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Sour orange fruit (*Citrus aurantium*) seeds: Humble seeds bursting with goodness

Marzieh Rownaghi, Mehrdad Niakousari*, Mohammad-Taghi Golmakani, Seyed Mohammad Hashem Hosseini

Department of Food Science & Technology, College of Agriculture, Shiraz University, Shiraz, Iran

ABSTRACT

Physicochemical properties of sour orange (*Citrus aurantium*) seeds including its length, weight, density (bulk, tapped, and true), porosity, 1000-grains weight, approximate analysis were assessed. Color parameters (L*, a*, b*), total phenolic and ascorbic acid contents were evaluated. While soaking seeds in water, prior to oil extraction by n-hexane, an aqueous oily layer (AQO) was formed where its characteristics was determined. The oil content of seeds was extracted by conventional (Soxhlet) and two fairly novel methods (ohmic-assisted extraction (OAE) and ultrasound-assisted extraction (UAE)). To facilitate the oil extraction by OAE, a novel approach was established. In the latter, brine was added to n-hexane to ensure a suitable conductivity was established between the two platinum electrodes. The extraction yield and specific energy consumption for each method were evaluated and oil characteristics including color profile (in terms of ΔE), fatty acid composition, refractive index, total phenolic contents, antioxidant activity and anisidine value were assessed. The highest and lowest oil yield of about 50% and 3% was obtained using Soxhlet and maceration, respectively. The lowest specific energy consumption of 5 kWh/kg was achieved when oil extraction was performed by OAE. The extracted oil contains substantial amount of unsaturated and saturated fatty acids. Interestingly, the highest total phenolic content (about 0.9 mg GAE/ g oil) was associated with the aqueous oil. The extraction of valuable components from a waste in a fruit processing plant not only reduces the environmental impact of waste disposal but also present a new valorization method in the food industry.

Keywords: Aqueous oil; Edible oil; Extraction; Ohmic; Sour orange seeds; Ultrasound

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1. Introduction

Citrus aurantium also known as bitter (sour) orange is a cross-breed between of citrus maxima (pomelo) and citrus reticulata (El-Adawy et al., 1999). Apart from being consumed as a source of freshly squeezed juice, the fruit is extensively used to produce pasteurized bottled juice in Iran. In the latter process, large amount of seeds as well as peels are generated as waste (by-product). On average each mature fruit contains at least 10-15 seeds, weighing about 0.1-0.2 g for each seed when dried. Due to the growing demands for edible oil and other ingredients, the efficient recovery of these components from agricultural wastes such as fruit seeds and peels might be a suitable mean to overcome this scarcity. The demand for new sources of edible oils and proteins are on the rise. Hence, citrus fruit wastes found in abundance can represent a suitable choice to be explored (El-Adawy et al., 1999). The genus *Citrus* belongs to the Rutaceae family includes various fruits such as oranges, grapefruits, and lemons will be able to grow almost all over the world in tropical and subtropical climates. Their fruits are processed to produce juice, jam, or marmalade and approximately 34% of their product is used to produce juice. Wastes generated during processing, including peels, seeds, and pomaces may possess a high potential in producing the value-added products (Kumar et al., 2020). While citrus peel is a good source of pectin, essential oil, and limonene which are presently extracted industrially, the seeds are considered as a waste and need to be safely disposed of. It is worth mentioning that flavonoids in citrus are in the forms of polymethoxylated and glycosylated flavones. This may be used as a mean for the fraud prevention, particularly in

*Corresponding author.
E-mail address: niakosar@shirazu.ac.ir (M. Niakousari).
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the import-export businesses (Bocco et al., 1998). Seeds are a good source of unsaturated fatty acids (about 36%) and proteins (about 14%) with appropriate amino acid profiles, high in glycine, cysteine and methionine, and low in tryptophan and lysine in comparison to soybeans (Matthaus & Ozcan, 2012; El-Adawy et al., 1999; Ahmad & Mariod, 2022). It is believed that the seeds have great potential as a new ingredient source in the food industry for manufacturing of added value products such as edible oil (with excellent characteristics) and protein. A review article published by Zayed et al. (2021) provides comprehensive information about citrus fruits and their seeds with concentration on orange. But, this recent article also does not concern much about the sour orange seeds and its components. As Fars province, is a major sour orange growing part of the country and home to the only sour orange juice processing plant, it is believed introducing new seeds' processing method to extract oil and assess the seeds and oil characteristics both in terms of academia and industry is a novel research to pursue. The information generated in this work complements those reported by Zayad et al. (2021) particularly on sour orange seeds which are missing from their article. In the present study, these potentials were explored. Seeds were assessed for their physicochemical characteristics, edible oil and aqueous oil were extracted and analyzed, too. To our knowledge no article deals with extraction of aqueous oil by maceration and by ohmic assisted extraction system. Furthermore, no publication was cited to compare the specific energy consumption of the extraction processes. Therefore, in the present study the optimum method in terms of yield and energy consumption was selected.

