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Chemical evaluation of *citrus* seeds, an agro-industrial waste, as a new potential source of vegetable oils

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RESUMEN

Evaluación química de algunas semillas de cítricos, un residuo agroindustrial como nueva fuente potencial de aceites vegetales.

Aceites de semillas de siete y diez variedades de frutas cítricas turcas y de Vietnam respectivamente, fueron examinadas por su composición en ácidos grasos, tocoferoles y esteroles. El contenido de aceite de las muestras varió entre 32.1 g/100 g y 58.8 g/100 g. El principal ácido graso de los aceites extraídos de las semillas fue oleico (12.8 a 70.1%), seguido por linoleico (19.5-58.8%) y palmítico (5.1 a 28.3%). Los ácidos esteárico, vacénico, linolénico y araquídico se encontraron en niveles bajos. El contenido total de compuestos de vitamina E activa en los aceites varió entre 0.8 y 21.0 mg/100 g. Los isómeros predominantes fueron α- y γ-tocoferol, con aproximadamente la misma cantidad, entre 0.4 y 17.5 mg/100 g. El contenido de esteroles totales de los aceites se encontró entre 1310.54 y 3986.58 mg/kg, con β-sitosterol como el esterol predominante representando más del 70% de la cantidad total de esteroles. Otros esteroles, campesterol (8.03-15.26%), estigmasterol (2.55-7.69%), Δ5-avenasterol (1.80-5.67%), colesterol (0.83 a 2.70%) y clerosterol (0.93-1.78%) se detectaron en la mayoría de los aceites. Los resultados del presente estudio indican que los aceites de semillas de cítricos se consideran una fuente potencial de aceite debido a la importante composición de ácidos grasos, tocoferoles y esteroles, y podría ser utilizado para aplicaciones alimenticias y para la producción de posibles productos de valor añadido.

PALABRAS CLAVE: Aceites – Ácidos grasos – Citrus spp. – Esteroles – Rutaceae – Semillas –Tocoferoles.

SUMMARY

Chemical evaluation of *citrus* seeds, an agro-industrial waste, as a new potential source of vegetable oils.

The seed oils from seven Turkish and ten Vietnamese varieties of *Citrus* fruits were examined for their fatty acid composition, tocopherols and sterol contents. The oil contents of the samples varied between 32.1 g/100 g and 58.8 g/100 g. The major fatty acid of the extracted seed oils was oleic (12.8-70.1%), followed by linoleic (19.5-58.8%) and palmitic (5.1-28.3%). Stearic, vaccenic, linolenic and arachidic acids were found at low levels. The total content of vitamin E active compounds in the oils ranged between 0.8 and 21.0 mg/100 g. The predominant isomers were α- and γ-tocopherol, with approximate equal amounts between about 0.4 and 17.5 mg/100 g. The total sterol contents of the oils were found between 1310.54 and 3986.58 mg/kg, with β-sitosterol as the predominant sterol that accounted for more than 70% of the total amount of sterols. Other sterols, campesterol (8.03-15.26%), stigmasterol (2.55-7.69%), Δ5-avenasterol (1.80-5.67%), cholesterol (0.83-2.70%) and chlerosterol (0.93-1.78%) were detected in most of the oils. The results of the present study indicate that the seed oils of *Citrus* fruits are considered to be a potential oil source due to their fatty acid composition and important tocopherol and sterol, and might be used for edible applications as well as the production of potential value-added products.

KEY-WORDS: Citrus – Oil – Fatty acids – Seeds – Sterols – Rutaceae – Tocopherols.

1. INTRODUCTION

The *Citrus* species, belonging to the family Rutaceae, is an annual plant that is widely distributed in the Mediterranean countries of the Middle East and Southern Europe but also grows abundantly in other warm climates worldwide. The plants of most species of *Citrus* are large evergreen shrubs or small trees, and their fruits are among the most important tree fruit crops in the world. *Citrus* fruits are of great economic importance because of their varied uses (Saidani *et al.*, 2004).

Citrus species are of prime economic importance in both fresh and processed fruit markets. Consequently, large amounts of *Citrus* seeds are discharged at processing plants. At present *Citrus* fruits are processed to produce juice, jam or marmalade and the wastes of this industry such as peels, seeds and pulps represent about 50% of the raw processed fruit (Ben Gern, 1967). This not only wastes a potentially valuable resource, but also aggravates already serious disposal problems. So, expansion in the *Citrus* industry in recent years attracts attention to the further use of the *Citrus* seeds as a potential source for other nutrients. Obviously, such an objective would improve the utilization of the

available resources and result in the production of various products for food or feed (Abdel-Rahaman, 1980; Lazos and Servos, 1988).

