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Physico-Chemical Characteristics of Citrus Seeds and Seed Oils from Pakistan

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Abstract The physico-chemical characteristics of the seeds and seed oils of four *citrus* species, Mitha (*Citrus limetta*), Grapefruit (*Citrus paradisi*), Mussami (*Citrus sinensis*), and Kinnow (*Citrus reticulata*) were investigated. The hexane-extracted oil content of citrus seeds ranged from 27.0 to 36.5%. The protein, fiber and ash contents were found to be 3.9–9.6%, 5.0–8.5%, and 4.6–5.6%, respectively. The extracted oils exhibited an iodine value of 99.9–110.0; refractive index (40 °C), 1.4639–1.4670; density (24 °C), 0.920–0.941 mg/mL; saponification value, 180.9–198.9; unsaponifiable matter, 0.3–0.5%; acid value (mg KOH/g of oil), 0.5–2.2 and color (1-in. cell) 1.4–3.0R + 15.0–30.0Y. The oils revealed a good oxidative stability as indicated by the determinations of specific extinctions at 232 and 270 nm (2.3–4.4 and 0.6–0.9, respectively), *p*-anisidine value (2.2–3.2) and peroxide value (1.6–2.4 mequiv/kg of oil). The citrus seed oils mainly consisted of linoleic acid (36.1–39.8%). Other prominent fatty acids were palmitic acid (25.8–32.2%), oleic acid (21.9–24.1%), linolenic acid (3.4–4.4%), and stearic acid (2.8–4.4%). The contents of tocopherols (α, γ, and δ) in the oil were 26.4–557.8, 27.7–84.1, and 9.1–20.0 mg/kg, respectively. The results of the present study demonstrated that the seeds of citrus species investigated are a potential source of valuable oil which might be utilized for edible and other industrial applications.

Keywords Citrus seeds · Oil extraction · Characterization · Oxidative stability · GC/MS · Fatty acids · Tocopherols

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Introduction

The genus *Citrus*, belonging to the Rutaceae or Rue family consists of about 140 genera and 1,300 species including those of some important fruits species such as *C. sinensis* (Orange), *C. paradisi* (Grapefruit), *C. limon* (Lemon), *C. reticulata* (tangerine), *C. grandis* (shaddock), *C. aurantium* (sour orange), *C. medica* (Citron), and *C. aurantifolia* (lime). The plants of most species of *Citrus* are large evergreen shrubs or small trees, 5–15 m tall [1].

Citrus are recognized as one of the world's major fruit crops. These are produced in many countries all around the world with tropical or subtropical climate. Brazil, USA, Japan, China, Mexico, Pakistan, and countries of the Mediterranean region are the major citrus producers. Citrus production worldwide is around 105 million metric

tons (MMT) per annum with Brazil being the largest producer of 19.2 MMT followed by the United States. However, the United States leads the world with an average yield of 30 tons per hectare followed by Brazil and China with 20–25 and 18–20 tons, respectively. Pakistan with an annual production of 1.76 MMT of citrus fruits stands among the ten top citrus producing countries of the world [2, 3].

In addition to large scale consumption, the citrus fruits are mainly processed to produce juice and the waste of this industry such as peels, seeds and pulps which represent about 50% of the raw processed fruit are a potential source of valuable by-products [4]. Citrus fruits are of high-economic value because of their multiple uses, such as in the food industry, cosmetics and folk medicine [5–7].

The citrus seeds, commonly considered as agro-industrial waste, are a potential source of oil. Reda et al. [8] studied the characteristics of *Citrus limonia* and *C. limon* seed oils from Brazil. Habib et al. [9] investigated the chemical composition of Egyptian citrus seeds as potential sources of vegetable oils. Ajewole and Adeyeye [10] reported the characterization of Nigerian citrus seed oils. Trandjiiska and Nguyen [11] studied the triacylglyceride composition of seed oils of citrus fruits from Vietnam. Literature revealed that citrus seeds oils are a good source of unsaturated fatty acids (FAs) [7].

The global demand for vegetable oils and fats (approx. 125 million tons per annum) has increased due to rapid industrial and uncontrolled human population growth. The outcome, not only in the form of expenditures of huge amounts of valuable foreign exchange for importing vegetable oils and fats, nevertheless, has resulted in a deficiency in people's fat intake in many countries of the developing world. In view of the rapidly growing edible and oleo-chemical industrial demands, the search for some alternative sources of additional vegetable oils and fats with nutritional and pharmaceutical attributes has to play a vital role [12].

As a result of large scale citrus fruit consumption and processing, a huge quantity of citrus seeds, generally discarded as an agro-industrial waste is generated every year which could be favorably utilized for production of oil and value-addition. This means there is a need to carry out a comprehensive study to extract and characterize citrus seed oils. To our best understanding, no such detailed studies on the composition and characteristics of citrus seeds and seed oils from sub-continental regions and in particular from Pakistan have yet been reported. The main objective of the present study was to investigate the detailed physico-chemical characteristics and composition of the seeds and seed oils of commonly cultivated species of citrus fruits native to Pakistan.

Materials and Methods

Citrus Seeds

Mature fruit samples (10 kg) of each of the four citrus species, Mitha [Sweet lemon] (*Citrus limetta*), Grapefruit (*C. paradisi*), Mussami [Orange] (*C. sinensis*) and Kinnow [Mandarin orange] (*C. reticulata*) were purchased from the local market of Faisalabad, Pakistan. The fruits samples were further identified and authenticated by Professor Dr Muhammad Ashraf, Department of Botany, University of Agriculture, Faisalabad, Pakistan. The fruits were cut into small pieces with a sharp knife and seeds were collected manually. The seeds were washed with tap water, and then dried at 40 °C in an oven (EYELA, VOC-300 SD, Tokyo, Japan) for 24 h.