2. Material and Methods

2.1. Materials

Unprocessed wet seeds were provided Mahram plant in Fars province, Iran. Folin-ciocalteu phenol reagent, 1,1- diphenyl-2-picrylhydrazyl (DPPH), gallic acid, N-hexane, ethanol, methanol, sodium hydrogen carbonate, acetic acid, orthophosphoric acid, acetyl-chloride, isooctane, p-anisidine, glacial acetic acid, sodium chloride, sodium hydroxide was purchased from Sigma Chemical Company (Germany).

2.2. Seed preparation

Unprocessed wet sour orange seeds were rinsed and separated from the pulp. It was then air dried for about 24 hours under a flow of ambient air. To better extract the material from the seed, the outer shell was detached from the inner core and packaged for the future analysis. The inner core was grounded using a mortar and pestle. The powder was then passed through a sieve with a 20 mesh size. The powder was packaged in dark poly ethylene bags and stored under refrigerated condition for further analysis (Dehdivan & Panahi, 2017).

2.3. Seed physicochemical assessment

2.3.1. Bulk, tapped and true densities

The bulk density of whole seed and inner core is the ratio of 0.6 g of an untapped seed (or powder) to its volume occupied in the 10 mL graduated cylinder. The tapped density is the proportion of the 0.6 g seed (or powder) and its volume occupied in the 10 mL graduated cylinder after they mechanically tapped (Honarpour et al., 2012). True density represents the fraction of putting the 0.6 g powder or whole seed to the displaced volume of toluene in which the seed or powder keeps inert, it is calculated by dividing the mass of a solid by the volume taken up by the solid.

2.3.2. Porosity

Porosity is represented as interstitial voids in particles (Armstrong et al., 1989). It is calculated using Eq. (1):

$$\text{Porosity} = 1 - \frac{\text{bulk density}}{\text{true density}} \quad (1)$$

2.3.3. Thousand grain weight (TGW)

The TGW for the seeds (expressed in gram) was evaluated by dividing the weight of 50 grains by the number of grains, multiplying by 1000 (Deivasigamani & Swaminathan, 2018).

2.3.4. Approximate analysis of inner core

Moisture content (wet basis) was also determined using a laboratory oven (Parsian Teb, Tehran, Iran) set at 100°C for 3 hours. Protein content was assessed by the Kjeldahl method. As we are dealing with a plant protein, protein content was calculated by multiplying the total nitrogen content by a factor of 5.5. The ash content was measured by incinerating the powder in the furnace (Metallurg Co, Tehran, Iran) at 600°C for 6 hours. The oil was extracted by n-hexane for 4 hours using a Soxhlet system. Finally, the amount of carbohydrate is obtained by subtracting the total amount of oil, ash, protein, and fat from 100 (Afify et al., 2017; AOAC, 2005).

2.3.5. pH and dry matter

In all processes it is important to figure out what fraction of a solid may get dissolved in a solvent and what is the pH of the resulting solution. To evaluate the pH of the inner core and outer shell, 5 g of each part was added to 100 cm^3 distilled water (pH = 7.05). After mixing for about 15 min, the pH reading was made (AZ Bench Top 86502 pH meter). The mixture was left stirred for 24 hours at ambient temperature. The pH measurement was made again after period of mixing. Some matters dissolved in this pH in the solvent (water). To find out the amount of material dissolved, the supernatant was separated by centrifugation and filtering. The liquid was placed in an oven at 105°C and left it to reach a constant weight. This was followed by cooling in a desiccator for about 20 min. The weight recorded and the percentage of the dry matter in the inner core and outer shell were evaluated.

