Comparing the effects of conventional and microwave roasting methods for bioactive composition and the sensory quality of cold-pressed orange seed oil

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Abstract This study aims to report the composition of bioactives and volatile aromatic compounds, and determine the descriptive sensory properties of cold-pressed orange seed oil. The effects of oven pre-roasting and microwave pre-roasting of the seeds before cold pressing were compared. Thirteen sensory parameters were used to define the oil samples. The major bioactive components of the orange seed oils were naringin, hesperidin, and *trans*-ferulic acid. Flavonoids constituted the main phenolic class with 78.5% and 74.4%, followed by phenolic acids with 21.4% and 25.5% in the oven and microwave pre-roasted oil samples. The mean concentration of hesperidin and naringin varied from 903.4 to 909.6 mg/kg and from 234.3 to 299.8 mg/kg, respectively. The results showed for the first time in the literature that orange seed oil contains some volatile aromatic compounds and glycosylated flavanones that could have functional properties. Hence, cold-pressed orange seed oil could be suggested as the new potential health-promoting oil.

Keywords Cold press · Oil · Orange seed · Quality · Sensory · Volatile

Introduction

Citrus fruits are among the most important and common fruits in production and trade worldwide and a variety of citrus species are grown in more than 100 tropical and subtropical countries all over the world, it is believed that they are spread throughout the world from Southeast Asia (Nayak et al. 2015; Matheyambath et al. 2016). The most widely known members of the *Citrus* genus are lemon (*Citrus limon*), orange (*Citrus sinensis*), tangerine (*Citrus reticulata*), grapefruit (*Citrus paradisi*), pomelo (*Citrus grandis*) and key lime (*Citrus aurantifolia*) (Anwar et al. 2008).

Although most citrus species are consumed fresh, they also have important economic value for their fixed and essential oils. The oil content of lemon, orange and grapefruit seeds obtained as the byproduct of citrus processing was reported to be 34–45%, 27–52% and 36–49%, respectively (Saidani et al. 2004; Anwar et al. 2008). The considerable variation in oil content may have been due to several factors such as geographical location, season, and environmental factors. Citrus seeds are considered valuable and cheap oil sources for food, cosmetics, pharmacology, aromatherapy, and soap and detergent manufacturing (Matthaus and Özcan 2012; Malacrida et al. 2012).

Cold pressing is a special oil production system that is based on pressing very clean, pure, homogeneous and safe oil-bearing seeds or kernels under suitable and mild operation conditions in spite of low yield. Cold pressed oils are preferred for their special flavor/aroma and bioactive components that are retained during processing. Furthermore, these oils are definitely not refined and contain no chemicals or additives. They are sold as they are extracted only after centrifugation or filtration. Generally, cold-pressed oils are used for nutraceuticals, food enrichment,

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encapsulated products, and cosmetics. Cold pressing has also been applied for the valorization of various oil-bearing seeds and kernels generated as byproducts of food-processing operations (Aydeniz et al. 2014; Dündar Emir et al. 2014; Yilmaz et al. 2015a, b; Yilmaz and Aydeniz Güneşer 2017). A few pre-treatments such as pre-roasting and dehulling were used for the seeds or kernels prior to cold pressing to determine their bioactive compound composition and flavor/aroma quality and to enhance the yield of the cold-pressed oils (Aydeniz et al. 2014; Dündar Emir et al. 2014; Yilmaz et al. 2015a).

Although past studies on cold-pressed citrus seed oils are scarce, a few studies have stated that citrus seed oils are rich sources of glycosylated flavanones, which have anti-inflammatory and antihypertensive properties (Manners 2007; Benavente-García and Castillo 2008). Hence, the objective of the present novel study was to investigate the effect of pre-treatments such as microwaving and oven roasting prior to cold pressing on the compositions of bioactives (flavonoid, phenolic acid and pigments) and volatile compounds in orange seed oils. Furthermore, we also aimed to determine the sensory descriptive properties of the cold-pressed oils. This study may prove useful to orange juice processors and cold press oil facilities who could valorize the otherwise-wasted citrus seeds into a highly valued cold press oil product, and manufacturers may eventually find this new and unexplored oil source of potential interest for various functional food, cosmetic, and pharmaceutical applications.

Materials and methods

Plant material and reagents

The fruit seeds of orange (*Citrus sinensis* var. Dörtiyol) were kindly provided by Anadolu Etap Penkon Co. Inc. (Mersin, Turkey) during 2014 harvest and processing season. All solvents and reagents used in the experiments were of analytical and/or chromatographic grade and purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA). The flavonoid standards eriocitrin ($\geq 98\%$), rutin hydrate ($\geq 94\%$), naringin ($\geq 95\%$), hesperidin ($\geq 80\%$), neohesperidin ($\geq 90\%$), naringenin ($\geq 98\%$), kaempferol ($\geq 97\%$), and the phenolic acid standards gallic acid (97%), 2-*trans*-hydroxybenzoic acid (97%), vanillic acid (97%), caffeic acid ($\geq 98\%$), syringic acid (analytical), *p*-coumaric acid ($\geq 98\%$), sinapic acid ($\geq 98\%$), *trans*-ferulic acid (99%), hydroxycinnamic acid (97%), (+)-catechin hydrate ($\geq 98\%$), and chlorogenic acid ($\geq 95\%$) were purchased from Sigma-Aldrich and Fluka Chemicals. The homologous series of C5–C30 straight-chain alkanes kit containing 24 standards used for

volatiles identification were purchased from Sigma Aldrich-Fluka (St. Louis, MO, USA).

