± 10.6	226.7^{b} ± 9.2	158.1^{b} ± 5.3	189.4^{b} ± 27.1	*	LRI, MS, Std
53	2169	Nonanoic acid	118.2^{b} ± 0.1	216.4^{a} ± 5.8	48.7^{c} ± 4.3	98.2^{b} ± 6.4	*	LRI, MS, Std
54	2314	Decanoic acid	268.9^{a} ± 2.9	133.1^{b} ± 1.7	199.7^{b} ± 4.8	nd	*	LRI, MS, Std
55	2420	Benzoic acid	nd	202.9 ± 0.1	75.4 ± 15.9	nd		LRI, MS, Std
		Total	2642.9 ± 29.3	2243.4 ± 30.4	1383.5 ± 48.9	801.5 ± 29.2		
Lactones								
56	1612	γ-Butyrolactone	165.4^{a} ± 3.8	152.6^{ab} ± 2	127.3^{b} ± 11.7	78.4^{c} ± 4.3	*	LRI, MS, Std
		Total	165.4 ± 3.8	152.6 ± 2	127.3 ± 11.7	78.4 ± 4.3		
Volatile Phenols								
57	1973	Phenol	22.4^{b} ± 5.3	44.1^{a} ± 7.6	25.2^{b} ± 8.8	39.1^{a} ± 9.6	*	LRI, MS, Std
58	2189	Carvacrol	nd	nd	nd	109.5 ± 0.9		LRI, MS, Tent
59	2277	2,4-Ditertbutyl phenol	98.1^{b} ± 6.1	130.7^{ab} ± 9.5	63.9^{c} ± 10.8	151.3^{a} ± 9.7	*	LRI, MS, Tent
		Total	120.5 ± 11.4	174.7 ± 17.1	89.1 ± 10.6	299.9 ± 20.3		
Furans								
60	1740	5-Phenyl-2-furanone	nd	169.3 ± 7.6	78.9 ± 8.2	nd		LRI, MS, Tent
		Total		169.3 ± 7.6	78.9 ± 8.2			
		General total	59,207.6 ± 251.1	34,971.5 ± 208.7	24,953.8 ± 419.3	30,674.1 ± 188.9		

Different letters (a, b, c, d) on the numbers in same row indicate significant differences ($p < 0.05$) between different GSO samples and asterisk represent the significance level

potentially aroma active property. A similar conclusion was revealed by the calculated OAV data for the Okuzgozu samples which are exhibited higher OAVs. Some of esters, such as ethyl octanoate, ethyl decanoate, hexyl acetetate, and some alcohols and aldehydes were responsible for the aroma characteristics of GSOs regarding the OAVs.

Alcohols

In all CP and SX extracts, although the number of ester compounds identified in GSOs was higher, alcohols were the most abundant aroma group regarding their concentrations. A total of 13 and 12 alcohol compounds were detected in CP and SX extracts, respectively. As a result of applied high temperature during SX method, total concentration of alcohols were almost doubled in each cultivar. This result of the present work is demonstrated a clean