Plant seeds are important sources of oils for nutritional, industrial, and pharmaceutical applications. Lipids are important for the development of cells as structural components, functional compounds and for the storage of energy. The study of oilseeds for their minor constituents is useful in order to use both oil and the minor constituents effectively (Kamel *et al.* 1982; Aitzetmüller, K. 1993). Some seed oils from other plants are already used for several purposes like blending with modified nutritional values, as ingredients in paint and varnish formulations, lubricants, pharmaceuticals, organic pesticides, plastics, dispersants, textiles, soaps, surface coating and oleo-chemicals, as well as oils for cosmetic purposes (Muuse *et al.*, 1992; Watkins, 1999; Hosamani and Sattigeri, 2000). Two of the main constituents of *Citrus* seeds are seed meal, consisting of protein and seed oil. *Citrus* seeds contain about 36% oil and 14% protein (Braverman, 1949; El-Adawy *et al*., 1999). The high oil content makes the seed material interesting for the production of oil. The fatty acid composition of some *Citrus* seed oils has been identified by Saidani *et al*., 2004; El-Adawy *et al.,* 1999; Abdel-Rahaman *et al.,* 1980; Lazos and Servos, 1988; Habib *et al*., 1986.

Previous studies on some Rutaceae seed oils have been reported by several authors (Henderson and Kesterson, 1963; Abdel-Rahaman, 1980; Habib *et al*., 1986; Lazos and Servos, 1988; Trandjiiska and Nguyen, 1989; Helmy, 1990; Ajewole and Adeyeye, 1993; Hassanein, 1999). Limited studies on the occurrence and variability of have been conducted (Abdel-Rahaman, 1980; Habib *et al*., 1986; Lazos and Servos, 1988; Trandjiiska and Nguyen, 1989; Saidani *et al*., 2004; Reda *et al*., 2005). In many plants the seed oil composition differs considerably from lipids contained in green photosynthetic tissue. Unususal fatty acids are often present in significant percentages in the seed oil (Smith, 1970).

The aim of this study was to investigate and compare the chemical properties of the seed oils of some *Citrus* species provided from several locations in Turkey and Vietnam concerning the fat content and the composition of fatty acids, tocopherols, and sterols.

2. MATERIALS AND METHODS

2.1. Seeds

About 20 kg of fruit from each variety of *Citrus* species were collected by hand from *Citrus* trees growing in several locations of Turkey (*Citrus nobilis* Lour. var. *nobilis*, *Citrus sinensis* Osb (hamlin), *Citrus sinensis* Osb (Sour orange), *Citrus aurantium* var. *amara* (bitter), *Citrus limon* (Interdonate), *Citrus limon* (kutdiken) and *Citrus paradise*) and Vietnam (*Citrus sinensis* (L.) Osb., *Citrus nobilis* Lour. var. *nobilis*, *Citrus limonia* Osb., *Citrus aurantifolia* (Christm & Panz) Sw (lime), *Citrus japonica* Thunb, *Citrus nobilis* var. *chrysocarpa* Lamk, *Citrus nobilis* var *microcarpa* Hassk, Buoi Da Gai, *Citrus grandis* (L) Osb. var. *grandis* and *Citrus grandis* (Buoi Dien) in December, 2006. The skin and pulp were removed from the seeds, and the seeds were washed and cleaned in an air screen cleaner to remove immature and broken seeds. Air conditioning was used for the drying of the seeds. The skins of maturated seeds were decorticated by hand and then stored in polypropylene bags at 4 °C temperature.

2.2. Chemicals

Petroleum ether (40-60 °C) was of analytical grade (> 98%; Merck, Darmstadt, Germany). Heptane and tert-butyl methyl ether were of HPLC grade (Merck, Darmstadt, Germany). Tocopherol and tocotrienol standard compounds were purchased from Cal Biochem (Darmstadt, Germany). Betulin, β-sitosterol, campesterol, and stigmasterol were obtained from Aldrich (Munich, Germany).

2.3. Oil content

The oil content was determined according to the method ISO 659:1998 (ISO, 1998). About 2 g of the seeds were ground in a ball mill and extracted with petroleum ether in a Twisselmann apparatus for 6 h. The solvent was removed by a rotary evaporator at 40 °C and 25 Torr. The oil was dried by a stream of nitrogen and stored at –20 °C until use.

2.4. Fatty Acid Composition

The fatty acid composition was determined following the ISO standard ISO 5509:2000 (ISO 2000). In brief, one drop of the oil was dissolved in 1 mL of *n*-heptane, 50 μg of sodium methylate were added, and the closed tube was agitated vigorously for 1 min at 24 °C. After the addition of 100 μL of water, the tube was centrifuged at 4500 g for 10 min and the lower aqueous phase was removed. Then 50 μL of HCl (1 mol with methyl orange) was added, the solution was mixed, and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure; Merck, Darmstadt, Germany) were added, and after centrifugation at 4500 g for 10 min, the top n-heptane phase was transferred to a vial and injected into a Varian 5890 gas chromotograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 μm). The temperature program was as follows: from 155 °C; heated to 220 °C (1.5 °C min^{-1}), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 36 cm s^{-1} hydrogen; split ratio 1:50; detector gas 30 mL min^{-1}

hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 μL. The peak areas were computed by the integration software, and percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization.

