



Quality Properties, Fatty Acid Composition, and Mineral Contents of Some *Citrus* Seeds and Oils Extracted by Solvent Extraction

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Abstract

In this study, while the oil yields of *Citrus* seeds were found to be between 27.61% (mandarin) and 36.42% (bitter orange), the crude protein contents of seeds ranged between 10.54% (bitter orange) and 14.21% (lemon). Total phenolic and total flavonoid amounts of *Citrus* seeds were determined to range from 411.43 mg GAE/100 g (lemon) and 814.84 mg GAE/100 g (bitter orange) to 97.84 mg/100 g (grapefruit) and 126.48 mg/100 g (lemon). In addition, the antioxidant activity values of *Citrus* seeds ranged between 53.27% (mandarin) and 74.21% (lemon). Results showed some differences depending on fruit type. In general, the sugar content of bitter orange was found to be low. However, the most abundant sugar in all samples was sucrose. Oleic and linoleic acid amounts of *Citrus* seed oils were detected to range from 21.84% (bitter orange) and 27.58% (grapefruit) to 33.94% (lemon) and 38.67% (mandarin). According to the results, the minerals found in the greatest amounts in the seeds were phosphorus, magnesium, potassium, and calcium, followed by sodium, iron, zinc, manganese, and copper.

Keywords Lemon · Mandarin · Bitter orange · Grapefruit · Sugars

Introduction

Citrus plants, belonging to Rutaceae family, are widely distributed in Mediterranean countries of the Middle East and Southern Europe and grow in other subtropical and tropical climates (Saidani et al. 2004; Özcan and İnan 2022). As a result of the production of commercially produced *Citrus* juice, marmalade, and jam, a large number of seeds are formed as a by-product. It is thought that these seeds can be used as a source of oil (Abdel-Rahman 1980; El-Safy et al. 2012; Malacrida et al. 2012; İnan et al. 2018; Özcan et al. 2021). *Citrus* seed not only wastes a potentially valuable resource but has also aggravated serious disposal problems (Malacrida et al. 2012; Lazos and Servos 1988). The high

oil content makes the seed material interesting for the production of oils. Lipids are important for the development of cells as structural components, as functional compounds, and for the storage of energy (Aitzetmüller 1993). Recently, there has been a great deal of attention on the use of by-products and waste from the food industry (Schieber et al. 2001; Duman et al. 2016). Waste from industrial processing includes peels, seeds, and pulps, representing 45% to 58% of the raw processed fruit (El-Adawy et al. 1999). It is important to determine the potential of *Citrus* seed utilization in human and animal diets (Akpatá and Akubor 1999). The main constituents of *Citrus* seeds are seed meal, consisting of protein and seed oil (El-Adawy et al. 1999). *Citrus* seed oils have tocopherols, unsaturated fatty acids, carotenoids, and phenolics (Anwar et al. 2008; Malacrida et al. 2012; Matthaus and Özcan 2012). El-Adawy et al. (1999) reported that *Citrus* seeds contain 26%–42% oil and potassium, calcium, sodium, iron, and magnesium in significant amounts. *Citrus* fruits are rich in bioactive properties that are beneficial for human health (Zou et al. 2016). The aim of this study was to determine the bioactive compounds, sugar content, fatty acid composition, and mineral contents of some *Citrus* seeds and oils extracted by solvent extraction.

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Material and Methods

Materials

In this study, the seeds of lemon, mandarin, orange, bitter orange, and grapefruit provided from Mersin, a province of Turkey, were used. All *Citrus* seeds were manually separated from fruit pulps and washed with distilled water. After they were dried at room temperature, the dried kernels were ground in a laboratory-type mill.

Methods

Moisture Content

The moisture content of *Citrus* seeds was measured at 105 °C until constant weight using the oven method (Nüve FN055, Ankara, Turkey).