Table 2 Aroma profiles of GSOs obtained by SX method

No	LRI	Compound name	Concentration (μg/kg)				*p*	Identification
			Okuzgozu	Sangiovese	Emir	Moscatello		
Alcohols								
1	1170	3-Penten-2-ol	$264.7^{b} \pm 7.1$	$265.6^{b} \pm 6.9$	$306.2^{b} \pm 3.2$	$484.4^{a} \pm 7.5$	*	LRI, MS, Std
2	1211	3-Hexanol	$12{,}369.7^{ab} \pm 5.1$	$15{,}336.3^{ab} \pm 0.8$	$12{,}721.8^{b} \pm 8.0$	$17{,}378.2^{a} \pm 12.6$	*	LRI, MS, Tent
3	1217	2-Hexanol	$14{,}987.5^{ab} \pm 6.6$	$17{,}863.9^{ab} \pm 3.8$	$14{,}335.3^{b} \pm 9.3$	$20{,}972.6^{a} \pm 11.9$	*	LRI, MS, Std
4	1342	3-Methyl cyclopentanol	$884.1^{ab} \pm 3.9$	$832.9^{b} \pm 0.8$	$590.8^{c} \pm 7.8$	$1067.9^{a} \pm 11.7$	*	LRI, MS, Tent
5	1359	1-Hexanol	$2553.1^{a} \pm 4.6$	$1869.5^{b} \pm 0.8$	$1001.1^{c} \pm 7.2$	$1411.1^{bc} \pm 11$	*	LRI, MS, Std
6	1391	2-Butoxyethanol	nd	92.8 ± 1.6	48.4 ± 5.2	nd		LRI, MS, Std
7	1430	1-Octen-3-ol	$516^{a} \pm 3.1$	$482^{a} \pm 3.2$	$247.6^{c} \pm 5.6$	$337.1^{b} \pm 4.6$	*	LRI, MS, Std
8	1759	α-cumyl alcohol	nd	nd	nd	245.0 ± 0.3		LRI, MS, Std
9	1776	2-Phenyl-2-propanol	$107.4^{a} \pm 6.7$	$120.3^{a} \pm 7.3$	$121.3^{a} \pm 2.8$	nd	ns	LRI, MS, Tent
10	1786	Butoxy ethoxy ethanol	nd	166.6 ± 5.2	nd	297.8 ± 3.3		LRI, MS, Tent
11	1861	Benzyl alcohol	$200.3^{c} \pm 8.8$	$421.2^{a} \pm 7.8$	$236.7^{c} \pm 8.7$	$316.4^{b} \pm 7.7$	*	LRI, MS, Std
12	1923	Phenyl ethyl alcohol	$8453.2^{a} \pm 3.5$	$6315.8^{b} \pm 9.9$	$1206.2^{c} \pm 10.9$	$831.1^{c} \pm 1.6$	*	LRI, MS, Std
		Total	$40{,}337.0 \pm 49.4$	$43{,}766.8 \pm 68$	$30{,}815.3 \pm 68.6$	$43{,}515.5 \pm 64.5$		
Esters								
13	1126	Isoamyl acetate	$2099.4^{a} \pm 27.6$	$448.3^{b} \pm 3.7$	$630.5^{b} \pm 1.6$	$839.2^{b} \pm 5.2$	*	LRI, MS, Tent
14	1226	Butyl butanoate	$1425.7^{a} \pm 13.2$	$275.7^{b} \pm 4$	$417.6^{b} \pm 8.6$	$523.5^{b} \pm 8.8$	*	LRI, MS, Std
15	1240	Ethyl hexanoate	3993.3 ± 3.8	903.9 ± 4.8	nd	nd		LRI, MS, Std
16	1276	Hexyl acetate	1081.3 ± 11.9	nd	114.9 ± 10.2	nd		LRI, MS, Std
17	1430	Ethyl octanoate	$8569.7^{a} \pm 6.9$	$1016.8^{b} \pm 3.2$	$458.7^{b} \pm 2.7$	$1037.6^{b} \pm 3.5$	*	LRI, MS, Std
18	1526	Methyl benzoate	nd	nd	104.3 ± 2.2	nd		LRI, MS, Std
19	1650	Ethyl benzoate	nd	nd	860.6 ± 7.6	613.2 ± 3.5		LRI, MS, Std
20	1652	Ethyl decanoate	$9402.2^{a} \pm 10.7$	$706.2^{b} \pm 5.1$	nd	$418.9^{b} \pm 0.3$	*	LRI, MS, Std
21	1686	Diethyl succinate	417.6 ± 5.2	185.3 ± 2	nd	nd		LRI, MS, Std
22	1786	Phenyl ethyl acetate	$1492.4^{a} \pm 2.6$	$187.7^{b} \pm 2.1$	nd	$139.6^{b} \pm 6.5$	*	LRI, MS, Std
23	1835	Ethyl dodecanoate	836.2 ± 3.8	nd	nd	nd		LRI, MS, Tent
24	2130	Phenoxy ethyl acetate	$104^{b} \pm 12$	$81.5^{b} \pm 4.9$	$2941.6^{a} \pm 5.4$	$77.5^{b} \pm 4.8$	*	LRI, MS, Tent
25	2259	Ethyl palmitate	361.7 ± 11.7	166.4 ± 5.2	nd	nd		LRI, MS, Std
26	2511	Ethyl linoleate	760.3 ± 7.4	nd	nd	nd		LRI, MS, Std
		Total	$31{,}072.6^{a} \pm 125.5$	3971.9 ± 35.0	5528.1 ± 38.3	3649.4 ± 32.5		
Aldehydes								
27	1078	Hexanal	$3525.7^{a} \pm 2.6$	$1598^{d} \pm 0.4$	$2147.3^{c} \pm 3.4$	$2762.9^{b} \pm 4$	*	LRI, MS, Std
28	1291	Octanal	818.4 ± 8.2	nd	nd	nd		LRI, MS, Std
29	1334	(*E*)-2-Heptenal	346.8 ± 6.3	nd	403.6 ± 7.1	nd		LRI, MS, Std
30	1395	Nonanal	$1609.4^{a} \pm 4.5$	$799^{b} \pm 2.0$	$458.5^{c} \pm 6.2$	$490.7^{bc} \pm 12.4$	*	LRI, MS, Std
31	1414	(*E*,*E*)-2,4-Hexadienal	nd	59.4 ± 8.5	nd	nd		LRI, MS, Std
32	1483	(*E*,*E*)-2,4-Heptadienal	nd	nd	219.8 ± 4.4	nd		LRI, MS, Std
33	1532	2-Nonenal	$502.8^{a} \pm 9.7$	$490.7^{a} \pm 0.2$	$434.4^{a} \pm 3.1$	$480.7^{a} \pm 0.2$	ns	LRI, MS, Std
34	1650	Benzene acetaldehyde	237.3 ± 5.6	200.5 ± 5.7	nd	nd		LRI, MS, Std
35	1702	(*E*,*E*)-2,4 Nonadienal	386.9 ± 6.9	nd	117 ± 2.4	nd		LRI, MS, Std
36	1805	(*E*,*E*)-2,4-Decadienal	98.2 ± 4.0	109.1 ± 5.7	nd	nd		LRI, MS, Std
37	2545	Vanillin	nd	63.4 ± 8.7	nd	nd		LRI, MS, Std
		Total	7635.3 ± 55.5	3320.1 ± 31.3	3780.7 ± 26.5	3734.4 ± 16.6		
Terpenes								
38	1459	α-Cubebene	nd	nd	178.2 ± 12.3	nd		LRI, MS, Tent
39	1493	α-Copaene	nd	nd	169.3 ± 5.3	nd		LRI, MS, Std
40	1537	Linalool	nd	nd	nd	770.2 ± 1.3		LRI, MS, Std