2.5. Tocopherols

For the determination of tocopherols, a solution of 250 mg of oil in 25 mL of n-heptane was used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with an L-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm), and a D-2500 integration system. Samples in the amount of 20 μL were injected by a Merck 655-A40 autosampler onto a Diol phase HPLC column 25 cm × 4.6 mmID (Merck, Darmstadt, Germany) used with a flow rate of 1.3 mL min^{-1}. The mobile phase was n-heptane/tert-butyl methyl ether (99 + 1, v/v (Balz *et al*., 1992).

2.6. Sterols

The sterol composition of the oils was determined following ISO/FIDS 12228:1999 (E) (ISO, 1999). In brief, about 250 mg of oil were saponified with a concentration of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction in an aluminium oxide column (Merck, Darmstadt, Germany) in which fatty acid anions were retained and sterols passed through. The sterol fraction was separated from the unsaponifiable matter by stationary phase for thin-layer chromatograph (Merck, Darmstadt, Germany), re-extracted from the TLC material, and then the composition of the sterol fraction was determined by GLC using betulin as internal standard. The compounds were separated on an SE 54 CB (Macherey-Nagel, Düren, Germany; 50 m long, 0.32 mm ID, 0.25 μm film thickness). Further parameters were as follows: hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320 °C, temperature program, 245 °C to 260 °C at 5 °C min^{-1}. Peaks were identified either by standard compounds (β-sitosterol, campesterol, stigmasterol), by a mixture of sterols isolated from rape seed oil (brassicasterol), or by a mixture of sterols isolated from sunflower oil (Δ7-avenasterol, Δ7-stigmasterol, and Δ7-campesterol).

2.7. Statistical analyses

Results of the research were analyzed for statistical significance by analysis of variance (Püskülcü and İkiz, 1989). This research was performed in three duplicates.

3. RESULTS AND DISCUSSION

3.1. Oil contents

The oil contens of the seeds of *Citrus* varieties from different locations in Turkey varied between 45.1% (*Citrus limon* (kütdiken)) and 58.8% (*C. aurantium* (Bitter orange) (Table 1). *C. aurantium* subsp *amara* had the highest oil content (58.8%), followed by *C. sinensis* (bitter) (57.4%) and *C. sinensis* Osb (sour) (56.5%). In addition to these, the oil contents of *Citrus limon* (kütdiken), *C. limon* (interdonate) and *C. nobilis* var. *nobilis* had almost the same amounts. The results reveal that the seed from *C. limon* has the lowest oil contents.

The oil contents of the seeds from Vietnam *citrus* were established between 32.1% (*C. aurantifolia*) and 54.8% (*Citrus grandis*). The oil contents of *C. aurantifolia, C. limonia, C. grandis* and *C. nobilis* were found to be lower than those of the other Vietnam *citrus* seeds. The oil contents of the seeds from Turkey *Citrus* were found high compared with the samples from Vietnam.

It is apparent that *C. aurantium* (bitter orange), *C. sinensis* (sour), *C. sinensis* (Silifke), Buoi Da Gai and *C. nobilis* Lour var. *nobilis* could be characterized by their high content of oil %. Abdel-Rahaman (1980) has established that the seeds of different *Citrus* species grown in Egypt contained

Table 1
Oil contents of *citrus* seeds

Samples	Oil content [g/100 g]
Turkey	
Citrus sinensis	47.7 ± 0.1*
Citrus nobilis, var. *nobilis*	46.6 ± 0.3
Citrus sinensis (bitter)	57.4 ± 0.3
Citrus sinensis (sour)	56.5 ± 0.5
Citrus aurantium subsp *amara*	58.8 ± 0.5
Citrus limon (ınterdonato)	45.7 ± 0.2
Citrus limon (kütdiken)	45.1 ± 0.2
Citrus paradisi	49.3 ± 0.4
Vietnam	
Citrus limonia	33.9 ± 0.3
Citrus aurantifolia	32.1 ± 0.2
Citrus japonica	40.1 ± 0.2
Citrus nobilis, var. *chrysocarpa*	36.3 ± 0.1
Citrus nobilis, var, *microcarpa*	40.6 ± 0.3
Citrus grandis	54.8 ± 0.5
Citrus grandis, var. *grandis*	38.6 ± 0.3
Citrus grandis	35.5 ± 0.2
Citrus nobilis, var. *nobilis*	52.6 ± 0.4

*mean (n = 3) ± standard deviation.

between 24.3% (Shaddock) and 38.6% (bitter orange) oil. Ajewole and Adeyeye (1993) determined that *citrus* seed oil contents ranged between 24% and 41%. According to previous reports, seed oil contents of *Citrus* established the range of 24-65% (Saleem *et al*., 1977; Kamel *et al*., 1982; Habib *et a.,*1986; Tarandjiiska and Nguyen, 1989; El-Adawy *et al.* 1999; Reda *et al*., 2005). Also, Saidani *et al*., (2004) determined 78.9% oil in *Citrus limon* (lemon) seeds. When our results were compared with some oil-seed crops, the seed oil content (32.1-58.8%) in the present analysis of *Citrus* species was higher than those of Date pit (8.70%) (Allam, 2001), Mahaleb cherry (18.5%) and Blackthorn (16.5%) (Yücel, 2005), Cotton seeds (15.0-24%), soybean (17-21%), grape seeds (6-20%), olive (20-25%) (Saleem *et al*., 1977) and turpentine (38.4-45.1%) (Matthaus and Özcan, 2006). This agro-industrial waste is considered a potential oil source and could be processed commercially in the future due to its high oil content and economical advantages.