Orange seed preparation and cold pressing

First, orange seeds were manually separated from other particulate matter/materials and washed with distilled water. Then, the seeds were air dried and deep-frozen at $-20\text{ }^{\circ}\text{C}$ until they were cold pressed. The orange seeds were divided into two lots: one lot underwent regular oven pre-roasting by direct roasting at $150\text{ }^{\circ}\text{C}$ for 30 min in a conventional oven (Inoksan FPE 110, Bursa, Turkey) and the other part was pre-roasted in a microwave oven (Model MD 1505, BEKO Electrical Appliances, China) at a frequency of 360 W for totally 30 min by applying 3 min-pause mode, 3 min-run mode action. After the treatments, the orange seeds were allowed to cool to an ambient temperature and the moisture contents of the seeds were measured (Ohaus MB45 moisture analyzer, Switzerland) and adjusted to 11% by adding water. Briefly, the volume of water added was calculated by determining the actual and desired moisture contents. Water was added to the seeds placed in a vessel, which was then sealed for 48 h for moisture penetration/retention (Aydeniz et al. 2014). Finally, the seeds were cold pressed by using a laboratory-scale cold press machine (Koçmaksan, ESM 3710, İzmir, Turkey). Fine particles and residual water in the cold-pressed oil were first separated by filtration (Miroil RB22Fs, Allentown, USA) and then by centrifugation at (Sigma 2–16 K, Postfach, Germany) $6800 \times g$ for 10 min. The oil samples were transferred into colored glass bottles, flushed with nitrogen and stored at $-18\text{ }^{\circ}\text{C}$ until analysis.

Polyphenol extraction

Polyphenolic compounds from the orange seed oil were extracted according to the method described by Vallverdú-Queralt et al. (2014). Briefly, all oil samples were allowed to pass through a SePak C–18 cartridge (Waters, Milford, MA, USA) using vacuum in the manifold. The seed oil was homogeneously dissolved in hexane (3 g oil in 3 mL hexane), loaded on the preconditioned cartridge, and rinsed with 5 mL hexane to remove non-polar fractions. Hexane residues in the cartridge were evaporated under nitrogen flow. The retained fraction of phenolics was eluted with 10 mL of methanol and all phenolic extracts were filtered through a PTFE (polytetrafluoroethylene) membrane (0.45 μm pore size, Fisher Scientific) before injection to an HPLC chromatogram. Samples were stored at $-18\text{ }^{\circ}\text{C}$ and analyzed within 24 h. Flow through each cartridge was maintained constant and simultaneous on the manifold.

Analysis and identification of phenolic compounds

The identification of the individual phenolic acids and flavonoids was performed according to the method described by Moulehi et al. (2012) with minor modifications. Separation was performed on a C18 reversed phase column (Zorbax Eclipse Plus, 250 mm × 4.6 mm id × 5 µm) and analyses were performed at 25 °C with a 0.5 mL/min flow rate and the injection volume was 20 µL. Chromatographic analysis was performed using simultaneous monitoring of phenolic extracts at 280 nm. The mobile phase A consisted of 100% acetonitrile, mobile phase B was a ultra pure water with 0.2% (v/v) sulphuric acid (95–97%, analytical). Separation was performed by using the following gradient programme: 80%A/20%B for 0–18 min, 70%A/30%B for 18–24 min, 60%A/40%B for 24–30 min, 50%A/50%B for 30–36 min, 40%A/60%B for 36–40 min, 45%A/55%B for 40–45 min, 35%A/65%B for 45–50 min, 20%A/80%B for 50–52 min, 10%A/90%B for 52–54 min, 100%B for 54–70 min, 45%A/55%B for 70–72 min, 80%A/20%B for 72–74 min. Individual phenolic compounds were identified based on their retention time and quantified from peak area at 280 nm by comparing a standard curve prepared from the corresponding standards. All analyses were performed in triplicate. A sample chromatogram of the phenolic compounds is presented in Figure 1S.

Pigments analysis

The carotenoid contents expressed as total carotenoid, β-carotene, and lutein in the cold-pressed orange seed oil samples were analyzed as described by Franke et al. (2010). The extraction procedure was performed using 0.5 g of each oil sample that was mixed with 2 mL petroleum ether: acetone (1:1, v/v) until dissolved (Heidolph Reax Top, Heidolph, Germany). Absorbance at 445 nm was measured using a spectrophotometer (Agilent 8453 UV–Visible Spectrophotometer, Waldbronn, Germany) and petroleum-ether:acetone was taken as blank. The carotenoid content was determined using the equation given below, and the following specific absorption coefficients in petroleum ether were used.

$$X(\text{mg}/100 \text{ g}) = \frac{A * y(\text{mL}) * 10^6}{A\%1\text{cm} * 1000 \text{ g}}$$

X = Total carotenoid content (mg/100 g), A = Absorbance value at 445 nm, y = Volume of extraction solution (mL), A%1 cm = Specific absorption coefficients for carotenoids.