Reagents and Standards

All reagents (analytical and HPLC) used were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Pure standards of tocopherols [DL-α-tocopherol, (+)-δ-tocopherol, (+)-γ-tocopherol], and FA methyl esters (FAMEs) were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Oil Extraction

Samples of dried citrus seeds were crushed using a commercial blender (TSK-949, Westpoint, France). A hundred grams of the well crushed seeds for each citrus species in each of the batch were fed into a Soxhlet extractor fitted with a 1-L round bottomed flask and a condenser. The extraction was executed on a water bath for 6 h with 0.50 L of *n*-hexane. The solvent was distilled off under vacuum using a rotary evaporator (EYELA, N-N Series; Rikakikai Co Ltd., Tokyo, Japan). Except for a small quantity (used for tocopherol analysis), the recovered oils were further degummed.

Degumming of Oil

The oil to be degummed was heated (70 °C) on a water bath, and hot water was added to give a final volume of 18%. The mixture was mixed for 10 min with the aid of a glass rod. After cooling, the oil was centrifuged (3,000 rpm i.e., $1221 \times g$) for 12 min in 100 cm^3 tubes in an automatic refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). The degummed and centrifuged oil was left in contact (stirred) with the anhydrous sodium sulfate for

approx. 5 min, filtered through a filter paper by gravity in a drying oven (EYELA, VOC-300 SD, Tokyo, Japan) at 50 °C, and kept in separate sealed polyethylene terephthalate bottles under refrigeration (4 °C), until used for further analysis.

Analysis of Oilseed Residues

The oilseed residues (meals), left after the extraction of oil from the seeds were analyzed for protein, fiber, and ash contents. Protein content (N × 6.25) was determined according to AOAC method 954.01 [13], using a Kjeldahl apparatus. The fiber content was determined according to the ISO method 5983 [14]. Finely ground sample of meal (2.5 g) was freed from fat by extracting it with 15 mL of *n*-hexane. The test portion was boiled with a sulfuric acid solution (0.255 mol/L), followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol/L), followed by separation, washing, and drying. The dried residue was weighed and ashed in a muffle furnace (EYELA, TMF-2100, Tokyo, Japan) at 600 °C, and the loss in mass was determined.

Ash content was determined according to ISO method 749 [15]. Two grams of the test portion was taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace (EYELA, TMF-2100, Tokyo, Japan) at 550 °C, until constant mass was achieved.

Analysis of Extracted Oils

Physical and Chemical Parameters of Oils

Determinations of density, refractive index, iodine value (IV), peroxide value (PV), acidity, saponification value and unsaponifiable matter of the extracted oil were made following AOCS official methods Cc 10a–25, Cc 7–25, Cd 1–25, Cd 8–53, F 9a–44, Cd 3–25, and Ca 61–40, respectively [16]. The color of the oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom), using a 1-in. cell. Specific extinctions at 232 and 270 nm were determined using a spectrophotometer (U-2001; Hitachi, Instruments Inc., Tokyo, Japan). Samples were diluted with *iso*-octane to bring the absorbance within limits (0.2–0.8) and $[\varepsilon^{1\%}_{1cm}(\lambda)]$ were calculated following an IUPAC method II D.23 [17]. The determination of *p*-anisidine value was made following an IUPAC method II. D. 26 [17]. The oil samples dissolved in *iso*-octane were allowed to react with *p*-anisidine for 10 min to produce colored complex and the absorbance values were noted at 350 nm, using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan).

GC/MS Fatty Acid Composition

Fatty acid methyl esters were prepared by standard IUPAC method 2.301 [17] and analyzed by gas chromatography/mass spectrometry (GC/MS), using Agilent-Technologies (Little Falls, CA, USA) 6890N Network GC system, equipped with an Agilent-Technologies 5975 inert XL Mass selective detector and Agilent-Technologies 7683B series auto injector. FAMEs were separated on Agilent-Technologies RT-2560 capillary column (100 m × 0.25 mm, film thickness 0.20 μm). A sample of 1.0 μL was injected in the split mode (split ratio 1:100). Helium at a flow rate 1.2 mL/min was used as a carrier gas. Column oven temperature was programmed from 150 to 250 °C at 4 °C/min, initial and final hold up time was 1 and 5 min, respectively. For GC/MS detection, an electron ionization system, with ionization energy of 70 eV, was used. Injector and MS transfer line temperatures were set at 250 and 260 °C, respectively. Scanning mass range was 30–550 *m*/*z*.

FAMEs Identification and Quantification

The identification of the unknown FAMEs was based on the comparison of their relative retention times with those of authentic standards of FAMEs (Sigma Chemical Co., St Louis, MO, USA). FAMEs were further identified and authenticated using their MS spectra compared to those from the NIST mass spectral library of the GC/MS system. The quantification was done by Agilent-Technologies data handling software (ChemStation 6890). The FA composition was reported as a relative percentage of the total peak area.