3.2. Fatty acid composition

The fatty acid compositions of the seed oils from both the Turkish and Vietnamese *citrus* species are given in Table 2. According to Table 2, there is a wide variation in fatty acid compositions of *citrus* seeds belonging to these countries.

The major fatty acid compositions of the Turkish *citrus* seed oil are oleic, linoleic and palmitic acids. Turkish *citrus* oil contained a considerable amount of oleic acid (18.3-70.1%) and linoleic acid (19.5-58.9%). Palmitic and stearic acids were detected between 5.1% to 28.3% and 0.3% to 5.9%, respectively. A lower amount of vaccenic acid (0.6 to 1.3%) was found in Turkish *citrus* oils. The high content of saturated fatty acids, consisting of palmitic acid, which amounted to between 5.1% (*C. aurantium* (bitter) to 28.3% (*C. nobilis*) is nutritionally unfavorable.

Lower amounts of vaccenic and stearic acids were found. Linoleic (23.5% to 46.4%) and oleic acids (12.8% to 36.3%) were the most predominantf fatty acids found in the Vietnamese *citrus* seeds. Palmitic and stearic acids were detected in the Vietnam oils at 9% to 27.6% and 2.1% to 5.5% of the total fatty acids, respectively. As another quantitatively interesting saturated fatty acid, the Vietnamese oil contained linolenic acid in a range from 0.5% (*C. nobilis* var. *nobilis*) to 8.2% (*C. japonica*). The major fatty acids in the Vietnamese *citrus* oil had similar levels to those of the Turkish *citrus* oil.

The constituents of total lipids and neutral lipid classes, i.e., hydrocarbons, wax esters, sterol esters, triacylglycerols, free fatty acids, 1,3-diacylglycerols, 1,2-diacylglycerols, free sterols, alcohols, and monoacylglycerols of the three species of *Citrus*

Table 2
Fatty acid compositions of *citrus* seed oils [%]

Samples	Palmitic	Stearic	Oleic	Vaccenic	Linoleic	Linolenic	Arachidic	Total
Turkey								
Citrus sinensis	26.6 ± 0.3	4.9 ± 0.2	22.4 ± 0.6	1.3 ± 0.1	39.9 ± 0.5	3.7 ± 0.2	0.4 ± 0.2	99.2
Citrus nobilis, var. *nobilis*	28.3 ± 0.5	5.9 ± 0.3	18.3 ± 0.4	1.2 ± 0.2	42.1 ± 0.7	3.2 ± 0.1	0.5 ± 0.2	99.5
Citrus sinensis (bitter)	9.7 ± 0.1	3.2 ± 0.2	23.3 ± 0.5	0.6 ± 0.1	58.3 ± 0.7	2.6 ± 0.3	0.0 ± 0.1	97.7
Citrus sinensis (sour)	11.9 ± 0.2	3.2 ± 0.2	19.7 ± 0.5	1.0 ± 0.2	58.9 ± 1.0	0.4 ± 0.1	0.2 ± 0.2	95.3
Citrus aurantium subsp *amara*	5.1 ± 0.2	0.3 ± 0.1	66.9 ± 1.2	1.2 ± 0.2	23.9 ± 0.4	0.1 ± 0.2	0.1 ± 0.2	97.6
Citrus limon (ınterdonato)	5.4 ± 0.1	1.4 ± 0.2	63.6 ± 1.5	1.3 ± 0.3	26.8 ± 0.4	0.1 ± 0.1	0.1 ± 0.1	98.7
Citrus limon (kütdiken)	9.0 ± 0.2	5.0 ± 0.3	38.5 ± 0.9	1.1 ± 0.2	44.5 ± 0.5	0.4 ± 0.1	0.5 ± 0.1	99.0
Citrus paradisi	5.8 ± 0.1	1.6 ± 0.1	70.1 ± 1.4	1.3 ± 0.1	19.5 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	98.5
Vietnam								
Citrus limonia	23.6 ± 0.4	4.2 ± 0.2	9.5 ± 0.3	1.5 ± 0.3	43.7 ± 0.4	5.9 ± 0.2	0.4 ± 0.2	98.8
Citrus aurantifolia	22.6 ± 0.7	4.0 ± 0.3	17.7 ± 0.4	1.6 ± 0.4	44.3 ± 0.3	8.1 ± 0.3	0.4 ± 0.1	98.7
Citrus japonica	20.9 ± 0.3	4.5 ± 0.5	18.3 ± 0.5	1.0 ± 0.7	34.2 ± 0.3	8.2 ± 0.3	0.5 ± 0.2	96.6
Citrus nobilis, var. *chrysocarpa*	21.9 ± 0.5	5.4 ± 0.5	19.1 ± 0.5	2.4 ± 0.4	44.6 ± 0.5	4.9 ± 0.2	0.5 ± 0.1	98.8
Citrus nobilis, var. *microcarpa*	23.4 ± 0.4	4.8 ± 0.3	22.7 ± 0.4	1.8 ± 0.3	41.7 ± 0.5	3.8 ± 0.2	0.4 ± 0.2	98.6
Citrus grandis	27.6 ± 0.5	3.8 ± 0.3	26.6 ± 0.3	1.3 ± 0.2	35.2 ± 0.4	4.3 ± 0.1	0.4 ± 0.1	99.2
Citrus grandis, var. *grandis*	27.0 ± 0.3	4.3 ± 0.2	26.7 ± 0.4	1.1 ± 0.2	35.9 ± 0.5	3.9 ± 0.2	0.3 ± 0.2	99.2
Citrus grandis	18.7 ± 0.4	2.1 ± 0.1	22.8 ± 0.2	1.9 ± 0.1	33.5 ± 0.3	4.8 ± 0.2	0.1 ± 0.1	83.9
Citrus nobilis, var. *nobilis*	9.0 ± 0.2	5.5 ± 0.2	36.3 ± 0.4	0.9 ± 0.2	46.4 ± 0.7	0.5 ± 0.1	0.6 ± 0.2	99.2