Protein Content

The protein contents of seed samples were determined by the Dumas nitrogen analyzer (Velp NDA 701, Italy). Crude protein was calculated by using a nitrogen conversion factor of 6.25 (Greenfield and Southgate 1992).

Oil Extraction

The extraction of ground *Citrus* seeds was carried out in a Soxhlet apparatus using petroleum ether at 60 °C for 6 h. Solvent was removed from the oil by a rotary evaporator.

Extraction Procedure

In the extraction of seed samples, the modified method described by Mraïhi et al. (2013) was used. After 15 ml of a methanol:water (80:20, v/v) mixture was added to the ground sample (2 g), the mixture was sonicated in an ultrasonic bath for 30 min at room temperature. Then it was washed with 5 ml of hexane. The extracts were centrifuged for 10 min at 5000 rpm. After these steps were repeated twice, the supernatants were collected, and the extract was evaporated under vacuum on a rotary evaporator at 37 °C. The solutions prepared according to this method were filtered through a 0.45-µm filter and injected into a high-performance liquid chromatograph for analysis.

Total Phenolic Content

The total phenolic contents of the *Citrus* seed extracts were recorded using Folin–Ciocalteu reagent (Yoo et al. 2004). After 1 ml of Folin–Ciocalteu was added to the sample, the mixture was stirred by vortex for 5 min, and then 10 ml of

7.5% Na₂CO₃ was added. The solution was mixed again, and the final volume was made up to 25 ml with deionized water. The total phenolic amount of the samples was determined at 750 nm and given in milligrams of gallic acid equivalent (GAE) per 100 grams (fresh weight [fw]).

Total Flavonoid Content

The colorimetric method described by Hogan et al. (2009) was applied in order to determine the total flavonoid contents of seed extracts. After 1 ml of extract was mixed with 0.3 ml NaNO₂, 0.3 ml of AlCl₃ and 2 ml of NaOH were added to the solution. Then the absorbance values of the mixture were recorded at 510 nm. The results obtained are described as milligrams of catechin per gram (fw).

Antioxidant Activity

For the antioxidant activity of *Citrus* seed extracts, 1,1-difenil-2-pikrilhidrazil (DPPH) was applied (Lee et al. 1998). After the citrus seed extract was shaken with 2 ml of a DPPH-methanolic solution, the mixture was stirred vigorously by vortex and allowed to rest at room temperature for 30 min. Absorbances obtained were determined at 517 nm in a spectrophotometer.

Sugar Analysis

The modified phenol-sulfuric acid method was performed, and the amounts of some monosaccharides and disaccharides in seeds were determined (Enzo et al. 2006). The absorbances of samples was measured at 490 nm.

Fatty Acid Composition

The fatty acid methyl esters described in standard method (ISO-5509 1978) were determined by a gas chromatograph (Shimadzu GC-2010) consisting of a flame-ionization detector and capillary column. For the analysis of fatty acids, the carrier gas and total flow rate were nitrogen with a flow rate of 1.51 ml/min and 80 ml/min, respectively. The split rate was 1/40. The temperature of the injection block and detector was calibrated to 260 °C. In addition, column temperature was calibrated at 120 °C for 5 min, increased to 240 °C by 4 °C/min, and held at 240 °C for 25 min (AOAC 1990).

Mineral Content

About 0.5 g of ground *Citrus* seed sample was burned by using 5 ml of 65% HNO₃ and 2 ml of 35% H₂O₂ in a microwave system. Minerals in seeds were determined by

inductively coupled plasma atomic emission spectroscopy (Varian-Vista, Australia) (Skujins 1998).

Statistical Analyses

Version 9.0 of JMP statistical software (SAS Inst. Inc., Cary, NC, USA) was used for analysis of variance of results. The results were stated as mean \pm standard deviation of independent *Citrus* seed types (Püskülcü and İkiz 1989).