Tocopherol Content

Tocopherols (α, γ, and δ) were analyzed using an HPLC following the Current Protocols in Food Analytical Chemistry method [18]. Oil (0.1 g) and 0.05 g ascorbic acid were placed in a 16 × 125-mm test tube. Five milliliters of 90.2% ethanol and 0.5 mL of 80% aqueous KOH solution were added to the test tube and vortexed for 30 s. The test tube was flushed with nitrogen, capped and incubated in a water bath (70 °C) for 30 min with periodical vortexing. The tubes were placed in an ice bath for 5 min, then 3 mL deionized water and 5 mL *n*-hexane were added and vortexed for 30 s followed by centrifugation for

10 min at 1,000×*g* at room temperature. The upper hexane layer was transferred to another test tube. The aqueous layer and the residue were re-extracted by repeating the same procedure. The upper hexane layers from both the extractions were pooled and evaporated to dryness under stream of nitrogen. One milliliter of mobile phase was added to the tube and vortexed 30 s to re-dissolve the extract and then transferred to an HPLC sample vial. A 20-μL sample was injected into a Supelcosil (Supelco Inc., Supelco Park, Bellefonte, KY, USA) LC-Si column (250 mm × 4.6 mm). The chromatographic separation was performed by isocratic elution of the mobile phase constituting of ethyl acetate/acetic acid/hexane (1/1/198, v/v/v) at a flow rate of 1.5 mL/min. Detection was monitored at 295 nm. Tocopherols (α, γ, and δ) were identified by comparing their relative retention times with those of pure standards and were quantified on the basis of peak area of the unknowns with those of pure standards (Sigma Chemical Co., St Louis, MO, USA). Quantification was based on an external standard method. A D-2500 (Hitachi Instruments, Inc., Tokyo, Japan) Chromatointegrator model with a built-in computer program for data handling was used for quantification.

Statistical Analysis

Three seed samples for each species of citrus fruit were assayed. Each sample was analyzed individually in triplicate and data is reported as mean ($n = 3 \times 3 \times 1$) ± SD ($n = 3 \times 3 \times 1$). Data were analyzed by one-way analysis of variance (ANOVA), using Minitab 2000 Version 13.2 statistical software (Minitab Inc., USA) at 5% significance level. A probability value of $P \leq 0.05$ was considered to denote a statistical significance difference.

Results and Discussion

The proximate composition of seeds of four *Citrus* species: *Citrus limetta* (Mitha), *C. paradisi* (Grapefruit), *C. sinensis* (Mussami) and *C. reticulata* (Kinnow) are presented in Table 1. The hexane-extracted oil content of citrus seeds ranged from 27.0–36.5%. Statistical analysis of the data showed the oil content varied significantly ($P < 0.05$) with in the species analyzed. The oil concentration was highest (36.5%) in seeds of Grapefruit, whereas, those of Mussami seeds were lowest in oil yield (27.0%). Saleem et al. [19] reported the oil yields of citrus (*C. sinensis*, *C. reticulata*, *C. paradisi*) seeds were 32.4–36.4%.

The oil contents of citrus seeds determined in the present analysis from Pakistan were found to be quite comparable with those reported for Nigerian citrus (*C. sinensis*, *C. paradisi*, *C. aurantium*, *C. reticulata*, *C. aurantifolia* and tangelo (a hybrid between *C. paradisi and Citrus reticulate*) seeds (24.3–41.1%) [10], Tunisian citrus (Blood orange, Bitter orange and Bergamot) seeds (26.1–36.1%) [7], and Brazilian rangpur lime (*C. limonia* Osbeck) and Sicilian lemon (*C. limon*) seeds (32.0–38.3%) [8]. However, the present oil yield was lower than those reported for Egyptian citrus (Orange, Mandarin, Lime, Grapefruit) seeds (40.2–45.5%) [9], and Tunisian sweet orange (51.8%) and lemon seeds (78.9%) [7]. El-Adawy et al. [4] reported the lipid contents of Egyptian citrus (Citron, Orange, and Mandarin) seeds ranging from 38.9 to 42.6%. The range of oil content (27.0–36.5%) of citrus seeds in the present analysis was found to exceed those of three conventional oilseed crops: cotton (15.0–24.0%), soybean (17.0–21.0%), and olive (20.0–25.0%) [20].

Proximate analysis of the citrus oilseed residues (Table 1) revealed that the protein contents (3.90–9.56% of the seeds) vary significantly ($P < 0.05$) among the species investigated. The species Kinnow (*C. reticulata*), exhibited the highest protein contents (9.7%), whereas, Grapefruit (*C. paradisi*) had the lowest protein level (3.9%). El-Adawy et al. [4] and Habib et al. [9] reported the protein contents of Egyptian citrus seeds to be 15.9–19.9% and 13.8–17.4%, respectively. Akpata and Akubor [21] estimated the protein contents (3.1–3.2%) for sweet orange (*C. sinensis*) seeds. Hamid et al. [22] evaluated the protein contents of sweet orange (*C. sinensis*) seeds to be 10.0%. The ash and fiber contents of the seeds of different citrus species ranged from 4.6–5.6% and 5.0–8.5%, respectively. El-Adawy et al. [4] and Habib et al. [9] reported the ash