namely *Citrus sinensis, Citrus paradisi, Citrus aurantium* were investigated by thin layer and gas chromatography. Palmitic, oleic and linoleic acids were the major components in all the lipids and lipid classes studied (Waheed *et al.,* 2009). *Citrus* seed oils had high amounts of unsaturated fatty acids which consisted mainly of linoleic (33.21 to 38.44%) followed by oleic (22.25-26.0%) and at the last linolenic (2.58-9.56%) acids (El-Adawy *et al*., 1999). Although, *citrus* seed oil had the lowest contents of oleic and linoleic acids compared to the other *citrus* seed oils; it contained the highest amount of linolenic acid (9.6%). El-Adawy *et al*., (1999) determined 24.7-29.5% palmitic and 4.3-5.3% stearic acids as the major fatty acids in all *citrus* seed oils (citron, orange, mandarin and mixed seed oils). Lazos and Servos (1988) identified palmitic (25.44%), stearic (5.31%), oleic (24.64%), linoleic (39.27%) and linoleinic (4.53%) acids in orange seed oil. French (1962) determined the fatty acids 28% palmitic, 5.4% stearic, 22.6% oleic, 37.2% linoleic and 6.5% linolenic in *citrus* seed oils. Habib *et al.* (1986) reported that orange, mandarin, lime and grapefruit contained palmitic (28.81%, 18.08%, 19.08% and 42.64%), stearic (2.60%, 7.79%, 0.84% and 0.83%), oleic (23.72%, 16.76%, 17.07% and 12.18%), linoleic (30.98%, 13.53%, 18.62% and 34.49%) and linolenic (6.50%, 42.27% and 1,41%) acids, respectively. These results are in good agreement with the findings reported by El-Adawy *et al.* (1999), Lazos and Servos (1988) and Habib *et al.* (1986). Shibahara *et al.* (1987) determined 11.7-21.5% palmitic, 1.0-1.7% stearic, 3.9-27.5% oleic, 3.9-22.3% cis-oleic, 18.0-36.2% linoleic and 7.2-16.0% linolenic acids in the pulp lipids of lemon, grapefruit, sweet orange and Japanese mandarin. Palmitic acid was found to be the dominant saturated fatty acid, but this fatty acid in Turkish *citrus* oil was in lower concentrations than those reported by French, 1962; Lazos and Servos, 1988; El-Adawy *et al*., 1999 and Habib *et al.,* 1986. The level of total unsaturates in the current study of *citrus* seed oils was noted to be lower than those investigated for Nigerian *citrus* (*Citrus sinensis*, *Citrus paradise, Citrus aurantium, Citrus reticulate, Citrus aurantifolia*), tangelo seed oils (67.3-86.20%) (Ajewola and Adeyeye, 1993) and Brazilian rangpur lime (*Citrus limonia* Osbeck) and Sicilian lemon (*Citrus limon*) seed oils (71.80-73.0%) (Reda *et al*., 2005). Comparing the chemical properties of *citrus* seed oil with those of other vegetable oils, it is similar to olive oil, almond, peanut, date pit and caper seed oils (Allam, 2001; Matthaus and Ozcan, 2005).

The *citrus* seed oils investigated in the present study exhibited high degrees of unsaturation. The major fatty acids found in the oils from both countries were oleic, linoleic and linolenic acids. The data indicated that some of the cultivars of *citrus* grown in both countries are significantly different from each other in the levels of individual fatty acids. It is well known that the fatty acid composition of *citrus* seeds is influenced by the climate in which they are grown. Based on the results obtained the fatty acid composition of *citrus* seed oil showed that it probably falls in the linoleic-oleic acid oils category.