Table 2 continued

No	LRI	Compound name	Concentration (μg/kg)				*p*	Identification
			Okuzgozu	Sangiovese	Emir	Moscatello		
41	1705	α-Caryophyllene	nd	nd	836.4 ± 7.1	nd		LRI, MS, Std
42	1715	Germacrene	nd	nd	nd	238.1 ± 0.4		LRI, MS, Tent
43	1771	Citronellol	94 ± 15.5	nd	nd	nd		LRI, MS, Std
44	1781	Δ-Cadinene	nd	nd	397.1 ± 7.4	255.9 ± 2.0		LRI, MS, Std
45	1835	(*E*)-Calamenene	nd	nd	378.6 ± 2.9	nd		LRI, MS, Tent
		Total	94 ± 15.5	nd	1959.4 ± 34.9	1264.2 ± 3.7		
Ketones								
46	1245	Acetoin	247.7 ± 11	316.8 ± 10.7	nd	nd		LRI, MS, Std
47	1285	2-Octanone	nd	295.4^{b} ± 7.4	170^{c} ± 4.3	414.4^{a} ± 5.2	*	LRI, MS, Std
48	1415	2-Nonanone	984.3^{b} ± 1	1463.2^{a} ± 2.6	988.3^{b} ± 1.5	1189.6^{b} ± 1.1	*	LRI, MS, Std
49	1645	Acetophenone	341.5^{a} ± 13.9	153.8^{b} ± 6.7	140.3^{b} ± 4.41	184.7^{b} ± 1.72	*	LRI, MS, Std
		Total	1573.5 ± 25.9	2229.2 ± 27.4	1298.6 ± 10.2	1788.7 ± 8	*	
Carboxylic acids								
50	1686	Isovaleric acid	nd	446.8 ± 11.1	nd	161.7 ± 3		LRI, MS, Tent
51	1730	Pentanoic acid	289.6^{a} ± 6.4	101.7^{b} ± 10.3	119.9^{b} ± 3.9	nd	*	LRI, MS, Std
52	1855	Hexanoic acid	3151.2^{a} ± 6.5	1620.8^{b} ± 7.1	1488.7^{b} ± 9.4	1173.5^{b} ± 0.7	*	LRI, MS, Std
53	1960	Heptanoic acid	235 ± 13.4	432.7 ± 6.5	nd	nd		LRI, MS, Std
54	1990	Octanoic acid	983.7^{a} ± 9.5	273.3^{cd} ± 2.9	375.1^{bc} ± 0.3	486.1^{b} ± 5.4	*	LRI, MS, Std
55	2169	Nonanoic acid	338.9^{a} ± 12.4	213.1^{b} ± 1.2	193.4^{b} ± 2.4	162.3^{b} ± 3.4	*	LRI, MS, Std
56	2314	Decanoic acid	391.7^{b} ± 9.4	136.1^{cd} ± 1	324.8^{a} ± 8.8	91.2^{c} ± 3.4	*	LRI, MS, Std
57	2449	Dodecanoic acid	151.8 ± 12.2	128.3 ± 4.2	nd	nd		LRI, MS, Std
		Total	5542 ± 70.4	3352.3 ± 44.2	2894.9 ± 16.8	2074.8 ± 15.8		
Lactones								
58	1612	γ-Butyrolactone	675.9 ± 8.5	699.7 ± 10.4	170.7 ± 4.1	251.6 ± 2.6	*	LRI, MS, Std
59	1784	Δ-Valerolactone	nd	nd	85.2 ± 3.8	nd		LRI, MS, Std
60	1998	Pantolactone	77.2^{b} ± 10.7	142.1^{a} ± 0.8	126.9^{a} ± 2.5	46.8^{c} ± 2.2	*	LRI, MS, Std
		Total	753.1 ± 19.1	841.7 ± 11.2	382.9 ± 10.3	298.4 ± 4.8		
Volatile Phenols								
61	1973	Phenol	53.6^{a} ± 5.9	43.2^{b} ± 0.3	44.1^{b} ± 0.2	36.1^{c} ± 1.2	*	LRI, MS, Std
62	2189	Carvacrol	155.9^{a} ± 4.8	70.7^{b} ± 0.5	142.4^{a} ± 6	nd	*	LRI, MS, Std
63	2277	2,4-Ditertbutyl phenol	69.4 ± 12.7	217.7 ± 2.1	nd	nd		LRI, MS, Std
		Total	278.8 ± 23.5	331.6 ± 3	186.4 ± 6.2	36.1 ± 1.2		
Furans								
64	1235	2-Pentyl furane	nd	nd	549.6 ± 6.6	nd		LRI, MS, Std
65	1740	5-Phenyl-2-furanone	287^{a} ± 3.8	184.2^{b} ± 3.1	200.1^{b} ± 0.3	nd	*	LRI, MS, Std
		Total	287 ± 3.8	184.2 ± 3.1	749.7 ± 6.9			
Pyranones								
66	1947	Maltol	nd	52 ± 2.2	56.1 ± 3.5	nd		LRI, MS, Std
67	2295	Pyranone	nd	1721.5 ± 0.9	717.7 ± 4	nd		LRI, MS, Std
		Total		1773.5 ± 3.1	773.8 ± 7.5			
		General Total	87,463.5 ± 226.6	59,771.2 ± 291.8	50,571.2 ± 56.7	56,361.5 ± 154.4		