Table 1 Proximate composition (%) of seeds of different citrus species

Constituents	Mitha (*Citrus limetta*)	Grapefruit (*Citrus paradisi*)	Mussami (*Citrus sinensis*)	Kinnow (*Citrus reticulata*)
Oil content	29.76 ± 0.59^{c}	36.54 ± 0.36^{a}	27.00 ± 0.81^{d}	31.15 ± 0.62^{b}
Protein content (N × 6.25)	6.43 ± 0.18^{b}	3.90 ± 0.15^{d}	5.56 ± 0.25^{c}	9.56 ± 0.13^{a}
Fiber content	5.00 ± 0.20^{d}	8.50 ± 0.20^{a}	6.90 ± 0.17^{b}	6.50 ± 0.14^{c}
Ash content	5.50 ± 0.11^{a}	5.03 ± 0.15^{b}	4.60 ± 0.13^{c}	5.60 ± 0.09^{a}

Values (expressed on dry weight basis) are mean ± SD of three seeds of each citrus species, analyzed individually in triplicate

Different letters in superscript indicate significant differences within citrus species

Table 2 Physico-chemical characteristics of seed oils of different citrus species

Constituents	Mitha (*Citrus limetta*)	Grapefruit (*Citrus paradisi*)	Mussami (*Citrus sinensis*)	Kinnow (*Citrus reticulata*)
Iodine value (g of I/100 g of oil)	110.00 ± 3.20^{a}	101.50 ± 2.50^{b}	99.85 ± 1.80^{b}	104.80 ± 3.00^{ab}
Refractive index (40 °C)	1.4670 ± 0.03^{a}	1.4639 ± 0.02^{a}	1.4645 ± 0.02 ^{a}	1.4658 ± 0.01^{a}
Density (mg/mL) (25 °C)	0.941 ± 0.05^{a}	0.932 ± 0.03^{a}	0.920 ± 0.04^{a}	0.927 ± 0.03^{a}
Saponification value (mg of KOH/g of oil)	180.90 ± 2.70^{b}	198.85 ± 3.10^{a}	189.50 ± 3.70^{ab}	186.00 ± 4.20^{ab}
Unsaponifiable matter (%)	0.31 ± 0.04^{c}	0.39 ± 0.03^{b}	0.50 ± 0.04^{a}	0.48 ± 0.05^{a}
Color (red units)	3.00 ± 0.10^{a}	1.40 ± 0.10^{c}	3.00 ± 0.09^{a}	2.50 ± 0.12^{b}
Color (yellow units)	30.00 ± 0.60^{a}	15.00 ± 0.75^{d}	22.00 ± 0.90^{b}	20.00 ± 1.10^{c}
Acid value (mg KOH/g of oil)	2.18 ± 0.06^{a}	0.66 ± 0.03^{c}	0.50 ± 0.04^{c}	1.30 ± 0.05^{b}

Values are mean ± SD of three seed oils of each citrus species, analyzed individually in triplicate

Different letters in superscript indicate significant differences within citrus species

contents of Egyptian citrus seeds, 3.1–3.4% and 2.2–3.5%, respectively.

The results of various physico-chemical characteristics of the extracted seed oils of different citrus species are depicted in Table 2. The citrus species investigated, exhibited no significant ($P > 0.05$) variations with regard to the values of refractive index (40 °C) and density (24 °C), which ranged from 1.4639 to 1.4670 and 0.920–0.941 mg/mL, respectively. The present results were quite comparable with those of reported by Habib et al. [9] for Egyptian citrus seed oils, refractive index (1.4650–1.4681) and density (0.913–0.933). However, values of refractive index and density were slightly varied to those of investigated by El-Adawy et al. [4] for Egyptian citrus seed oils, 1.4672–1.4684 and 0.884–0.962, respectively [4].

The refractive indices (1.4639–1.4670) determined in the present analysis of citrus seed oils, agreed well with those reported for cottonseed (1.458–1.466), mustard seed (1.461–1.469), groundnut (1.460–1.465), almond kernel (1.462–1.465), kapok seed (1.460–1.466) oils, but were somewhat lower than those of low-, and high-erucic acid rapeseed (1.465–1.469), soybean (1.467–1.470), sunflower (1.467–1.469), safflower and grape seed (1.473–1.477) oils [23]. Pure oils have a characteristic range of refractive index and density. Thus the degree of variation of typical oil from true values of refractive index and density may indicate its relative purity.

The color of citrus seed oils (1.4–3.0R + 15.0–30.0Y), which varied significantly ($P < 0.05$) within the species analyzed, were lower than those reported by El-Adawy et al. [4] for red values (2.6–7.4 R). The results indicate that these oils could be employed for edible applications after slight processing. Color development in vegetable oils is mainly attributed to the presence of various pigments such as chlorophyll and carotenoids which are removed along with the oil during extraction. Such pigments are effectively eliminated during the refining and bleaching processing of oils. The vegetables oils with minimum color are more acceptable for edible and domestic applications.

The saponification numbers (180.9–198.9 mg of KOH/g of oil) determined in the present analysis of citrus seed oils, differed significantly ($P < 0.05$) among the species analyzed and higher than those reported by Saleem et al. (171.9–184.9) [19]. The saponification numbers were comparable with those of Egyptian citrus seed oil (189.6–196.8) [9] and Nigerian citrus seed oil (186.0–196.0) [10]. El-Adawy et al. [4] reported the saponification values of citrus seed oils fell within the range of 187.2–190.2. When compared with some conventional and non-conventional oilseed crops, the saponification values in citrus seed oils were found to be quite similar to those of corn (187–195), cottonseed (189–198), olive (184–196), pumpkin (185–198), soybean (188–195) and rice bran (179–195) oils [23].