3.3. Tocopherols

In addition to essential fatty acids, seed oils are excellent sources of Vitamin E (tocopherols). Tocopherols are natural antioxidants with biological activity. The main biochemical function of tocopherols is believed to be the protection of polyunsaturated fatty acids against peroxidation (Beringer and Dompert, 1976; Kamal-Eldin and Andersson, 1997). The group of vitamin E active compounds comprises, in addition to the tocopherols, four tocotrienols and plastochromonal-8, which also have antioxidative and biological activities, but less than the tocopherols.

The tocopherol contents of *citrus* seed oils belonging to Turkish and Vietnamese varieties are given in Table 3. The total amount of tocopherols of Turkish *citrus* oil ranged between 2.0 mg/100g (*C. nobilis*) and 21.0 mg/100g (*C. paradisi*). Turkish *citrus* oil was characterized by much higher amounts of α- and γ-tocopherols. The highest α-tocopherol contents were in *C. paradisi* (17.5 mg/100g), *C. limon* (Kütdiken) (13.0 mg/100g) and *C. limon* (ınterdonato) (10.9 mg/100 g). Very high levels of γ-tocopherol were only present in *C. aurantium* (3.1 mg/100g). The α-tocopherol contents of bitter and sour *C. sinensis* oil were found high compared with other Turkish *citrus* oils.

α-Tocopherol is the predominant component in Vietnamese *citrus* oils, followed by γ-tocopherol and γ-tocotrienol. The α-tocopherol contents of these oils ranged between 0.4 mg/100g (*C. japonica*) and 9.7 mg/100g (*C. aurantifolia*). Also, γ-tocopherol contents were established between 0.0 mg/100g (*C. nobilis* var. *chrysocarpa*) and 1.8 mg/100g (*C. aurantifolia*).

The tocopherol concentration in the different seed oils ranged from 396.8 mg/kg (*P. terebinthus* 1) to 517.7 mg kg^{-1} (*P. terebinthus* 6), with a mean value of 465.7 mg/kg. (Matthaus and Ozcan, 2006). In an earlier report (Baraud *et al*., 1969) on the tocopherol content of hazelnuts (*C. avellana*) grown in France, it was shown that the levels were relatively low (ranging from 335 to 420 mg/kg oil) but significantly higher in hybrids of *C. avellana* (range 602 to 690 mg kg^{-1}). In the tocopherol fraction (55.5 mg kg^{-1} in chokeberry oil (70.6 mg kg^{-1}). γ-Tocopherol was the main component in black currant oil (55.4 mg kg^{-1}) and rose hip oil (71.0 mg/kg) (Zlatanov, 1999). Matthaus and Özcan (2006) established 134.9 mg kg^{-1} α-tocopherol, 135.6 mg/kg γ-tocopherol and 100.0 mg kg^{-1} γ-tocotrienol as the mean in turpentine oils. The α-tocopherol levels of *citrus* oils were generally lower than those of soybean (9-352 mg kg^{-1}) and palm oils (4-185 mg kg^{-1}) (Rossell, 1991). The

Table 3
Tocopherol contents of *citrus* seed oils (mg/100g)

Samples	α-Tocopherol	α-Tocotrienol	β-Tocopherol	γ-Tocopherol	Plastochromanol-8	γ-Tocotrienol	Total
Turkey							
Citrus sinensis	9.3 ± 0.4	ND	ND	0.5 ± 0.3	ND	0.2 ± 0.1	10.0
Citrus nobilis, var. *nobilis*	0.8 ± 0.2	ND	ND	0.9 ± 0.2	ND	0.3 ± 0.1	2.0
Citrus sinensis (bitter)	15.6 ± 0.3	0.2 ± 0.1	0.3 ± 0.2	1.5 ± 0.4	ND	0.2 ± 0.1	17.8
Citrus sinensis (sour)	15.7 ± 0.5	0.1 ± 0.1	0.3 ± 0.1	1.2 ± 0.2	ND	0.2 ± 0.2	17.5
Citrus aurantium subsp *amara*	9.9 ± 0.2	0.1 ± 0.1	0.4 ± 0.2	3.1 ± 0.3	ND	0.2 ± 0.1	13.7
Citrus limon (ınterdonato)	10.9 ± 0.2	0.1 ± 0.1	0.3 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	12.3
Citrus limon (kütdiken)	13.0 ± 0.3	0.1 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.3 ± .02	14.2
Citrus paradisi	17.5 ± 0.4	0.7 ± 0.2	0.2 ± 0.1	1.8 ± 0.2	ND	0.3 ± 0.1	20.5
Vietnam							
Citrus limonia	6.2 ± 0.8	ND	ND	0.5 ± 0.2	ND	1.0 ± 0.3	7.7
Citrus aurantifolia	9.7 ± 0.3	ND	ND	1.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.3	13.2
Citrus japonica	0.4 ± 0.1	ND	ND	1.3 ± 0.1	ND	ND	1.7
Citrus nobilis, var. *chrysocarpa*	4.2 ± 0.2	ND	ND	ND	ND	ND	4.2
Citrus nobilis, var. *microcarpa*	1.4 ± 0.2	ND	ND	0.6 ± 0.2	ND	0.2 ± 0.1	2.2
Citrus grandis	4.3 ± 0.2	0.4 ± 0.2	ND	1.1 ± 0.1	ND	0.4 ± 0.2	6.2
Citrus grandis, var. *grandis*	6.5 ± 0.3	ND	0.2 ± 0.1	0.8 ± 0.3	ND	0.3 ± 0.1	7.8
Citrus grandis	6.3 ± 0.5	ND	ND	1.6 ± 0.2	1.4 ± 0.2	ND	9.3
Citrus nobilis, var. *nobilis*	8.1 ± 0.5	0.7 ± 0.3	0.3 ± 0.1	1.7 ± 0.4	ND	0.3 ± 0.1	11.1