The chlorophyll pigment as pheophytin-a was determined according to AOCS method Cc 13i-96 by measuring the absorbance at 630, 670 and 710 nm (AOCS 1998).

Analysis and quantification of volatile aromatic compounds

The volatile compounds in the cold-pressed orange seed oil samples were analyzed according to the technique described by Krist et al. (2006). The volatile compounds were extracted from the oil samples by the SPME technique. First, 2 g of the oil sample was weighed in a SPME vial (Supelco, Bellefonte, USA) and 1 g NaCl and 20 µL internal standard (IS) solution (1 µL of 2-methyl-3-heptanone dissolved in 10 mL methanol) were added. Then, the vial was closed and vortexed (Heidolph Reax Top, Heidolph, Germany) for 1 min. The vial was then put into a water bath at 40 °C for 20 min for retaining the volatile compounds in the headspace of the vial. Thereafter, an SPME needle (2 cm to 50/30 lm DVB/Carboxen/PDMS, Supelco, Bellefonte) was inserted carefully into the vial and the fiber was exposed to the headspace of the vial for 10 min at 40 °C in a water bath to allow for the adsorption of volatile compounds. The SPME-fiber-collected volatile compounds present in the headspace were injected into a GC–MS (Agilent 6890 N/Agilent 5875C mass spectrometer, Agilent technologies, Wilmington, DE, USA), immediately. The separation was carried out on a nonpolar HP5 MS column (30 m × 0.25 mm i.d. × 0.25 µm film thickness, J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a flow rate of 1.2 mL/min and a split rate of 1:2. The GC temperature was programmed as follows: heating at 38 °C for 1 min; then, heating from 40 to 220 °C at 5 °C/min rate; and finally held at the final temperature for 20 min. Furthermore, the MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 35–350 amu; scan rate, 4.45 scans/s. Identification and characterization of headspace volatiles of seed oils was based on the comparison of the mass spectra of unknown compounds with those in the National Institute of Standards and Technology and Wiley Registry of Mass Spectral Data databases and the Retention (Kovats) index. Methyl pentanoic acid and 2-methyl-3-heptanone were used as internal standards (IS) for acidic and neutral-basic characters compounds, respectively. Each analytical sample was measured in triplicate. The quantification of all volatile compounds was based on the relative abundance calculated by the equation given below.

$$\text{Mean relative abundance (lg/kg)} = \frac{\text{Concentration of IS}}{\text{peak area of compound/peak area of the IS}}$$

A sample GC/MS total ion chromatogram of volatile aromatics present in orange seed oil samples is shown in Fig. 2S.

Sensory descriptive analysis

The technique of quantitative descriptive analysis and methods used in previous studies were applied to determine the sensory properties of the orange seed oil samples (Meilgaard et al. 1991; Aydeniz et al. 2014; Yilmaz et al. 2015a; Aydeniz Guner and Yilmaz 2017a, b). The panelists (8 female, 4 males, aged 24–43) were trained for at least 15 h on different days, and they worked with the panel leader/moderator to find the reference standards (Table 1S) for the sensory descriptive terms of the orange seed oil samples. Thereafter, the panel assessed the appearance, smell, and taste/odor properties on a 15-cm line scale anchored with 0 (none) to 15 (extreme) for the quantification process.

Different evaluation sessions were scheduled for consecutive days at room temperature and during the day. During each session, the oil samples coded with different three-digit numbers were served randomly to the panel with warm water in individual glasses closed with lids. All sensory evaluations were triplicated for each replicate of the orange seed oil samples.

Statistical analyses

All the cold press oil productions (oven pre-roasting and microwave pre-roasting group) were replicated twice. The oil samples analyses were performed for three times, and the results were reported as mean values $\pm$ SD for Table 1, as mean values $\pm$ SE for Table 2. Statistical analyses were conducted with Minitab ver. 16.1.1 package program at 95% confidence level and Tukey's test (Minitab 2010). Sensory analysis data was compared with non-parametric Kruskal–Wallis and Dunn's test.

Results and discussion

Phenolic composition

This is the first study to describe the phenolic composition of cold-pressed orange seed oils (Table 1). Eight flavonoids and five phenolic acids were quantified in the samples. Flavonoids were found to be most abundant, while hesperidin and naringin were the major phenolic compounds in the oil samples from both the sets. Except for the levels of eriocitrin and rutin, there was no significant difference between the two oil samples in terms of the flavonoid content. The total amount of flavonoids in the microwave pre-roasted orange seed oil sample was higher than that in the oven pre-roasted sample. Hence, the microwave treatment of the seeds prior to cold pressing is believed to have enhanced the flavonoid content of the oil

samples. The most interesting sources of glycosylated flavanones among the citrus seeds have been reported to be naringin and neohesperidin found in bergamot; eriocitrin and hesperidin found in lemon; naringin and neoeriocitrin found in sour orange, and narirutin and hesperidin found in sweet orange (Robards et al. 1997; Bocco et al. 1998). Our findings concur with this previously documented observation in the literature.