The unsaponifiable matter (0.3–0.5%) of the citrus seed oils were slightly lower than those reported by Saleem et al. (0.5–0.7%) [19] and Habib et al. (0.9–1.3%) [9]. The unsaponifiable matter of the citrus seed oils were also lower than those of cottonseed (0.5–1.5%), olive (0.7–2.5%) and corn (0.5–2.8%) oils, but within the range of cocoa butter (0–0.5%), coconut (0–0.5%), palm (kernel) (0.2–0.8%), tea seed (0.1–1.0%), groundnut (0.2–0.8%), safflower (0.3–1.5%), palm fruit (0.3–1.2%), high-erucic acid rapeseed (0.2–2.0%) and low erucic acid rapeseed (0.2–1.8%) oils [23].

The acid value of the investigated citrus seed oils (0.5–2.2 mg KOH/g of oil) were comparable with that of Brazilian citrus seed oils (<2.0) [8], but higher than that (0.2–1.2) reported for Egyptian citrus seed oils [9]. El-Adawy et al. [4] reported acid value of citrus seed oils to be 0.67–1.12. Within the species analyzed, Mussami and Grapefruit seed oils had lower acid values, which may be correlated to their better resistance to hydrolysis. Generally, a higher acid value of the oils indicates a higher

Table 3 Oxidative state of seed oils of different citrus species

Constituents	Mitha (*Citrus limetta*)	Grapefruit (*Citrus paradisi*)	Mussami (*Citrus sinensis*)	Kinnow (*Citrus reticulata*)
Conjugated dienes $\varepsilon^{1\%}_{1cm}(\lambda_{232})$	3.14 ± 0.10^{b}	2.27 ± 0.12^{d}	4.40 ± 0.09^{a}	2.64 ± 0.05^{c}
Conjugated trienes $\varepsilon^{1\%}_{1cm}(\lambda_{270})$	0.83 ± 0.06^{a}	0.86 ± 0.04^{a}	0.64 ± 0.03^{b}	0.81 ± 0.05^{a}
Peroxide value (mequiv/kg of oil)	1.97 ± 0.08^{b}	1.55 ± 0.12^{c}	1.67 ± 0.10^{c}	2.40 ± 0.15^{a}
p-Anisidine value	2.85 ± 0.05^{ab}	2.23 ± 0.12^{b}	2.53 ± 0.08^{a}	3.15 ± 0.10^{ab}

Values are mean ± SD of three seed oils of each citrus species, analyzed individually in triplicate

Different letters in superscript indicate significant differences within citrus species

magnitude of hydrolytic deterioration thus leading to a generation of objectionable flavor and odors.

The IV of citrus seed oils in the present study, ranged from 99.9–110.0 g of I/100 g of oil varied significantly ($P < 0.05$) among the species analyzed. Saleem et al. [19] reported lower IV (82.7–98.8) of citrus seed oils. The IV (99.9–110.0 g of I/100 g of oil) of Pakistani citrus seed oils is comparable with those reported for Nigerian citrus seed oils (100–114) [10] and Brazilian citrus seed oils (104.2–105.0) [8]. However, considerably higher IV were observed in Pakistani citrus seed oils than those of Egyptian citrus (82.5–99.2) [9] and (91.5–102.5) [4] seed oils. The IV of the investigated citrus seed oils, exceeded to those of olive (75–94) and palm oils (50–55), were found to be within the range of cotton (99–119), mustard (92–125), kapok (90–110), high-erucic acid rape (94–120), and sesame (104–120) seed oils [23].

Seed oils of different citrus species from Pakistan had relatively low oxidative measures (Table 3). The specific extinctions at 232 and 270 nm, which revealed the oxidative deterioration and purity of the oil [17, 24], of citrus seed oils, were 2.3–4.4 and 0.6–0.9, respectively.

The PV for citrus oils (1.6–2.4 mequiv/kg of oil) was lower than that reported for Egyptian citrus seed oils (5.9–6.4) [4]. Our present results are in good agreement to those reported for Brazilian citrus seed oils (1.90–2.0 mequiv/kg of oil) [8]. The *p*-anisidine values for citrus seed oils were 2.2–3.2, indicating a high resistance to secondary oxidation. Rancid and off-flavors are generally produced due to aldehydes of short or middle chain [25]. No earlier reports are available on the quantification of specific extinctions and *p*-anisidine values of citrus seed oils with which to compare the results of our present analysis.

Table 4 depicts the FA composition of seed oils of different citrus species indigenous to Pakistan as determined by GC/MS (Figs. 1, 2). The contents of palmitic (C16:0) and stearic (C18:0) acids ranged from 25.8 to 32.2% and 2.8 to 4.4%, respectively. A small amount of

Table 4 Fatty acid composition (g/100 g) of seed oils of different citrus species