2.3.6. Color measurement

Color profile of inner core expressed as lightness (L*), yellowness (b*), and redness (a*) was assessed using a Hunter Lab colorimeter system as described by Baeghbali et al. (2019).

2.3.7. Total phenolic content (TPC)

Conferring to Stankovi (2011) method with some modification, an extract from the inner core was prepared using ethanol:water (50:50) with a 1:20 sample/solvent ratio in a reflux mode at 55°C. The extraction was carried out for 24 hours. The content of the flask was centrifuged for 10 min. The supernatant containing bioactives and the solvent was separated. To obtain the solid containing bioactives, the solvent was evaporated in an oven at 45°C for 24 hours. The total polyphenol content (TPC) of the extract from the inner core was determined using the Folin-Ciocalteau technique based on the reduction reaction defined by Safdar et al. (2017). The value was expressed based on the Gallic acid standard curve. Briefly, 0.5 ml of methanolic solution of extract (1 mg/mL) was mixed with 2.5 mL of Folin 10% and 2.5 mL of sodium hydrogen carbonate 7.5%. Next, the mixtures were incubated for 35 min at 25°C, and absorbance was measured at 765 nm with a UV-VIS Spectrophotometer (Stankovi, 2011).

2.3.8. Ascorbic acid

Based on Bassi et al. (2018) method with some variation, ascorbic acid was extracted by mixing 1.5 g of the inner core powder (or outer shell, if appropriate) with 2.5 mL extracting solution containing 92 mL deionized H_2O containing 8% (v/v) acetic acid and 3% (w/v) metaphosphoric acid. The process was carried out on a magnetic stirrer for about 1 min at room temperature. This was followed by centrifugation of the mixture (1000 g, 5 min) and separation of the supernatant by filtration through a 0.45 µm PTFE filter. The HPLC system involved a liquid chromatography Knauer, Smart line pump (1000), equipped with a UV detector 2500 Knauer set at 245 nm. The separation was carried out isocratically using a Knauer reverse phase C18 column model (vertex plus 250×4.6 mm Eurospher 100-5) with guard cartridge, Column oven model CTO-6A Knauer. The system was used at oven temperature (20ºC) Knauer. Samples were eluted from the column at a flow rate of 1ml/min. Solvents were degassed under a vacuum. A mobile phase of HPLC grade 0.2% Orthophosphoric acid. Both solvent and standard solutions were filtered through a 0.22 µm membrane filter. A sample volume of 50 microliters was injected into HPLC (Bassi et al., 2018).

2.4. Inner core oil extraction

10 g of the inner core powder was submerged in distilled water at a ratio of 1:20, and incubated in a shaker incubator for 24 hours at room temperature. An aqueous oil layer (AQO) was formed where its characteristics was determined. The inner core powder was filtered, dried at room temperature, and kept at 4°C prior to oil extraction using n-hexane. The oil was then extracted by 3 methods, namely Soxhlet apparatus, ultrasound- and ohmic-assisted extractor at 60 ± 5°C.

2.4.1. Soxhlet

As described in AOAC, (2005), oil was extracted using n-hexane as solvent at ratio of 1:8 (powder:solvent) for duration of 4 hours.

2.4.2. Ultrasound-assisted extraction (UAE)

A known quantity of powder and n-hexane at a ratio of 1:8 were placed in a volumetric flask. The flask was then connected to a condenser and heated in an ultrasound bath (Bandelin, Sonrex Digitec, DT255H) at about 65°C for 2 hours. The ultrasound waves were applied to the flask located within the bath as on-off basis (15 min on – 15 min off) (Gorji et al., 2016).