Various health benefits of citrus flavonoids have been discussed in the literature (Mir and Tiku 2015; Ferreira et al. 2016; Lei et al. 2016; Gao et al. 2018). For example, one study (Mir and Tiku 2015) showed that naringenin exhibits anti-inflammatory and anti-oxidative properties. In another study, hesperidin, eriocitrin, and eriodictyol were fed to mice through a high-fat diet, and the results showed that these flavanones had protective effects against inflammation and oxidative stress, and may prevent cardiovascular diseases (Ferreira et al. 2016). Similarly, citrus nobletin and tangeritin reduced plasma triglyceride levels and adipose tissue proliferation in hamsters (Lei et al. 2016). In an excellent recent review, citrus polymethoxyflavones and hydroxylated polymethoxyflavones showed potent bioactivities for cytoactivity and organ function by modulating the signaling cascade, gene transcription, and protein activity (Gao et al. 2018). Their anti-inflammatory and anti-microbial functions and their underlying mechanisms were also discussed in this review. Overall, citrus flavonoids are considered bioactive and functional compounds for food, pharmaceutical, and cosmetic uses. Hence, cold-pressed citrus seed oil could make an important niche in the functional oil market.

The phenolic acid composition of both the oil samples was also measured (Table 1). The order of abundance was *trans*-ferulic acid > rosmarinic acid > *trans*-2-hydroxycinnamic acid in both the samples. The total phenolic acid content of the oven pre-roasted oil sample was overall lower than that of the microwave pre-roasted sample. This proved the positive effect of microwave treatment on the retention of phenolic acid content. Phenolic acid has been shown to exhibit positive effects on health (Gao et al. 2018). Hence, the presence of these phenolic acid derivatives in cold-pressed orange seed oil samples could be useful in healthy food manufacturing.

The phenolic acid compositions of the methanolic extracts of lemon, bergamot, sour and sweet oranges, and tangerine seeds and peels have also been studied previously in addition to their antioxidant properties. The orange seed oil methanol extract contained around 20–30 times more naringin (10 mg/kg) and 3–4 times more hesperidin (220–280 mg/kg) than the seed extract (Bocco et al. 1998). In another study, bioactives of (sour orange) peel and juice were investigated, and *p*-coumaric and ferulic acids were found to be the major components (Karoui and Marzouk

Table 1 Flavonoid, phenolic acid and pigment composition of the orange seed oils

	Cold pressed orange seed oil	
	Oven pre-roasted	Microwave pre-roasted
Flavonoids (mg/kg)		
Catechin	14.87 ± 1.22 ^A	15.25 ± 0.35 ^A
Eriocitrin	31.01 ± 1.22 ^B	85.78 ± 3.80 ^A
Rutin	52.59 ± 0.39 ^B	76.48 ± 0.29 ^A
Naringin	234.28 ± 0.56 ^A	299.80 ± 27.20 ^A
Naringenin	10.38 ± 0.15 ^A	13.23 ± 1.66 ^A
Hesperidin	909.67 ± 5.63 ^A	903.40 ± 36.20 ^A
Neohesperidin	100.99 ± 0.81 ^A	125.90 ± 19.20 ^A
Kaempferol	8.64 ± 0.06 ^A	9.56 ± 0.83 ^A
Phenolic acids (mg/kg)		
Gallic acid	42.43 ± 3.57 ^A	29.41 ± 3.40 ^A
Syringic acid	6.93 ± 0.01 ^B	7.13 ± 0.01 ^A
<i>trans</i> -Ferulic acid	222.97 ± 2.76 ^B	364.30 ± 55.10 ^A
Rosmarinic acid	58.08 ± 0.48 ^A	77.90 ± 14.40 ^A
<i>trans</i> -2-Hydroxycinnamic acid	41.65 ± 0.40 ^A	47.22 ± 5.41 ^A
Pigments (mg/kg oil)		
Total carotenoid	7.64 ± 0.48 ^A	5.49 ± 0.14 ^B
β-Carotene	7.37 ± 0.46 ^A	5.30 ± 0.14 ^B
Lutein	7.35 ± 0.46 ^A	5.29 ± 0.14 ^B
Total chlorophyll (pheophytin a)	0.20 ± 0.02 ^B	0.34 ± 0.05 ^A

All values are mean values ± standard error

^{A,B}Different capital letters within the same row are significantly different ($p \leq 0.05$)

2013). Our reported values for *trans*-2-hydroxycinnamic and gallic acids in both the orange seed oil samples were in accordance with the values reported for the peel extract of sour orange. Furthermore, Nayak et al. (2015) reported that the microwave-assisted extraction method yielded the highest caffeic and ferulic acid from orange peels. These results support our finding that microwave pre-treatment has a positive effect on flavonoid and phenolic acid release into the oil. Lastly, in one of our previous studies, we determined the flavonoid and phenolic acid composition of the cold-pressed lemon seed oil samples and found that orange seed oil samples in this study had lower values of flavonoids than the lemon seed oil samples (Aydeniz Guneser and Yilmaz 2017a, b).