Fatty acids[a]	RT[b]	Mitha (*Citrus limetta*)	Grapefruit (*Citrus paradisi*)	Mussami (*Citrus sinensis*)	Kinnow (*Citrus reticulata*)
C16:0	17.1	26.49 ± 0.50^{c}	32.17 ± 0.40^{a}	29.62 ± 0.29^{b}	25.79 ± 0.31^{c}
C16:1 n-7	18.1	0.39 ± 0.10^{a}	0.20 ± 0.05^{b}	0.42 ± 0.07^{a}	tr
C18:0	19.9	2.75 ± 0.10^{c}	3.64 ± 0.15^{b}	3.90 ± 0.10^{b}	4.43 ± 0.15^{a}
C18:1 n-9	20.9	23.63 ± 0.60^{a}	21.93 ± 0.43^{b}	23.96 ± 0.50^{a}	24.05 ± 0.30^{a}
C18:1 n-7	21.0	2.10 ± 0.12^{a}	1.51 ± 0.12^{b}	1.51 ± 0.08^{b}	1.56 ± 0.05^{b}
C18:2 n-6	22.3	39.81 ± 0.35^{a}	36.10 ± 0.25^{b}	36.26 ± 0.60^{b}	39.55 ± 0.25^{a}
C 20:0	22.7	0.46 ± 0.10^{ab}	0.29 ± 0.05^{b}	0.40 ± 0.07^{ab}	0.55 ± 0.08^{a}
C18:3 n-3	24.0	3.86 ± 0.15^{b}	4.36 ± 0.12^{a}	3.44 ± 0.10^{c}	3.57 ± 0.10^{c}
Unidentified	28.3	0.51 ± 0.06^{a}	tr	0.49 ± 0.05^{a}	0.47 ± 0.10^{a}
TSFA	–	29.70	36.10	33.92	30.77
TUFA	–	69.79	64.10	65.59	68.73
TEFA	–	43.67	40.46	39.70	43.12

The retention time of the solvent peak (8.6 min) as in the chromatogram of Figs. 1, 2 is not shown here. Values are means ± SD of three seed oils of each citrus species, analyzed individually in triplicate. *tr* Denotes values below 0.10%

Different superscript letters indicate significant differences within citrus species

TSFA total saturated fatty acids, *TUFA* total unsaturated fatty acids, *TEFA* total essential fatty acids