Results and Discussion

Some Physicochemical Properties of Citrus Seeds

The physicochemical and bioactive properties of mandarin (satsuma), orange (Valencia), bitter orange, lemon (Interdonato), and grapefruit seeds are shown in Table 1. The moisture amounts of seed samples were calculated as being between 4.28% (mandarin) and 6.27% (bitter orange). While the oil yields of *Citrus* seeds were found to be between 27.61% (mandarin) and 36.42% (bitter orange), the crude protein contents of seeds ranged between 10.54% (bitter orange) and 14.21% (lemon). Also, the total phenolic amounts of *Citrus* seeds were determined to be between 411.43 mg GAE/100 g (lemon) and 814.84 mg GAE/100 g (bitter orange), while the total flavonoid amounts varied between 97.84 mg/100 g (grapefruit) and 126.48 mg/100 g (lemon). In addition, antioxidant activity values of *Citrus* seeds ranged between 53.27% (mandarin) and 74.21% (lemon). The results showed some differences depending on fruit type. A linear relationship was observed between total flavonoid contents and antioxidant activities of *Citrus* seeds.

At the same time, *Citrus* seeds are a good oil source. Therefore, it is thought that the seeds, which are considered waste in the production of *Citrus* fruit juice, can be used as oil raw material. El-Adawy et al. (1999) reported that the seeds of orange and mandarin contained 17.01% and 15.87% oil, respectively. Anwar et al. (2008) determined 6.42% protein and 29.76% oil in lemon seeds. Zhang et al. (2018) reported that phenolic con-

tents, flavonoid contents, and antioxidant activity values of *Citrus* seeds ranged between 1.09 mg GAE/g and 2.81 mg GAE/g, 5.96 mg RE/g and 18.23 mg RE/g, and 0.95 VCAC mg/g and 2.45 VCAC mg/g, respectively. In another study, total phenolic and flavonoid contents of *Citrus* seeds ranged between 3.15 mg GAE/g (*C. papaya*) and 6.48 mg GAE/g (*C. sinensis*) and between 0.191 mg QE/g (*C. papaya*) and 1.05 mg QE/g (*C. sinensis*), respectively (Kumar and Sharma 2021). *C. papaya* seed contained 0.107–3.179 mg GAE/g total phenolic content (Kothari and Seshadri 2010). Malacrida et al. (2012) determined 34.92% to 41.66% oil in *Citrus* seeds. Orange, lemon, and tangerine seed oils contained 1152.88 mg GAE/kg, 1196.71 mg GAE/kg, and 1007.77 mg GAE/kg total phenolic content, respectively (Malacrida et al. 2012). The antioxidant activity of several *Citrus* seeds was determined to be between 94.87 μ mol/g (*C. limetta*) and 97.82 μ mol/g (*C. sinensis*) (Kumar and Sharma 2021). Some differences have been observed with regard to comparisons of values from previous work on this subject. These differences probably depend on soil structure of the region, fertilizer, soil composition, environmental factors, and growing conditions (Konarski et al. 2006; Taşdemir et al. 2006).

Sugar Contents of Seeds of Several Citrus Fruits

The sugar contents of *Citrus* seeds are presented in Table 2. The fructose and glucose amounts of *Citrus* seeds were found to be 2.18% (bitter orange), 4.86% (grapefruit), 2.59% (bitter orange), and 5.64% (mandarin), respectively. Also, while galactose amounts of *Citrus* seeds varied between 1.09% (grapefruit) and 2.97% (orange), sucrose contents of *Citrus* seeds were reported to be between 3.64% (bitter orange) and 6.35% (mandarin). In general, the sugar content of bitter orange was found to be low. However, the most abundant sugar in all samples was sucrose. It is possible that this situation may have resulted from biochemical reactions in the seed during the development of the fruits. In a previous study, *Citrus* seeds contained 3.75–5.75 g/kg glucose and 4.09–6.03 g/kg fructose (Al-Juhaimi et al. 2016). In addition, during the growth of the plant, variables such as climatic factors, cultural (agricultural) factors, and har-