2.4.3. Ohmic-assisted extraction (OAE)

The ohmic-assisted extractor shown in Fig. 1 was designed and built at Shiraz University and patented in Iran. The system consisted of an extraction chamber equipped with two platinum electrodes each 1.5 cm in diameter and 23 cm apart. The voltage of 15.22 V/cm was applied to the electrodes via a VARIAC variable transformer. The voltage and temperature were controlled and monitored automatically using a computer controlled system. As before, a condenser was connected to the chamber to ensure total reflux of the solvent. However, due to the non-polarity of n-hexane, hence extremely low or non-conductivity of the solvent, an alternative method had to be developed. In this method, as in previous techniques, powder and n-hexane (ratio 1:8) were placed in the chamber, followed by addition of 92 mL of 0.5% brine (to enhance the conductivity). The temperature profile during this process was recorded and shown in Fig. 2. As part of this work, conductivity of various liquid/mixture including n-hexane, n-hexane-brine, brine, and distilled water were measured (Table 1) using an electrical conductivity meter (PrismaTech – BPTCond – 200). The extraction was then started while the flask contents were mixed on a magnetic stirrer. The process was completed in 2 hours. The temperature was kept at around 60°C throughout the process. After completing the extraction, two liquid layers were observed; the upper layer being mixture of oil and hexane as indicated by their color. The latter was separated from the lower layer containing brine and the powder residue. The brine-solid layer was also examined for the presence of oil, but no oil was detected in this layer (Seidi Damieh et al., 2016; Aamir & Jittanit, 2017).

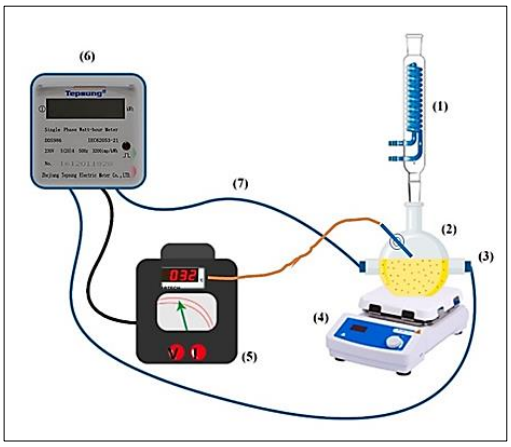

Fig. 1. Schematic depiction of ohmic-assisted extraction apparatus. (1) condenser, (2) sample flask, (3) platinum electrode, (4) magnetic stirrer, (5) Temperature and voltage control unit, (6) Watt meter, and (7) connecting wire.

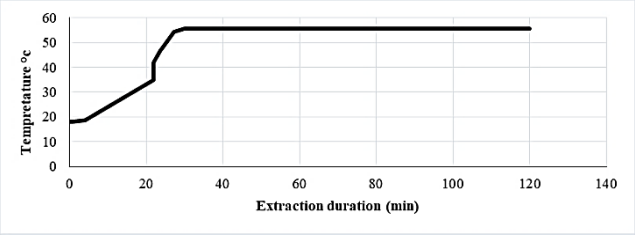

Fig. 2. Temperature profile during oil extraction by ohmic-assisted extractor.

Table 1. Electrical conductivity of solvents used in OAE system.

Solvent	Electrical conductivity (µs)
Hexane	1.855
Distilled water	19.428
NaCl (0.5%)	379.733
Hexane + 92 mL NaCl (0.5%)	10.597

2.4.4. *Separation of extracted oil from hexane*

In all three extraction methods, n-hexane was removed from the oil under partial reflux condition. To attain oil free hexane, the oil was left in an oven operating at around 45°C for about 2 hours. The oil was kept at freezing condition until further analysis.

2.4.5. *Fatty acid composition (FAC)*

The fatty acid composition (FAC) of the oil (different methods) or AQO was assessed by a gas chromatography-flame ionization detector (GC-FID, SP-3420A, Beijing Beifen-Ruili Analytical Instrument, Beijing, China) as described by Keramat et al. (2017) with some modification. The system was equipped with a split/split less injector, a flame ionization detector (FID) and a BPX70 capillary column (Bis-cyanopropylsiloxane-silphenylene, 30m×0.25 mm internal diameter; 0.25 µm film thickness, SGE Analytical Science, Mel-bourne, Australia). 10 mL mixture of acetyl-chloride: methanol (1:10) was added to the oil or AQO. The mixture was then placed in an oven set at 70°C for 1 hour. Afterwards, 5 mL of water and 1 mL of hexane were added to the tube containing the mixture and was shaken for 2 min. This was repeated twice. The whole mixture was finally centrifuged for 5 min. The upper layer was injected into the GC-FID system. The following conditions were maintained in the system; initial temperature = 90°C, final temperature = 210°C, heating rate = 3 °C/min, detector temperature = 300°C, injector temperature = 250°C, pressure of N_2 gas (as carrier gas) = 42.12 psi. FACs were distinguished concurring to the retention times of standard fatty acids injected beneath the same working conditions. The amounts of fatty acids were calculated by measuring their relative peak ranges (keramat et al., 2017).