Different letters (a, b, c, d) on the numbers in same row indicate significant differences ($p < 0.05$) between different GSO samples and asterisk represent the significance level

support for the lipoxygenase pathway related to high linoleic and linolenic acid content of GSO (Bombai et al. 2017). Increasing temperature during the SX extraction, enhanced the activity and availability of lipoxygenase enzyme in the seeds and resulted in the transformation of C_6 aldehydes into C_6 alcohols, namely lipoxygenase

Table 3 Odour activity values of GSOs obtained by CP method

No	Compound name	Odour treshold µg/kg	Odour Activity Value (OAV)				Odour descriptions
			Okuzgozu	Sangiovese	Emir	Moscatello	
Alcohols							
1	3-Penten-2-ol	400[1]	< 1	< 1	< 1	1.2	Perfumey, woody
2	3-Hexanol	400[2]	21.6	24.5	13.9	22.2	Cut grass
3	2-Hexanol	1508[1]	6.8	7.4	4.42	6.1	Fatty, fruity
4	2-Heptanol	65.2[1]	-	1.8	1.4	< 1	Mushroom, herb
5	1-Octen-3-ol	1[3]	165	280.9	140.1	85.1	Mushroom, earthy
6	Phenylethyl alcohol	1100[3]	2	1.5	< 1	< 1	Floral
Esters							
7	Isoamyl acetate	30[4]	30.2	17.6	8.7	13.7	Sweet banana
8	Ethyl hexanoate	14[4]	95.4	62.2	54.0	54.8	Sweet pineapple
9	Isoamyl butanoate	0.13[3]	969.2	-	-	-	
10	Hexyl acetate	2[4]	251.7	66.1	100	89.7	
11	Ethyl octanoate	5[2]	1443.4	113.2	232.2	359.5	Sweet, apple
12	Ethyl benzoate	20[4]	-	-	33.9	20.8	Fruity, pineapple
13	Ethyl decanoate	200[4]	429.5	1.6	4.1	3.3	Sweet, waxy, fruity
14	Phenyl ethyl acetate	480[3]	1.8	< 1	< 1	< 1	Floral, honey
Aldehydes							
15	Hexanal	300[5]	5.9	4.8	4.4	6.9	Green, fatty
16	(*E*)-2-Heptenal	13[5]	8.8	3.3	-	-	fatty, almond-like
17	Nonanal	2.8[5]	232.6	198.8	92.4	191.4	Fatty, citrus
18	(*E*,*E*)-2,4-Nonadienal	30[5]	4.8	-	-	-	Fried, fatty
Terpenes							
21	Linalool	6[3]	-	-	-	80.9	Flowery, coriander
20	(*E*)-caryophyllene	0.15[2]	-	-	810.4	-	Spicy
21	α-caryophyllene	160[3]	-	-	4.5	-	Woody
22	Citronellol	100[2]	1.2	< 1	-	-	Green, lemon
Ketones							
23	2-Octanone	5[3]	-	32.3	-	-	Earthy, cheese-like
24	2-Nonanone	41[6]	7.4	28.1	6.9	27.2	Sweet, fruity
25	Acetophenone	65[6]	-	2.2	-	2.9	Sweet, pungent, floral
Carboxylic acid							
26	Isovaleric acid	33.4[4]	8.0	5.9	4.3	1.8	Spicy, cheese