Table 1 Some physicochemical and bioactive properties of *Citrus* seeds

Sample	Moisture (%)	Oil (%)	Crude protein (%)	Total phenolic (mg GAE/100 g)	Total flavonoid (mg/100 g)	Antioxidant activity (%)
Mandarin (satsuma)	4.28 \pm 0.57* ^e	27.61 \pm 1.38 ^e	11.46 \pm 1.03 ^d	718.24 \pm 10.32 ^b	85.19 \pm 3.68 ^e	53.27 \pm 1.58 ^e
Orange (Valencia)	5.03 \pm 1.13 ^{c**}	31.84 \pm 2.21 ^c	13.38 \pm 1.24 ^b	687.61 \pm 27.19 ^c	121.57 \pm 5.76 ^b	70.16 \pm 3.87 ^b
Bitter orange	6.27 \pm 1.08 ^a	36.42 \pm 1.36 ^a	10.54 \pm 1.67 ^e	817.84 \pm 43.56 ^a	115.64 \pm 6.98 ^c	65.34 \pm 2.54 ^c
Lemon (Interdonato)	4.84 \pm 0.97 ^d	29.46 \pm 1.68 ^d	14.21 \pm 2.84 ^a	411.43 \pm 17.64 ^e	126.48 \pm 3.42 ^a	74.21 \pm 5.73 ^a
Grapefruit	5.63 \pm 0.76 ^b	35.57 \pm 1.97 ^b	12.85 \pm 1.36 ^c	634.51 \pm 29.32 ^d	97.84 \pm 2.76 ^d	57.18 \pm 2.93 ^d

*Standard deviation

**Values within each column followed by different letters are significantly different at $p < 0.05$

Table 2 Sugar contents of citrus seeds (%)

Sample	Fructose	Glucose	Galactose	Sucrose
Mandarin (satsuma)	3.17 ± 0.18*c	5.64 ± 1.28a	1.81 ± 0.13b	6.35 ± 1.19a
Orange (Valencia)	3.94 ± 0.56b**	4.18 ± 1.37c	2.97 ± 0.32a	5.71 ± 0.87b
Bitter orange	2.18 ± 0.71e	2.59 ± 0.93e	1.61 ± 0.17c	3.64 ± 1.21e
Lemon (Interdonato)	2.84 ± 0.64d	4.61 ± 0.86b	1.24 ± 0.28d	4.86 ± 1.32c
Grapefruit	4.86 ± 0.52a	3.28 ± 1.13d	1.09 ± 0.11e	3.91 ± 0.87d

*Standard deviation

**Values within each column followed by different letters are significantly different at $p < 0.05$

vest time are among those affecting the composition of the seeds.

Fatty Acid Compositions of Seed Oils of Citrus Fruits

Fatty acid compositions of *Citrus* seed oils are illustrated in Table 3. Linoleic, oleic, and palmitic acids were the abundant fatty acids in *Citrus* oils. While palmitic acid contents of *Citrus* seed oils varied between 17.54% (mandarin) and 21.14% (bitter orange), stearic acid amounts of oil samples were between 2.05% (bitter orange) and 5.42% (grapefruit). In addition, oleic and linoleic acid amounts of *Citrus* seed oils were detected to range from 21.84% (bitter orange) and 27.58% (grapefruit) to 33.94% (lemon) and 38.67% (mandarin). Also, arachidic and behenic acids were found at low levels (<0.51%). Statistically significant differences were observed among fatty acid amounts of *Citrus* oils depending on *Citrus* type ($p < 0.05$). As seen in Table 3, the lowest stearic acid and oleic acid amounts were found in bitter orange seed oils. In another study, citrus oils provided from Turkey contained 5.1%–28.3% palmitic, 0.3%–5.9% stearic, 18.3%–70.1% oleic, 19.5%–58.9% linoleic, and 0.1%–3.7% linolenic acids (Matthaus and Özcan 2012). Al-Juhaimi et al. (2016) determined 19.69%–26.29% palmitic, 21.3%–31.4% oleic, and 32.3%–43.7% linoleic acids in *Citrus* seed oils provided from Turkey and Saudi Arabia. Matthaus and Özcan (2012) reported that *Citrus* seeds provided from Turkey and Vietnam contained 45.1%–58.8% and 32.1%–54.8% oils, respectively. Malacrida et al. (2012) reported that orange, lemon, and tangerine seed oils contained, respectively, 26.42%, 21.03%, and 23.34% palmitic acid; 5.20%, 3.67%, and 5.26% stearic acid; 23.04%, 20.80%,