Pigment composition

Total carotenoid, β-carotene, lutein, and pheophytin-a contents of the oil samples are also presented in Table 1. Except for pheophytin-a, the concentrations of other carotenoid pigments were significantly lower in the microwave-treated sample. This might be due to the degradative effect of microwave on the carotenoids. The presence of natural pigments in cold-pressed oil samples could be considered good from the nutritional viewpoint and may

affect consumer preferences (Aydeniz Guneser and Yilmaz 2017a, b). Unfortunately, there is no existing study on cold-pressed orange seed oil samples in the literature to compare our current results; however, in one study, β-carotene and chlorophyll contents in the solvent extracted from Osage orange seed oil were reported to be 0.60 and 0.02 mg/kg of oil (Saloua et al. 2009). These values are fairly lower than the results found in this study. In another study, the carotenoid content in orange peels (151.6–218 µg/g dry weight) was found to be significantly higher than that in the orange juice vesicles (40–143.09 µg/g dry weight) (Xu et al. 2006). Clearly, carotenoid content in peels and juice vesicles is significantly higher than in seed oil. Malacrida et al. (2012) observed that lemon and tangerine seed oil samples have tenfold higher carotenoid levels (0.32 mg/kg oil) than seed oil samples obtained from the orange grown in Brazil. In general, total carotenoid and chlorophyll contents measured in orange seed oil samples in this study were higher than the values reported in the literature for orange seed and peel oil samples.

Volatile aromatics composition

Volatile aromatic compounds of the cold-pressed orange seed oil samples were investigated by the SPME-GC/MS

Table 2 Volatile aromatics composition of the orange seed oil samples

No.	RI ^a	Volatile compounds	Aroma/flavor description ^b	Concentration (µg/kg oil)	
				Oven pre-roasted	Microwave pre-roasted
1.	–	3-Methylbutanal	Fruity, sweet	nd	39.05 ± 6.97
2.	716	Acetoin	Creamy, buttery	24.23 ± 0.87	18.11 ± 2.83
3.	799	Hexanal	Green, grass	8.90 ± 0.03	16.30 ± 0.95
4.	813	Furfural	Sweet, caramel, baked bread	24.07 ± 5.97	45.36 ± 2.10
5.	825	Methyl pyrazine	Nutty, roasted cacao	nd	20
6.		2-Furan menthol	Roasted cacao, burnt	4.07 ± 0.77	7.96 ± 0.33
7.	877	Isoamyl acetate	Banana, fruity	2.80 ± 0.15	2.52 ± 0.53
8.	891	Butyrolactone	Creamy, fatty, caramel	0.38 ± 0.28	nd
9.	911	2,5-Dimethylpyrazine	Nutty, roasted	nd	4.14 ± 0.25
10.	914	Butyl isobutyrate	Pear, pineapple	0.89 ± 0.27	nd
11.	924	α-Thujene	Woody, green herb	2.86 ± 0.26	2.38 ± 0.01
12.	930	α-Pinene	Herbal, turpentine, woody	24.96 ± 0.22	17.68 ± 1.31
13.	942	Isopropyl pentanoate	Fruity, pear	11.52 ± 0.08	11.59 ± 0.15
14.	954	Benzaldehyde	Bitter almond	5.24 ± 1.12	6.50 ± 0.13
15.	972	β-Pinene	Woody, green pine	47.59 ± 2.58	31.34 ± 0.72
16.	991	β-Myrcene	Spicy, terpenic, herbal	124.89 ± 13.55	87.33 ± 6.50
17.	1000	α-Phellandrene	Terpenic, citrus	3.99 ± 0.22	3.57 ± 0.18
18.	1004	Octanal	Waxy, fatty	nd	2.03 ± 0.53
19.	1014	3-Carene	Sweet	2.89 ± 0.49	2.33 ± 0.19
20.		3-Methoxy-1-butanol	Alcohol	nd	2.70 ± 0.47
21.	1015	Hexyl acetate	Fruity, green apple	4.15 ± 0.63	6.51 ± 4.27
22.	1021	β-Cymene	Terpenic	22.27 ± 1.21	14.38 ± 0.73
23.	1027	D-Limonene	Fresh citrus	5902.07 ± 308.59	4568.84 ± 287.69
24.	1052	α-Ocimene	Fruity, floral	1.70 ± 0.07	1.19 ± 0.04
25.	1058	γ-Terpinene	Terpenic	32.11 ± 1.02	27.97 ± 1.97
26.		1-Octenol	Green herb, fatty, herbal	1.04 ± 0.18	0.55 ± 0.13
27.	1088	(Z)-Linalooloxide	Earthy, floral	1.88 ± 2.67	nd
28.	1089	α-Terpinolene	Herbal, woody, citrus	12.19 ± 2.51	10.14 ± 0.08
29.	1107	Phenylethyl alcohol	Floral, sweet, rose	0.52 ± 0.38	nd
30.	1139	(E)-Limonene oxide	Citrus	0.93 ± 0.16	1.32 ± 0.27
31.	1179	4-Carvomenthol	Woody, spicy	0.85 ± 0.17	0.77 ± 0.01
32.	1190	α-Terpineol	Floral, lilac	49.32 ± 8.79	44.17 ± 0.24
33.		Decyl acetate	Waxy, fatty, creamy	0.87 ± 0.29	0.70 ± 0.02