Thresholds from the references listed as; 1, Giri et al., 2010; 2, Vilanova et al., 2010; 3, Pino and Mesa, 2006; 4, Gómez-Míguez et al., 2007; 5, Matheis and Granvogl, 2016; 6, Du et al., 2010

pathway (LOX) (Podolyan et al., 2010). In accordance with the increasing volatile alcohol concentrations in SX extracts, OAVs of these aroma compounds increased as well. Among alcohols, although, 2-hexanol was found to be the most abundant alcohol compound by its concentration, 1-octen-3-ol was the most potential aroma contributor of GSOs providing mushroom-like and earthy odour. The OAVs of 1-octen-3-ol in all samples were dramatically increased in SX extracts. 1-Octen-3-ol possessed the highest OAV (517) in SX extracts of Okuzgozu sample followed by SX extract of Sangiovese GSO (482). The general increment of both aroma concentrations and OAVs of alcohol compounds in all samples can be associated with the accelerated LOX pathway under higher temperature conditions of SX extraction. 3-Hexanol and phenyl ethyl alcohol were second and third major volatile alcohols by their concentrations in all samples. Among four cultivars, Sangiovese seed oils possessed the highest amount of volatile alcohols having 25,485.6 and 43,766.8 µg/kg in CP and SX extracts, respectively. Although, the major alcohol compounds were similar in all samples, Italian varieties possessed higher concentrations of volatile alcohols and

Table 4 Odour activity values of GSOs obtained by SX method

No	Compound Name	Odour treshold µg/kg	Odour Activity Value (OAV)				Odour descriptions
			Okuzgozu	Sangiovese	Emir	Moscatello	
Alcohols							
1	3-Penten-2-ol	400[1]	< 1	< 1	< 1	1.2	Perfumey, woody
2	3-Hexanol	400[2]	30.9	38.3	31.8	43.4	Cut grass
3	2-Hexanol	1508[1]	9.9	11.8	9.5	13.9	Fatty, fruity
4	2-Butoxyethanol	4.59[7]	-	20.2	10.5	< 1	Green
5	1-Octen-3-ol	1[3]	517	482	248	337	Mushroom, earthy
6	Phenylethyl alcohol	1100[3]	7.7	5.7	1.1	< 1	Floral
Esters							
7	Isoamyl acetate	30[4]	69.9	14.9	21	27.9	Sweet banana
8	Ethyl hexanoate	14[4]	285.2	64.5	-	-	Sweet pineapple
9	Hexyl acetate	2[4]	540.5	-	57.4	-	Sweetish, perfumed
10	Ethyl octanoate	5[2]	1713.9	203.3	91.74	207.5	Sweetish, perfumed
11	Ethyl benzoate	20[4]	-	-	43.0	30.6	Fruity, pineapple
12	Ethyl decanoate	200[4]	47.1	3.5	-	2.1	Fruity, apple, solvent
13	Phenylethyl acetate	480[3]	3.1	< 1	-	< 1	Rose, honey, tobacco
Aldehydes							
14	Hexanal	300[5]	11.7	5.3	7.2	9.2	green, fatty
15	Octanal	56[5]	14.6	-	-	-	citrus-like, green
16	(*E*)-2-Heptenal	13[5]	26.6	-	31.1	-	fatty, almond-like
17	Nonanal	2.8[5]	574.8	285.4	163.7	175.3	fatty, citrus, waxy
18	(*E*,*E*)-2,4-Heptadienal	56[7]	-	-	3.9	-	nut, fat
19	2-Nonenal	140[5]	3.6	3.5	3.1	3.4	cucumber-like
20	(*E*,*E*)-2,4 Nonadienal	30[5]	12.9	-	3.9	-	fatty, soapy, sweet
21	(*E*,*E*)-2,4-Decadienal	0.2[3]	490.7	545.4	-	-	fatty, fried
22	Vanillin	20[3]	-	3.2	-	-	vanilla-like, sweet
Terpenes							
23	Linalool	6[3]	-	-	-	128.4	Flowery, coriander
24	α-Caryophyllene	160[3]	-	-	5.22	-	Woody
Ketones							
25	2-Octanone	5[3]	-	59.1	34.1	82.8	Earthy, cheese-like
26	2-Nonanone	41 Du 6	24.1	35.7	24.1	29.1	Sweet, fruity
27	Acetophenone	65 Du 6	5.3	2.4	2.1	2.8	Sweet, pungent, floral
Carboxylic acids							
28	Isovaleric acid	33.4[4]	-	13.4	-	4.8	Spicy, cheese
29	Hexanoic acid	3000[2]	1.1	< 1	< 1	< 1	Cheese, rancid, fatty
30	Octanoic acid	500[2]	1.9	< 1	< 1	< 1	Sweat, cheese
Volatile Phenols							
31	Carvacrol	2.29[3]	68.1	30.9	62.2	-	Phenolic, spicy
	Furan						
32	2-Penthyl furan	9.06[7]	-	-	60.6	-	Sweet
Pyranone							
33	Maltol	2.50[7]	-	20.8	22.4	-	Sweet

Thresholds from the references listed as; 1, Giri et al. 2010; 2, Vilanova et al. 2010; 3, Pino and Mesa, 2006; 4, Gómez-Míguez et al. 2007; 5, Matheis and Granvogl, 2016; 6, Du et al. 2010; 7, Miyazawa et al. 2015

butoxyetoxy ethanol was the compound only detected in Italian varieties.