[a] Fatty acids are listed in order of elution on RT-2560 MS column

[b] Retention times in minutes

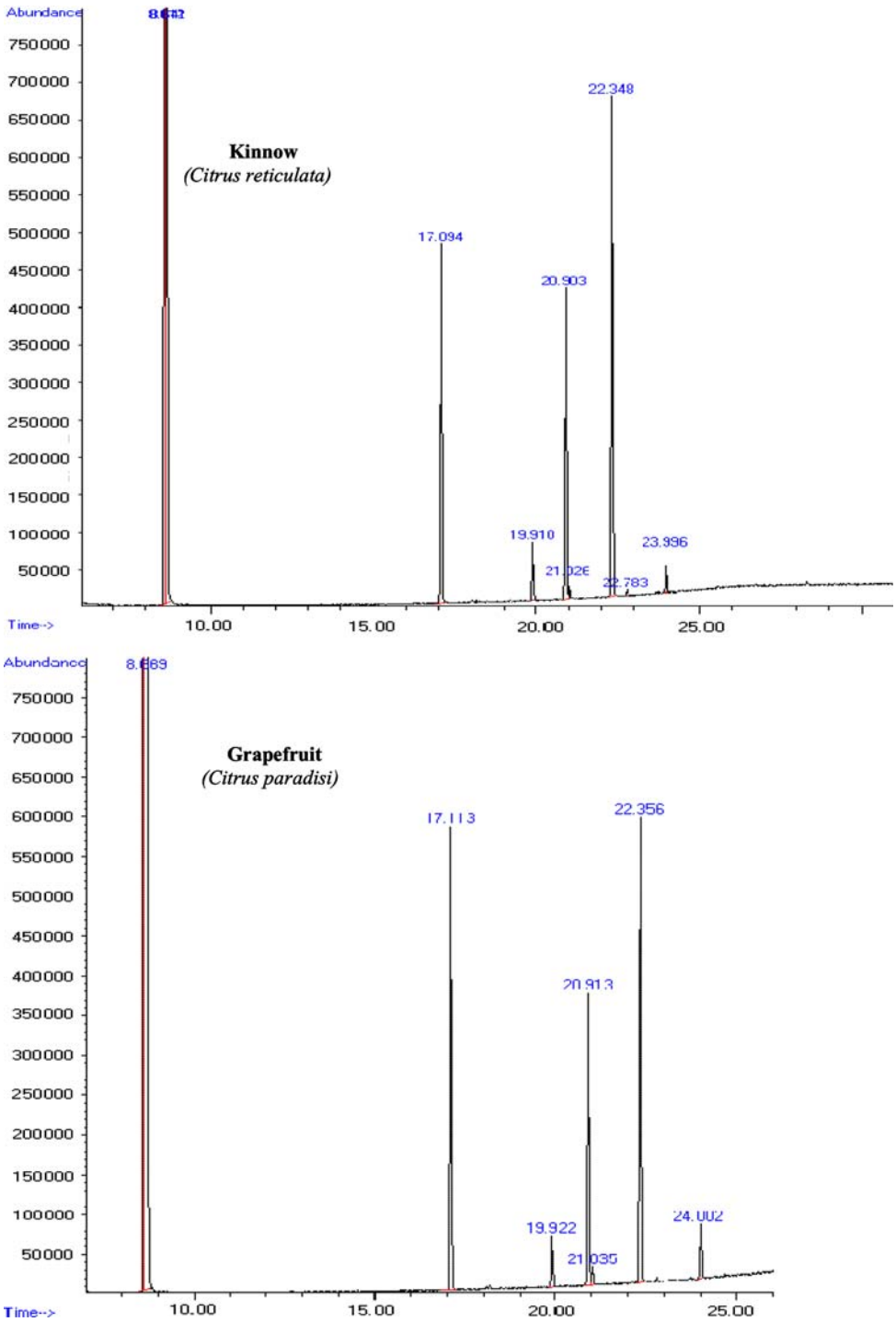

Fig. 1 Gas chromatography/mass spectrometry chromatogram of citrus seed oils

arachidic acid (20:0), 0.3–0.6% was also detected. The contents of total saturated FAs (TSFA), i.e. C16:0, C18:0, and C20:0, were higher (36.1%) in Grapefruit (*C. paradisi)* and lowest (29.7%) in Mitha (*Citrus limetta*) seed oils. Saleem et al. [19] reported TSFA contents of citrus seed oils to be 33.2–45.3%. The amounts (29.7–36.1%) of TSFA were comparable with those reported for Tunisian citrus seed oils (23.9–44.1%) [7] and Egyptian citrus seed oils (31.0–35.0%) [4]. The level of TSFA in the present analysis of citrus seed oils was higher than those investigated for Nigerian citrus seed oils (13.8–33.2%) [10] and Brazilian citrus seed oils (23.2–25.1%) [8]. However, the present data for TSFA were considerably lower than those reported for Egyptian citrus seed oils (22.5–58.4%) [9].

The citrus seed oils had a high level of linoleic acid (C18:2, n-6), which accounted for 36.1–39.0% of the total

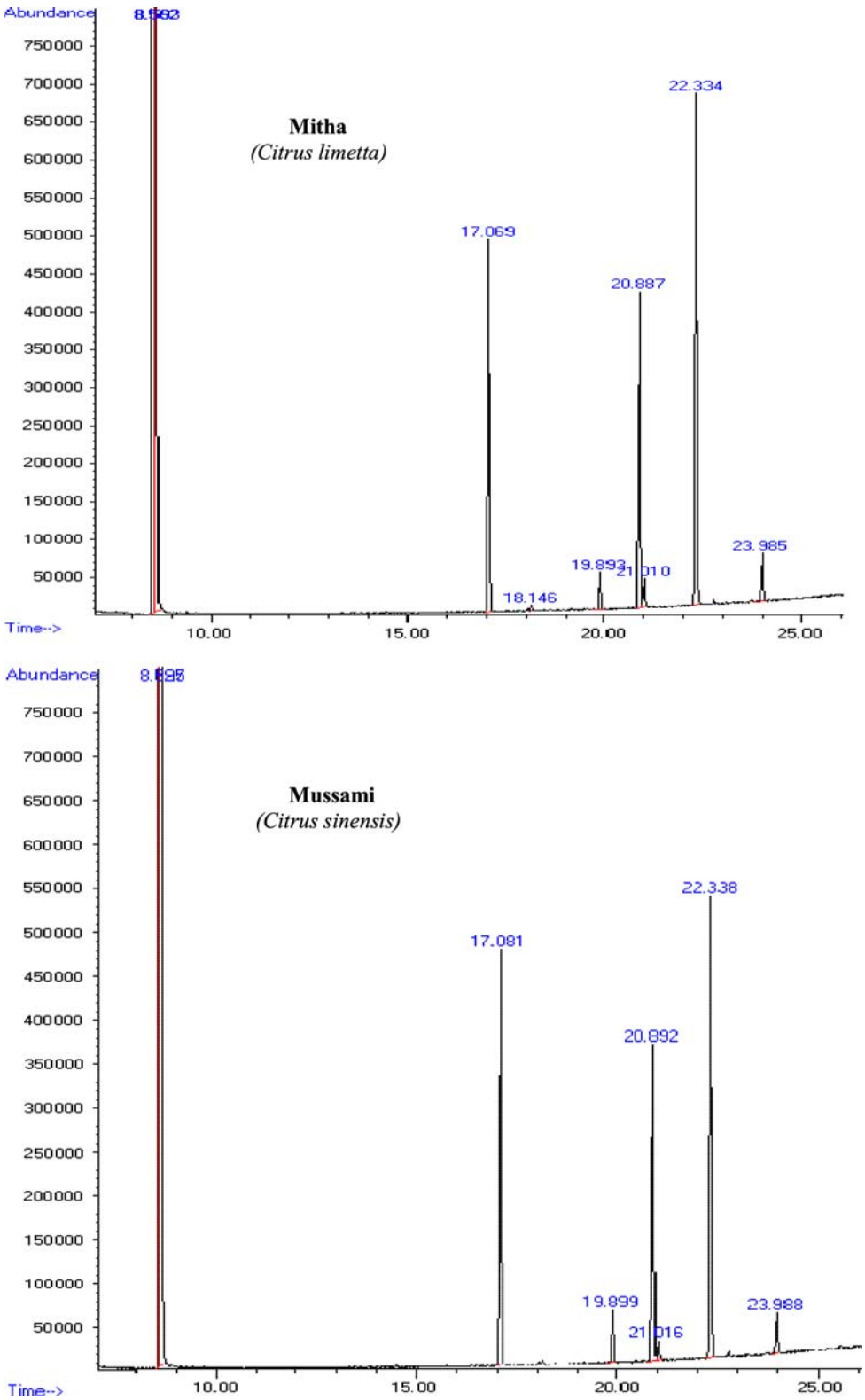

Fig. 2 Gas chromatography/mass spectrometry chromatogram of citrus seed oils

FAs, followed by oleic (C18:1 n-9) and linolenic (C18:3 n-3) acids, 21.9–24.1 and 3.4–4.4%, respectively. A small amount, 1.5–2.1 and traces–0.4%, of C18:1 n-7 and C16:1 n-7, respectively, was also detected. The citrus seed oils investigated revealed a high degree of unsaturation (64.1–69.8%). The contents of total unsaturated FAs (TUFA), that is, (C16:1 n-7, C18:1 n-9, C18:1 n-7, C18:2 n-6, C18:3 n-3) were noted to be highest (69.8%) in Mitha (*Citrus*