^aRI (Kovat Index) on HP 5 MS column. nd not detected

^bAromatic definitions of the volatile compounds are found from the web pages of <http://www.thegoodscentscompany.com/index.html#>, <http://www.flavornet.org/flavornet.html>

method, and 33 volatile compounds were quantified; they are described in Table 2. Orange seed oil contained higher levels of monoterpene hydrocarbons and monoterpene alcohols. D-limonene, β-myrcene, α-terpineol, β-pinene, γ-terpinene, β-cymene and furfural were quantified as the major volatile aromatic substances in the samples. D-limonene (4500–5900 µg/kg oil) was found to specifically be responsible for the aromatic citrus notes in both the oil samples. The volatile aromatic composition of cold-pressed

orange seed oil samples has been described for the first time in this study; hence, no comparison can be made with the literature articles at this stage.

Microwave treatment of the orange seeds before cold pressing led to an increase in the levels of hexanal, furfural, and 2-furan menthol and a decrease in the level of D-limonene (22%). Pyrazines compounds (methyl pyrazine, 2,5-dimethylpyrazine) known with roasted aroma notes, and octanal, 3-methoxy-1-butanol were observed

only in the microwave treated seed oils. It is expected that formation of pyrazines is a result of microwave treatment, just as we observed in previous studies (Aydeniz et al. 2014; Dündar Emir et al. 2014). The oils extracted from safflower, poppy seed, and capia pepper seeds that were microwave roasted before cold pressing contained pyrazine derivatives such as 2-methyl-5-pyrazine, 2,5-dimethyl pyrazine, 2,3-dimethyl 5-ethyl pyrazine, 2-ethyl-pyrazine, 3,5-dimethyl-2-ethylpyrazine, and 2-acetyl-6-methylpyrazine (Aydeniz et al. 2014; Dündar Emir et al. 2014; Yılmaz et al. 2015b).

Furthermore, the oven pre-roasted sample was richer in aromas associated with fruity, creamy, floral, herbal, woody, spicy, and terpenic notes than the microwave pre-roasted seed oil samples (Table 2). Qiao et al. (2008) investigated the aromatic compounds of fruit juice and peel oil of sweet orange using GC/MS and GC/O techniques. In total, 49 and 32 compounds were determined in orange juice and peel oil respectively. Ethyl butanoate, β -myrcene, octanal, linalool and decanal for fruit juice, and linalool, α -pinene and decanal for peel oil were reported as major aromatic components. Boussada and Chemli (2007) reported that D-limonene was the most abundant component in *Citrus aurantium* (bitter orange). In another study, Moufida and Marzouk (2003) determined the aromatic compounds in five varieties of citrus fruits: blood orange, sweet orange, lemon, bergamot, and bitter orange. The researchers showed that D-limonene was the major volatile component in all the citrus varieties and constituted 63%, 88%, and 90% of the volatile compounds in blood orange, sweet orange, and bitter orange, respectively. The profiles of volatile compounds obtained from the orange seed oil samples in the present study seem to be in good agreement with those reported for peel oils and juices by Moufida and Marzouk (2003), Boussada and Chemli (2007) and Qiao et al. (2008).

In a previous study, we reported the volatile composition of lemon seed oil samples (Aydeniz Guneser and Yılmaz 2017a, b). Although the volatile composition of the orange seed oil samples showed some similarities with those of the lemon seed oil samples, it was quite different from the reported findings of citrus peel oils (Kırbaşlar et al. 2006; Viuda-Martos et al. 2009). Hence, this study provides crucial and pioneering data in its field.

Sensory description

The results of the sensory descriptive analysis by the panel are presented in Fig. 1 in a spider web format. Among the 13 descriptive terms, differences existed only for ‘orange leaf’, ‘roasted’, ‘sweet aromatic’, and ‘throatcatching’ terms for the two samples. Microwave pre-roasted seed oil had significantly higher scores for ‘roasted’ and

Fig. 1 Descriptive sensory properties of the cold pressed orange seed oil samples

‘throatcatching’, and lower scores for ‘orange leaf’ and ‘sweet aromatic’ terms. The results for the rest of the defined terms were quite similar. Defined as the level of bright appearance, ‘clarity’ was almost equal and under 4.0 for both the samples, indicating that the oil samples were limpid. Aromatics associated with ‘orange leaf’ were higher in the oven pre-roasted sample, and most probably partially degraded or were lost during microwave pre-roasting in the other sample. The score for ‘roasted’ was significantly higher in the microwave-treated sample because of the extent of heat exposure. These sensory findings are in accordance with the results of the volatile analysis (Table 2), which indicated higher concentrations of pyrazines in the microwave-treated sample. ‘Raw vegetable’ is the flavor associated with uncooked green beans and common to most raw plant parts, and for both the samples, it was around 3.5–4.0. ‘Olive bagasse’ was defined as the aroma associated with fresh olive bagasse, and was found in low values (around 2.0). ‘Bitter’ was the most distinct characteristics of these oils with 9.9 and 10.5 scores, respectively. Clearly, microwave treatment had no effect on the bitter taste. In our previous study (Aydeniz Guneser and Yılmaz 2017a, b), cold-pressed lemon seed oil samples were also found to be bitter-tasting samples. Both the samples had considerable extents of ‘astringency’ (around 5.0), which is defined as the constriction felt in the mouth. Although the microwave-treated sample had a little higher ‘nutty’ score (4.67) than the oven pre-roasted sample (3.54), there was no statistically significant difference. ‘Sweet aromatic’ has been defined as the flavor associated with honey and was found to be significantly lower in the microwave pre-roasted sample. Both the samples were found to be ‘very fatty’ by the panel, as expected. Likewise, they gave higher scores for the ‘spicy’ term,