Esters

Esters were the second important aroma group of GSOs and their concentration apparently influenced by varietal distinctions and extraction methods. The high ester concentration of Okuzgozu samples in both extracts was one of the most notable finding of the study. This distinctness mainly resulted from the high level of ethyl octanoate and ethyl decanoate concentrations in Okuzgozu samples. In an earlier study, Cabaroglu et al. (2002) similarly mentioned about high ester concentration of Okuzgozu wines. Besides, the total concentration of esters was increased in SX samples due to the high temperature applied during the oil extraction process. The main reason of this increment may be associated to lipid oxidation of polyunsaturated fatty acids which are abundant in GSO. The correlation between lipid oxidation and ester formation in the presence of high temperature is explained in detail in an earlier study (Berdeaux et al. 2012). Similarly to increasing aroma concentration, it was observed that the OAVs of esters increased considerably in SX samples. Ethyl octanoate possesed the highest OAV in all varieties while Okuzgozu extracts exhibited much higher OAV with respect to varieties (1443.4 and 1713.9 in CP and SX extracts, respectively). Another important ester of Okuzgozu sample was the isoamyl butanoate. This compound was only found in CP extracts of Okuzgozu sample and can be a potential aroma contributor due to its low odour treshold value. Isoamyl butanoate, diethyl succinate, ethyl dodecanoate and ethyl linoleate were the ester compounds detected only in Okuzgozu samples of CP extracts, while interestingly isoamyl butanoate was not existed in SX extracts.

Aldehydes

Aldehydes were another important aroma group presented in the GSOs. In line with previous studies, their concentration dramatically increased due to accelerated lipid oxidation related to increased temperature in SX samples (Fullana et al. 2004). Depending on this heat difference between extractions, seven aldehydes (octanal, (*E*,*E*)-2,4-hexadienal, (*E*,*E*)-2,4-heptadienal, 2-nonenal, benzene acetaldehyde, (*E*,*E*)-2,4-decadienal and vanillin) were newly formed while hexanal, (*E*)-2-heptenal, nonanal, and (*E*,*E*)-2,4-nonadienal increased in their concentrations. Similarly to esters, aldehydes of Okuzgozu sample were highly influenced from heating process (2952.9 and 7635.3 µg/kg, CP and SX extracts, respectively). Among aldehydes, a total of nine aroma compounds exhibited an OAV greater than 1 in SX extracts, while the number potentially odour active compounds was only four in CP extracts. On the basis of OAVs, nonanal was the main contributor to GSO aroma in all samples and its OAV showed an increase in SX extracts of all varieties, except Moscatello.

Terpenes

Terpenes were the other important aroma group and the dramatical change in their concentrations were found to be another remarkable result of the present study. Total terpene concentration of white varieties in CP extracts was ten-fold higher (1881.6 and 1169.8 µg/kg, for Emir and Moscatello, respectively) when compared to red varieties (116.8 and 56.2 µg/kg for Okuzgozu and Sangiovese seed oils). Among terpenes, α-cubebene, α-copaene, α-caryophyllene and (*E*)-calamenene were determined only in Emir samples of both extracts. There were no any terpene compound existed in red varieties except citronellol. Similarly, white varieties possesed the highest OAV in their terpenes. (*E*)-Caryophyllene exhibited 810.4 OAV in Emir GSO of CP extracts providing a spicy odour (Jirovetz et al. 2003), while linalool was found as an important terpene compound of Moscatello GSO of SX samples due to its high OAV (128.4). Similarly to our findings, Sánchez-Palomo et al. (2005) determined a significant amount of linalool in Muscat grapes (Sánchez-Palomo et al. 2005). Terpenes are the predominant components generally responsible for the characteristic flowery aroma of grape while linalool contributes a particular pleasant coriander odour especially in Muscat varieties (Marais, 1983). These compounds mainly derive during maturation phase of grapes and highyl affected by the cultivar, climate, soil conditions, canopy management (Sánchez-Palomo et al. 2005).

Ketones

Another well-known secondary products of lipid oxidation, ketones, were exhibited higher concentrations in SX extracts as expected. Maltol, pyranone, 2-pentyl furan, Δ-valerolactone, and pantolactone were other heat derived products detected in SX extracts. Formation of those specific compounds not only resulted from lipid oxidation, but also Strecker degradation and Maillard reactions (Ho et al. 2007). 2-Nonanone was the only ketone exhibited an odour activity value greater than 1 in all samples providing sweet and fruity notes. Furthermore, almost all ketone compounds possessed higher OAVs in SX samples as a result of heating during extraction.

Lactones and other heat derived compounds

The high temperature applied during the SX extraction inevitably led to form new heat derived compounds such as lactones, furans and pyranones. The formation of these compounds are thought to occur due to the oxidation of polyunsaturated fatty acids (PUFAs) regarding to heating process (Şenyuva and Gökmen 2007). In particular, γ-lactones are known to be formed by heat-induced lipid oxidation reactions providing a deep fat fried character in the presence of unsaturation in the heated oil (Chang et al. 1978).

Additionally, some of heat derived compounds, furans and pyranones, known as oxygen-containing heterocyclic compounds, exhibited OAVs greater than 1 only in SX samples providing a sweet and caramelized odour. This clear evidence demonstrated the effect of heating process on GSOs.