ND: not detected.

current Recommended Dietary Allowance for Vitamin E intake is 10 mg (15 IU) for adult males and 7.6 mg (11.4 IU) for adult females (NRC, 1989). The amount of tocopherols in seed oils of *citrus* species is comparable to that found for other commonly used seed oils such as sunflower oil or rapeseed oil (AOCS, 1996). Variation for total tocopherol content was extremely wide, ranging from 68 mg/kg oil in *Diplotakis viminea* to 2479 mg kg^{-1} oil in *Schivereckia doerfleri*. The average tocopherol profile consisted of 65.4% γ-, 28.7% α-, 5.1% δ- and 0.8% β-tocopherol (Goffman *et al*., 1999).

All the seeds tested showed differences in their total tocopherol and tocotrienol contents. Tocopherol composition was dominated by α- and γ-tocopherol for the oils from both Vietnam and Turkey. It is obvious that α- and γ-tocopherols are the major vitamin E active components in all *citrus* seed oils (Beringer and Dompert, 1976; Kamal-Eldin and Andersson, 1997). The predominant tocopherols in *citrus* seed oils were α- and γ-tocopherol, which were found in different amounts. The contents of plastochromano-8 in the *citrus* seed oils from both countries was also in very small amounts. The tribe Rutaceae included two main groups for tocopherol composition, characterized by high concentrations of α- and γ-tocopherol, respectively.

3.4. Phytosterols

The sterol compositions of the *citrus* seed oils are presented in Table 4. The concentrations of total sterols in the Turkish *citrus* oils varied from 2038.1 mg/kg (*C. sinensis* (bitter)) to 3574.1 mg kg^{-1} (*C. limon* (interdonato). The composition of sterols in the Turkish *citrus* oil is dominated by β-sitosterol, which accounted for about 75% of the total sterols in the oil. This is typical of many vegetable oils in which β-sitosterol is predominant. Other sterols had campesterol (about 11% of the total sterols), Δ5-avenasterol (about 3%), and stigmasterol (about 4%). β-Sitosterol was the major sterol, present in all products and ranging from 74.9 mg/kg (*C. limon* (interdonato) to 78.3 mg kg^{-1} (*C. sinensis* (bitter). The campesterol contents ranged from 8.4 mg kg^{-1} (*C. sinensis* (bitter) to 15.3 mg/kg (*C. paradisi*).

The major sterols in the Vietnamese *citrus* oils were β-sitosterol, ranging from 71.7 mg/kg (*C. aurantifolia*) to 79.8 mg kg^{-1} (*C. grandis*); campesterol, ranging from 8.9 mg/kg (*C. nobilis* var. *nobilis*) to 14.6 mg kg^{-1} (*C. japonica*); stigmasterol, ranging from 2.8 mg kg^{-1} (*C. grandis*) to 18.3 mg kg^{-1} (*C. japonica*) and Δ5-avenasterol, ranging from 1.8 mg kg^{-1} (*C.nobilis* var. *chrysorca*) to 4.1 mg/kg (*C. nobilis* var. *nobilis*). Other minor components were Δ7-avenasterol, Δ7-stigmasterol