and 27.78% oleic acid; 40.19%, 44.31%, and 38.89% linoleic acid; and 3.92%, 8.96%, and 3.34% linolenic acid. Park et al. (2021) reported that lemon (*Citrus limon*) seed oil contained 11.68%–16.86% palmitic, 1.95%–3.41% stearic, 11.10%–18.65% oleic, 15.51%–27.03% linoleic, and 1.50%–5.54% linolenic acids. Results show some differences depending on *Citrus* seed type. These differences are probably due to genetics, species, growing conditions, climatic factors, and harvest times. The fatty acid composition of a plant oil is not stable every time. One notable difference is that linoleic acid is present in appreciable amounts in *Citrus* seed oils.

Mineral Contents of Some Citrus Fruit Seeds

Mineral contents of *Citrus* seeds are presented in Table 4. According to the results, minerals found in the greatest amounts in seeds were phosphorus, magnesium, potassium, and calcium, followed by sodium, iron, zinc, manganese, and copper. While the calcium content of seeds ranged between 287.18 mg/kg (lemon) and 867.84 mg/kg (orange), the potassium content ranged between 1218.93 mg/kg (orange) and 3976.24 mg/kg (lemon). In addition, phosphorus and magnesium amounts of *Citrus* seeds were established to be between 2817.54 mg/kg (orange) and 3467.29 mg/kg (grapefruit) and between 678.74 mg/kg (grapefruit) and 1084.59 mg/kg (lemon), respectively. Sodium content of seeds varied between 347.54 mg/kg (mandarin) and 634.18 mg/kg (grapefruit). The highest iron (47.88 mg/kg), zinc (39.67 mg/kg), manganese (21.51 mg/kg), and copper (7.09 mg/kg) contents were found in grapefruit, bitter orange, lemon, and lemon, respectively. In another study, orange seed cake contained 0.006 ppm calcium,

Table 3 Fatty acid composition of citrus seed oils (%)

Sample	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Behenic
Mandarin (satsuma)	17.54 ± 1.23*e	2.41 ± 0.08d	23.18 ± 1.45d	38.67 ± 2.38a	3.61 ± 0.17b	0.43 ± 0.03c	0.013 ± 0.003e
Orange (Valencia)	19.41 ± 0.87b**	4.18 ± 0.13c	26.51 ± 1.38b	34.51 ± 1.47d	2.89 ± 0.09e	0.51 ± 0.05a	0.016 ± 0.001c
Bitter orange	21.14 ± 1.27a	2.05 ± 0.17e	21.84 ± 2.27e	36.38 ± 1.59c	4.76 ± 0.11a	0.37 ± 0.01d	0.015 ± 0.001d
Lemon (Interdonato)	18.67 ± 1.34c	4.69 ± 0.32b	25.43 ± 1.29c	33.94 ± 0.84e	3.44 ± 0.07d	0.29 ± 0.03e	0.019 ± 0.003a
Grapefruit	18.14 ± 0.86d	5.42 ± 0.65a	27.58 ± 0.98a	37.13 ± 0.93b	3.59 ± 0.21c	0.48 ± 0.07b	0.018 ± 0.005b

*Standard deviation

**Values within each column followed by different letters are significantly different at $p < 0.05$

Table 4 Mineral contents of some citrus seeds (mg/kg)