In addition, principal component analysis (PCA) was applied to study the effect of extraction methods and cultivars on the aroma compounds of GSO samples using all quantified aroma compounds (Figs. 1 and 2). Regarding the PCA biplots, each compound were separately considered to identify the two principle factors for both CP and SX samples and they labeled with respect to their appearance number in the aroma tables (Table 1 and 2). All aroma compounds were counted as a single variable for the PCA analysis and the elucidated variance was 97.67% and 96.4% respectively for CP and SX samples. Figures 1 and 2 shows the differentiation of each volatiles with respect to grape seed varieties. In both plots, F1 accounted for the highest proportion of variance (66.97% and 67.47%), which is associated to varietal distinction. These high ratios, explained the full range of factors that composed the biplot graph. The narrowing of the angle between the vectors indicates the closeness of the properties between the variables, while expansion of the angle indicates the weakness of the relationship.

Expectedly, red (Okuzgozu and Sangiovese) and white samples (Emir and Moscatello) were divided into the two side of the PCA plot. Interestingly, all four GSO samples were placed at the four different side of PCA plot in both CP and SX samples. This means that different aroma compounds were characterized the different GSOs. In the PCA biplots right side of the graph was exhibited the red varieties, while white varieties were located at the left. These results clearly shows the discrimination between varieties as well as the effect of heating process on the volatile composition of GSOs in SX extracts.