Table 5 Tocopherol contents (mg/kg) of seed oils of different citrus species

Tocopherol	Mitha (*Citrus limetta*)	Grapefruit (*Citrus paradisi*)	Mussami (*Citrus sinensis*)	Kinnow (*Citrus reticulata*)
α-Tocopherol	26.40 ± 1.00[d]	380.00 ± 15.00[b]	220.00 ± 18.32[c]	557.82 ± 12.90[a]
γ-Tocopherol	58.03 ± 0.70[b]	43.41 ± 1.10[c]	27.72 ± 0.85[d]	84.10 ± 1.68[a]
δ-Tocopherol	17.27 ± 0.75[b]	9.08 ± 0.50[c]	16.73 ± 0.35[b]	20.02 ± 1.00[a]

Values are mean ± SD of three seed oils of each citrus species, analyzed individually in triplicate

Different letters in superscript indicate significant differences within citrus species

limetta) and lowest (64.1%) in grapefruit (*Citrus paradisi*) seed oils. Saleem et al. [19] reported that the contents of total unsaturates in citrus seed oils were 53.7 to 66.0%.

The concentration of TUFA (64.1–69.8%) in the present analysis of citrus seed oils from Pakistan, was comparable to those reported for Tunisian citrus seed oils (56.5–75.0%) [7], but lower than those of Nigerian citrus seed oils (67.3–86.2%) [10] and Brazilian citrus seed oils (71.8–73.0%) [8]. The present results of TUFA, were similar to those reported for Egyptian citrus seed oils (65.0–69.0%) [4], but varied from those of Egyptian citrus seed oils (32.4–78.3%) [9].

The contents (39.7–43.8%) of total essential FAs (TEFA), that is C18:2 n-6, C18:3 n-3, which have potential health benefits, were in good agreement to those reported for Egyptian citrus seed oils (40.8–43.0%) [4]. The level of TEFA in the present study differed slightly from those reported for Nigerian citrus seed oils (33.40–45.42%) [10] and Tunisian citrus seed oils (31.6–44.4%) [7], but varied greatly from Egyptian citrus seed oils (13.5–60.9%) [9] and Brazilian citrus seed oils (44.4–50.6%) [8]. The contents of TSFA (29.7–36.1%) and TUFA (64.1–69.8%) in the present analysis of citrus seed oils, was comparable with those of cotton seed oil, i.e., 25.0–31.0 and 61.0–80.0%, respectively; however, the values could not be compared with other common vegetable oils [23].

The data for the quantification of tocopherols (α, γ, and δ) of seed oils of different citrus species: *Citrus limetta* (Mitha), *C. paradisi* (Grapefruit), *C. sinensis* (Mussami) and *C. reticulata* (Kinnow) are presented in Table 5. The content (mg/kg) of α-tocopherol in the seed oils of citrus species investigated varied widely ($P < 0.05$) from 26.4 (Mitha) to 557.8 (Kinnow). The level of α-tocopherol in Grapefruit and Kinnow seed oils, 380.0 and 557.8 mg/kg, respectively, was higher than those reported for soybean (99), palm (89), groundnut (178), cottonseed (338), maize (282), and low erucic acid rapeseed (202) oils [23]. The amount of α-tocopherol in Mussami seed oil, i.e., 220 mg/kg, was comparable to that of low erucic acid rapeseed oil (202) [23].

The concentration of γ-tocopherol (27.7–84.1 mg/kg) in citrus seed oils tested was lower than the values reported for cottonseed (429), soybean (1021), groundnut (213), low erucic acid rapeseed (490), and maize (1034) oils but higher than palm (18) and sunflower (11) oils [23]. The content of δ-tocopherol (9.1–20.0 mg/kg), which exhibits the strongest antioxidant potency than either α-, or γ-tocopherol [26], was higher than those of sunflower (0.6), cottonseed (3.3), groundnut (7.6), and low erucic acid rapeseed (9) oils, but was lower than the levels in maize (54) and soybean (421) oils [23]. Of the citrus species investigated, the seed oil of Kinnow (*Citrus reticulate*), had the highest amounts of total tocopherols (661.9 mg/kg), whereas Mitha (*Citrus limetta*) seed oil generally offered the lowest level of tocopherol (101.7 mg/kg). As with many of the other traits, no previously reported data on the tocopherol contents of citrus seed oils are available in the literature.

Although Pakistan is an agricultural country, its indigenous resources are still unable to produce sufficient edible oils for the current need of 2.9 MMT, compared with 2.2 MMT in 2000. The annual seed oil production from local resources is about 0.8 MMT, which is mainly met through cottonseed, accounting for a 67% share, followed by canola (19.6%) and the remaining 13.4% principally from sunflower. Overall consumption of edible oils in Pakistan during recent years indicates a sizeable growth and per capita consumption in the country increasing from 15 kg in 2001 to 18.5 kg in 2006 [27, 28]. The results of our study demonstrated that the oils from citrus seeds native to Pakistan, being a rich source of essential FAs and important tocopherols, have good potential for edible and industrial applications. As Pakistan is one of the largest citrus fruits producers of the world, citrus seeds generally discarded as an agro-industrial waste could emerge as a valuable commodity for production of useful oil and other value-added products.

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