Sample	Ca	K	P	Mg	Mn	Na	Cu	Fe	Zn
Mandarin (satsuma)	758.11 ± 26.73 ^{a,b}	2987.10 ± 35.61 ^c	3127.46 ± 19.53 ^c	986.41 ± 12.76 ^b	15.41 ± 1.34 ^e	347.54 ± 9.56 ^e	6.44 ± 0.76 ^c	29.17 ± 3.45 ^e	21.74 ± 1.43 ^e
Orange (Valencia)	867.84 ± 32.56 ^{a,**}	1218.93 ± 43.68 ^e	2817.54 ± 27.71 ^e	864.54 ± 11.48 ^c	18.76 ± 2.53 ^c	456.41 ± 11.65 ^c	7.61 ± 1.13 ^a	44.56 ± 2.56 ^b	33.81 ± 4.52 ^c
Bitter orange	482.21 ± 17.38 ^c	1248.51 ± 53.76 ^d	3351.48 ± 48.95 ^b	776.87 ± 21.38 ^d	19.94 ± 3.67 ^b	367.81 ± 22.85 ^d	5.87 ± 0.73 ^d	32.68 ± 1.18 ^d	39.67 ± 1.67 ^a
Lemon (Interdonato)	287.18 ± 12.56 ^e	3976.24 ± 27.98 ^a	2936.71 ± 56.32 ^d	1084.59 ± 43.97 ^a	21.51 ± 1.38 ^a	579.63 ± 6.89 ^b	7.09 ± 0.86 ^b	35.71 ± 1.23 ^c	29.45 ± 2.38 ^d
Grapefruit	391.64 ± 16.89 ^d	3268.43 ± 44.75 ^b	3467.29 ± 43.76 ^a	678.74 ± 23.65 ^e	17.69 ± 3.76 ^d	634.18 ± 5.64 ^a	5.32 ± 1.07 ^e	47.88 ± 5.83 ^a	36.54 ± 1.65 ^b

*Standard deviation

**Values within each column followed by different letters are significantly different at $p < 0.05$

1.53 ppm magnesium, 0.02 ppm sodium, 7.33 ppm potassium, 0.02 ppm iron, and 0.01 ppm copper (Okoye et al. 2011). El-Safy et al. (2012) determined 36.56 mg/100 g calcium, 719.79 mg/100 g magnesium, 145.11 mg/100 g potassium, 2.12 mg/100 g manganese, 6.50 mg/100 g sodium, 3.72 mg/100 g copper, 6.40 mg/100 g iron, 15.71 mg/100 g zinc, and 390.70 mg/100 g phosphorus in orange seeds. In other study, orange seed cake contained 0.006 ppm calcium, 1.53 ppm magnesium, 0.02 ppm sodium, 7.33 ppm potassium, 0.02 ppm iron, and 0.01 ppm copper (Okoye et al. 2011). Al-Juhaimi et al. (2016) reported that *Citrus* seeds provided from Turkey and Saudi Arabia contained 5018–7619 mg/kg calcium and 7007–10334 mg/kg potassium. The results show some differences compared with results of previous studies. Differences in the mineral content of the seeds are probably due to the species, growing conditions, fertilization, soil nutrients, irrigation, climatic factors, and other agricultural factors.

Conclusion

The results showed some differences depending on fruit type. A linear relationship was observed between total flavonoid contents and antioxidant activities of *Citrus* seeds. At the same time, *Citrus* seeds are a good oil source. The greatest oil content was determined in bitter orange seeds. In general, the sugar content of bitter orange was found to be low. However, the most abundant sugar in all samples was sucrose. Linoleic, oleic, and palmitic acids were the abundant fatty acids in *Citrus* oils. All *Citrus* seed oils are rich in linoleic acid. Minerals found in the highest amounts in seeds were phosphorus, magnesium, potassium, and calcium, followed by sodium, iron, zinc, manganese, and copper, in descending order.

Conflict of interest M. M. Özcan, Ö. Öztürk, and V. Lemiasheuski declare that they have no competing interests.

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