indicating some common aromas found in spices to match with those of the samples. The oil samples had around 3.5–4.3 ‘menthol’ scores, which indicate the cooling sensation felt on the tongue. Usually, regular seed oils (soy, corn, sunflower, etc.) do not have any menthol definition, but citrus seed oils mostly have it (Aydeniz Güneser and Yılmaz 2017a, b). The burning sensation felt in the throat after swallowing the oil samples is defined as ‘throat-catching’, and the microwave pre-roasted sample had a higher score for the same, possibly due to the formation of the pyrazines in addition to the naturally occurring bitter phenolic compounds in the oil samples.

A clear relationship could be shown between the content of flavonoids and the sensory properties of the cold-pressed orange seed oil samples. The microwave pre-roasted seed oil sample particularly contained higher eriocitrin and rutin levels than the oven pre-roasted oil sample, thus supporting the fact that these oils have higher throatcatching and bitterness scores (Fig. 1). Similarly, clarity, roasted, nutty, raw vegetable, menthol, and spicy scores of the microwave-treated seed oil samples were a little higher than those of the oven pre-roasted sample. Since this study provides the first sensory descriptive data of cold-pressed orange seed oils, it is assumed to be most likely appreciated by those who are interested to produce and use this new and authentic oil for various useful applications.

Conclusion

Cold-pressed oils are becoming popular consumer items in functional food, cosmetic, and pharmaceutical markets. There are a few studies about the beneficial effects of orange seed oil, and this study provides new data about its flavonoid and volatile compound compositions, and an array of useful sensory descriptions. This study showed that the oil is rich in hesperidin, naringin, neohesperidin, *trans*-ferulic, gallic and *trans*-2-hydroxycinnamic acids. Among the 33 volatile compounds quantified, D-limonene and furfurals were found to be in the highest concentrations. The sensory panel identified bitter and astringent as the dominant sensory attributes. Hence, cold-pressed orange seed oil could be utilized in functional foods. However, further studies about its biochemical effects and applications in product formulations are needed.

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References

- Anwar F, Naseer R, Bhanger MI, Ashraf S, Talpur FN, Aladedunye FA (2008) Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. *J Am Oil Chem Soc* 85:321–330
- AOCS (1998) Official methods and recommended practices of the American Oil Chemists’ Society. American Oil Chemists’ Society, Champaign
- Aydeniz Güneşer B, Yılmaz E (2017a) Bioactives, aromatics and sensory properties of cold-pressed and hexane-extracted lemon (*Citrus limon* L.) seed oils. *J Am Oil Chem Soc* 94:723–731
- Aydeniz Güneşer B, Yılmaz E (2017b) Effects of microwave roasting on the yield and composition of cold pressed orange seed oils. *Grasas Aceites* 68:e175
- Aydeniz B, Güneser O, Yılmaz E (2014) Physico-chemical, sensory and aromatic properties of cold press produced safflower oil. *J Am Oil Chem Soc* 91:99–110
- Benavente-García O, Castillo J (2008) Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular and anti-inflammatory activity. *J Agric Food Chem* 56:6185–6205
- Bocco A, Cuvelier ME, Richard H, Berse C (1998) Antioxidant activity and phenolic composition of citrus peel and seed extracts. *J Agric Food Chem* 46:2123–2129
- Boussaada O, Chemli R (2007) Seasonal variation of essential oil composition of *Citrus aurantium* L. var. amara. *J Essent Oil Bear Plants* 10:109–120
- Dündar Emir D, Güneşer O, Yılmaz E (2014) Cold pressed poppyseed oils, sensory properties, aromatic profiles and consumer preferences. *Grasas Aceites* 65(3):e029
- Ferreira PS, Spolidorio LC, Manthey JA, Cesar TB (2016) Citrus flavanones prevent systemic inflammation and ameliorate oxidative stress in C57BL/6J mice fed high-fat diet. *Food Funct* 7:2675–2681
- Franke S, Fröhlich K, Werner S, Böhm V, Schöne F (2010) Analysis of carotenoids and vitamin E in selected oilseeds, press cakes and oils. *Eur J Lipid Sci Technol* 112:1122–1129
- Gao Z, Gao W, Zeng S-L, Li P, Liu EH (2018) Chemical structures, bioactivities and molecular mechanisms of citrus polymethoxyflavones. *J Funct Foods* 40:498–509
- Karoui IJ, Marzouk B (2013) Characterization of bioactive compounds in Tunisian bitter orange (*Citrus aurantium* L.) peel and juice and determination of their antioxidant activities. *BioMed Res Int* 345415:1–12
- Kırbaşlar Şİ, Boz İ, Kırbaşlar FG (2006) Composition of Turkish lemon and grapefruit peel oils. *J Essent Oil Res* 18:525–543
- Krist S, Stuebiger G, Bail S, Unterwieser H (2006) Detection of adulteration of poppy seed oil with sunflower oil based on volatiles and triacylglycerol composition. *J Agric Food Chem* 54:6385–6638
- Lei L, Li YM, Wang X, Liu Y, Ma KY, Wang L, Man SW, Zhang C, Huang Y, Chen ZY (2016) Plasma triacylglycerol-lowering activity of citrus polymethoxylated flavones is mediated by modulating the genes involved in lipid metabolism in hamsters. *Eur J Lipid Sci Technol* 118:147–156
- Malacrida CR, Kimura M, Jorge N (2012) Phytochemicals and antioxidant activity of citrus seed oils. *Food Sci Technol Res* 18:399–404
- Manners GD (2007) Citrus limonoids: analysis, bioactivity and biomedical prospects. *J Agric Food Chem* 55:8285–8294
- Matheyambath AC, Padmanabhan P, Paliyath G (2016) Encyclopedia of food and health, 1st edn. Elsevier Press, Oxford
- Matthaus B, Özcan MM (2012) Chemical evaluation of citrus seeds an agro-industrial waste, as a new potential source of vegetable oils. *Grasas Aceites* 63:313–320