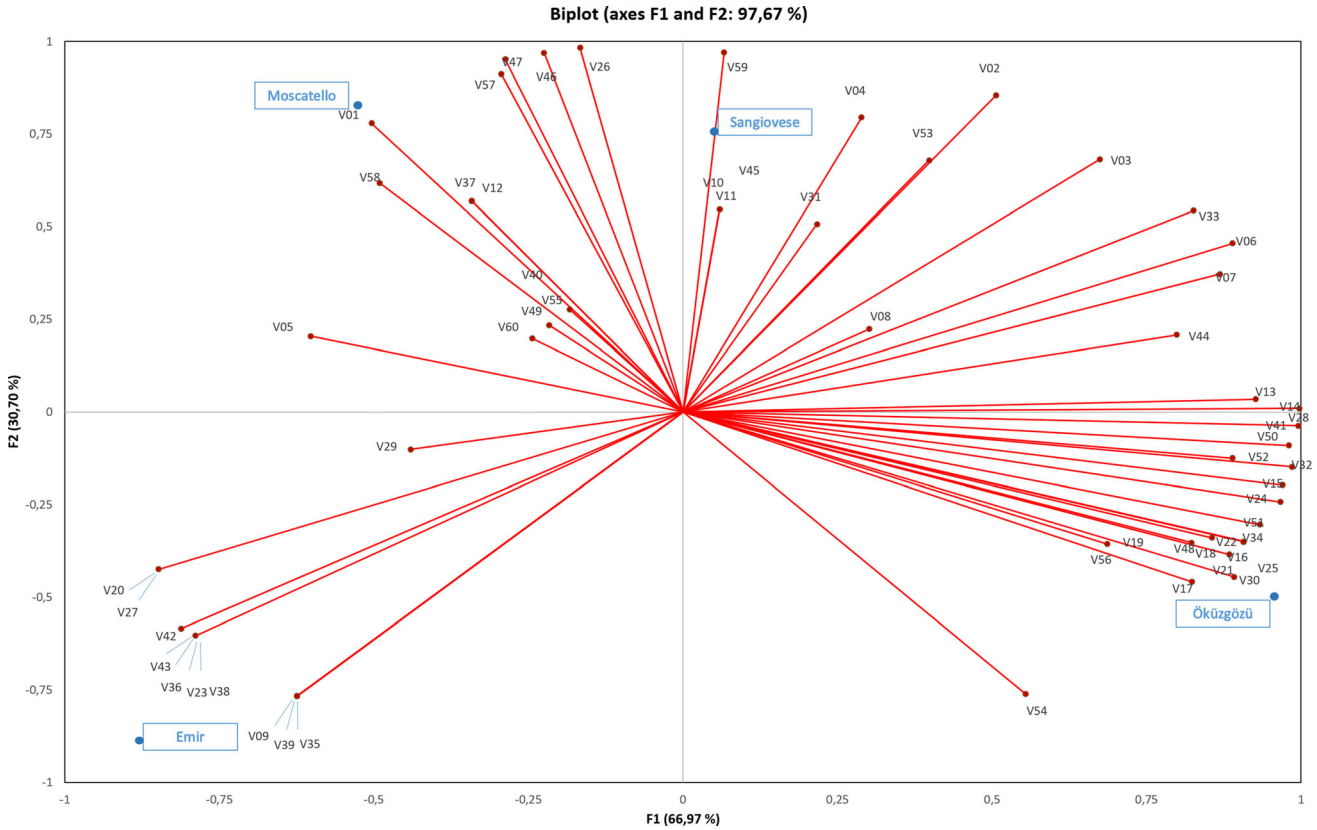

Fig. 1 PCA plot of aroma compounds isolated from CP grape seed oils

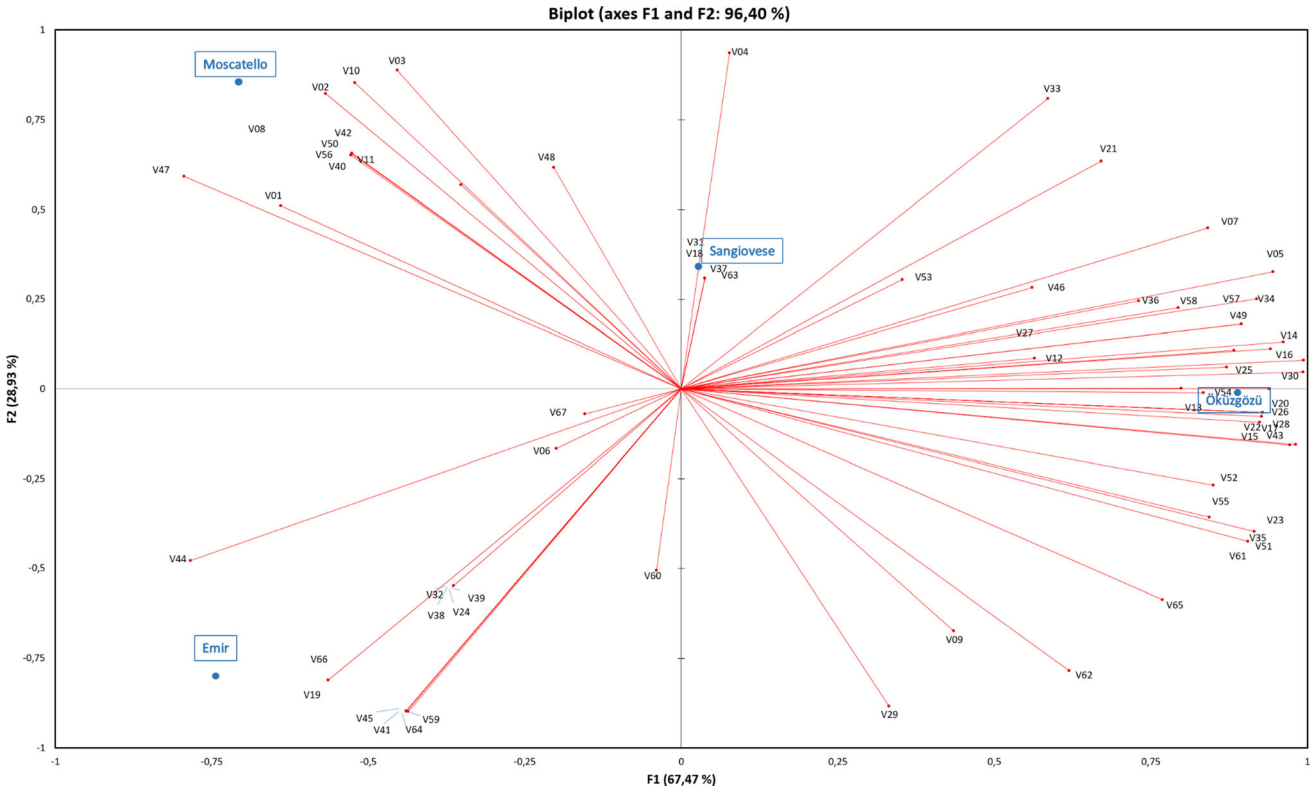

Fig. 2 PCA plot of aroma compounds isolated from SX grape seed oils

Conclusion

In this study, two different extraction methods (cold percolation and soxhlet extractions) were applied to Turkish and Italian grape seed samples for extracting oil and the effects of these methods and cultivars on the aroma and odor activity values were studied for the first time. It was determined that both the different extraction methods and the cultivar differences significantly affected the aroma of the GSO samples. Results showed that a remarkable discrimination were observed among grape cultivars. The clear differentiation between varieties and extraction methods was also supported by OAVs and PCA plots of aroma compounds. When the extraction methods were considered, it was determined that the maximum amount of total aroma compounds was found in the samples extracted by SX method which played an important role both in forming heat-derived new aroma compounds such as maltol, pyranone, 2-pentyl furan, Δ-valerolactone, pantolactone, octanal (*E*,*E*)-2–4-hexadienal, (*E*,*E*)-2–4-heptadienal, 2-nonenal, benzene acetaldehyde, (*E*,*E*)-2–4-decadienal and while they were not exist in CP method. Apart from the aroma compounds, grape seeds contained the oil in a range of 9.6–16.5% in dry weight and the results showed that the oil yields of grape seeds were also influenced by cultivars and extraction methods.

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Author's contributions OS: Formal analysis, Investigation, Methodology, Software, Validation, Visualization. HK: Conceptualization, Formal analysis, Investigation, Writing-original draft, Writing-review and editing. ADR: Breeding and canopy management of vineyards, Sampling the grape clusters, Destemming and extracting the seeds from berries, seed drying, Visualization. SS: Conceptualization, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

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Declarations

Conflicts of interest The authors declare that there is no conflict of interest.

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