Table 4
Sterol contents of *citrus* seed oils

	Cholesterol	24-methylen-cholesterol	Campes-terol	Campes-tanol	Stigmas-terol	Δ7-campesterol	Δ5,23-stigmas-tadienol	Chlerosterol	β-sitosterol	Sitostanol	Δ5-avenasterol	5,24-stigmas-tadienol	Δ7-avenastenol	Δ7-avenastenol	Total amount [mg/kg]
Turkey															
Citrus sinensis	1.4	0.2	8.0	0.3	4.3	0.5	0.0	1.6	77.9	0.6	4.1	0.4	0.4	0.2	2759.0
Citrus nobilis, var. *nobilis*	1.3	0.3	11.1	0.0	5.6	0.5	0.0	1.3	76.2	0.5	2.4	0.4	0.2	0.2	2769.0
Citrus sinensis (bitter)	1.2	0.2	8.4	0.0	2.7	0.2	0.0	1.2	78.3	0.3	5.7	0.7	0.6	0.6	2038.1
Citrus sinensis (sour)	1.4	0.2	10.1	0.0	2.7	0.0	0.0	1.1	77.9	0.3	5.0	0.7	0.2	0.3	2506.6
Citrus aurantium subsp *amara*	1.3	0.2	10.1	0.0	2.6	0.0	0.0	1.2	77.8	0.3	5.1	0.8	0.3	0.4	3198.6
Citrus limon (ınterdonato)	2.6	0.2	12.2	0.0	3.9	0.0	0.0	1.0	74.9	0.4	3.6	0.7	0.0	0.4	3574.1
Citrus limon (kütdiken)	2.7	0.1	10.4	0.0	4.4	0.2	0.0	1.1	75.6	0.4	2.5	0.8	1.1	0.7	3530.4
Citrus paradisi	0.8	0.2	15.3	0.0	3.5	0.2	0.0	1.3	75.1	0.3	2.1	0.5	0.5	0.3	2723.8
Vietnam															
Citrus limonia	2.0	0.2	10.7	0.2	6.5	0.6	0.0	1.2	74.1	0.8	2.4	0.4	0.5	0.4	3708.0
Citrus aurantifolia	1.6	0.2	13.3	0.2	7.6	0.4	0.0	1.1	71.7	0.6	1.9	0.4	0.5	0.5	3469.7
Citrus japonica	0.9	0.2	14.6	0.2	8.3	0.3	0.0	0.9	71.9	0.0	2.0	0.3	0.4	0.2	3986.6
Citrus nobilis, var. *chrysocarpa*	1.4	0.1	11.1	0.1	7.7	0.3	0.0	1.1	74.5	0.6	1.8	0.3	0.7	0.4	3725.3
Citrus nobilis, var. *microcarpa*	0.9	0.1	10.6	0.1	5.7	0.3	0.0	1.3	77.7	0.4	2.2	0.3	0.4	0.2	3429.6
Citrus grandis	1.5	0.3	11.7	0.1	3.2	0.5	0.0	1.7	75.3	0.6	3.8	0.8	0.5	0.3	2362.5
Citrus grandis, var. *grandis*	1.2	1.0	12.0	0.0	3.2	0.3	0.0	1.8	75.2	0.8	2.8	0.7	0.5	0.6	3143.6
Citrus grandis	1.2	0.2	10.7	0.0	2.8	0.3	0.0	1.5	79.8	0.4	2.2	0.4	0.0	0.2	3035.0
Citrus nobilis, var. *nobilis*	2.2	0.1	8.9	0.0	5.4	0.0	0.0	1.5	75.1	0.4	4.1	1.1	0.5	0.6	1310.5

and cholesterol. Overall, the predominant sterols were β-sitosterol, Δ5-avenasterol, stigmasterol and campesterol. El-Adawy *et al.* (1999) established 2.18%, 3.52%, 3,27% and 3.14% sterols in citron, orange, mandarin and mixed seed oils, respectively. Lazos and Servos (1988) determined 0.23 % cholesterol, 9.0% campesterol, 2.77% stigmasterol, 87.84% β-sitosterol and 0.16% 5-avenasterol in crude orange seed oil. Habib *et al.* (1986) reported that mandarin, lime and grapefruit contained 0.73%, 0.48% and 0.52% β-sitosterol, respectively. Hassanein (1999) established that plum, apricot and peach kernel oils contained 87.4, 87.8 and 84.4% β-sitosterol, 5.5%, 4.0% and 4.1% campesterol, respectively. β-Sitosterol contents were found in low amounts in all *citrus* seed oils compared with plum, apricot and peach kernel oil (Hassanein, 1999); but according to the results of Habib *et al.*, (1986) they were higher. According to Munshi *et al.,* (1982) the differences between the fatty acid compositions of triacylglycerols and sterol esters may be due to different phases of biosytnthesis of these compounds and the stages of biosynthesis and accumulation of fatty acids. *Citrus* seed oil can be utilized as a source of oil for human consumption and the production of potential value-added products. The composition of sterols in *Citrus* oil is dominated by β-sitosterol, which accounted for about 70% of the total sterols in the oil. This is typical of many vegetable oils. In general, the campestenol, stigmasterol, and sitostenol contents of the Vietnamese samples were higher than those of the Turkish *citrus* oils.

As a result of the high oil content, the seeds of *citrus* species seem to be an interesting source for the production of vegetable oil. The results indicate that the oil contains linoleic acid as the major fatty acid accompanied by oleic acid. The content and composition of tocopherols are comparable to those of other sources such as sunflower, corn or rapeseed oil, and therefore the use of *citrus* oil in nutritional or technological applications is possible.

In addition, minor compounds like vitamin E active compounds and sterols are available in relatively small amounts in comparison to other commonly used vegetable oils. Thus, tocopherols present in high concentrations in *citrus* seed oils are expected to offer some protection during storage and processing. Further studies on these unconventional tropical plant seeds will determine whether some of the seeds described may be used as an addition to indigenous food supplies.

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