- Meilgaard M, Civille GV, Carr BT (1991) Sensory evaluation techniques. CRC, Boca Raton
- Minitab (2010) Minitab statistical software (version 16.1.1.). Minitab, Inc., State College
- Mir IA, Tiku AB (2015) Chemopreventive and therapeutic potential of “naringenin”, a flavanone present in citrus fruits. *Nutr Cancer* 67:27–42
- Moufida S, Marzouk B (2003) Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange. *Phytochemistry* 62:1283–1289
- Moulehi I, Bourgou S, Ourghemmi I, Tounsi MS (2012) Variety and ripening impact on phenolic composition and antioxidant activity of mandarin (*Citrus reticulata* Blanco) and bitter orange (*Citrus aurantium* L.) seeds extracts. *Ind Crops Prod* 39:74–80
- Nayak B, Dahmoune F, Moussi K, Remini H, Dairi S, Aoun O, Khodir M (2015) Comparison of microwave, ultrasound and accelerated-assisted solvent extraction for recovery of polyphenols from citrus sinensis peels. *Food Chem* 187:507–516
- Qiao Y, Xie BJ, Zhang Y, Zhang Y, Fan G, Yao XL, Pan SY (2008) Characterization of aroma active compounds in fruit juice and peel oil of jinchen sweet orange fruit (*Citrus sinensis* (L.) Osbeck) by GC–MS and GC–O. *Molecules* 13:1333–1344
- Robards K, Li X, Antolovich M, Boyd S (1997) Characterisation of citrus by chromatographic analysis of flavonoids. *J Sci Food Agric* 75:87–101
- Saidani M, Dhifil W, Marzouk B (2004) Lipid evaluation of some Tunisian citrus seed. *J Food Lipids* 11:242–250
- Saloua F, Eddine NI, Hedi Z (2009) Chemical composition and profile characteristics of Osage orange *Maclura pomifera* (Rafin.) Schneider seed and seed oil. *Ind Crops Prod* 29:1–8
- Vallverdú-Queralt A, Regueiro J, Alvarenga JFR, Torrado X, Lamuela-Raventos RM (2014) Home cooking and phenolics: effect of thermal treatment and addition of extra virgin olive oil on the phenolic profile of tomato sauces. *J Agric Food Chem* 62:3314–3320
- Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA (2009) Chemical composition of mandarin (*C. reticulata* L.), grapefruit (*C. paradisi* L.), lemon (*C. limon* L.) and orange (*C. sinensis* L.) essential oils. *J Essent Oil Bear Plants* 12:236–243
- Xu CJ, Fraser PD, Wang WJ, Bramley P (2006) Differences in carotenoid content of ordinary citrus and lycopene-accumulating mutants. *J Agric Food Chem* 54:5474–5481
- Yilmaz E, Aydeniz Güneşer B (2017) Cold pressed versus solvent extracted lemon (*Citrus limon* L.) seed oils: yield and properties. *J Food Sci Technol* 54:1891–1900
- Yilmaz E, Arsunar ES, Aydeniz B, Güneşer O (2015a) Cold pressed capia pepperseed (*Capsicum annuum* L.) oils: composition, aroma and sensory properties. *Eur J Lipid Sci Technol* 117:1016–1026
- Yilmaz E, Aydeniz B, Güneşer O, Arsunar ES (2015b) Sensory and physico-chemical properties of cold press produced tomato (*Lycopersicon esculentum* L.) seed oils. *J Am Oil Chem Soc* 92:833–842