2.4.6. *Color profile evaluation*

The color profile of the oil or aqueous oil was measured based on the Hunter lab system. The L*a*b* space consists of a luminosity 'L*' or brightness layer, chromaticity layer 'a*' indicating where color falls along the red-green axis, and chromaticity layer 'b*' indicating where the color falls along the blue-yellow axis. 500 µL of oil was poured into a 1.5 cm diameter petri dish. A photograph was taken from the sample in a controlled environment in terms of lighting. The picture was transferred to Adobe Photoshop, Photo and Design Software. Color profile was recorded and using Eq. (2), the change in ΔE (total color difference) was evaluated. The profile for oil extracted by Soxhlet was selected as the reference (Baeghbali et al., 2019).

$$\Delta E = \sqrt{(L_s^* - L_r^*)^2 + (a_s^* - a_r^*)^2 + (b_s^* - b_r^*)^2} \quad (2)$$

where r and s are profile related to the oil (Soxhlet) and oil (ultrasound- and ohmic- assisted extractor) or aqueous oil, respectively.

2.4.7. *Specific energy consumption (SEC)*

The SEC for oil extracted by each method was evaluated according to the equation proposed by Alishahi et al. (2021) (Eq. 3). A watt meter was used to measure the electrical energy (in kWh) consumed during each extraction process. These values were compared and contrasted to select best method in terms of energy efficacy. It is noteworthy that no account was made for the water used in the condenser.

$$\text{Specific Energy Consumption (kWhkg}^{-1}) = \frac{\text{Total energy consumption (kWh)}}{\text{Weight of powder used (kg)}} \quad (3)$$

2.4.8. *Refractive index*

The refractive index of the oil or aqueous oil was measured using an Analog Abbe refractometer AR4. Prior to measurement, the unit was calibrated using distilled water. The measurement was performed by applying a drop oil or aqueous oil on the sample point. The lens of the refractometer was adjusted in a way that the visor was separated into two semi-circle of dark and light. The reading was then recorded (Ogbuneugafor et al., 2011).

Table 2. Seeds physical characteristics.

Sample	Weight (g)	Length (mm)	Density (kgm^{-3})			Porosity	1000-grain weight (g)
			Bulk	Tapped	True		
Inner core	0.076 ± 0.014	8.544 ± 1.310	0.275 ± 0.007	0.367 ± 0.014	1.830 ± 0.577	0.841 ± 0.044	----
Whole seed	0.111 ± 0.016	13.850 ± 1.319	0.256 ± 0.007	0.291 ± 0.002	0.973 ± 0.093	0.738 ± 0.024	112.670 ± 3.055

2.4.9. Oil Total phenolic content (OTPC)

2.4.9.1. Sample preparation

300 µl of methanol was added to 100 mg oil or AQO. The mixture was thoroughly mixed on a vortex. The resulting mixture was then centrifuged at 1000×g for 10 minutes. Supernatant was then withdrawn from the Eppendorf Microcentrifuge Tube. The latter procedure was repeated twice. The supernatants from both stages were collected. The total volume was adjusted to 1 mL by addition of methanol (Malacrida et al., 2012). The sample (Extract) is now ready for OTPC determination.

2.4.9.2. OTPC determination

The TPC of oil or AQO was determined using the Folin-Ciocalteu reagent (also known as Folin's phenol reagent) based on the reduction reaction as described by Safdar et al. (2017). The data was presented based on the Gallic acid standard curve. Briefly, 0.5 mL supernatant (as described above) was mixed with 2.5 mL Folin (10%) and 2.5 mL sodium hydrogen carbonate (7.5%). The resulting mixture was then incubated for 35 minutes at 25°C. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The OTPC was expressed as mg gallic acid equivalent (GAE)/g oil.

2.4.10. Antioxidant activity evaluation

The free radical scavenging activity of oil samples against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was expressed by the method proposed by Ogbuneugafor et al. (2011). Various concentrations of oil or AQO were prepared in methanol (25, 50, 75, 100 µg/ mL). 1 mL of each concentration and 1.0 mL of methanolic solution of DPPH were mixed. The mixture was shaken vigorously and incubated in dark for 20 min. The absorbance was measured against the reagent blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated by Eq. (4):

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Blank absorbance-Sample absorbance}}{\text{Blank absorbance}} \times 100 \quad (4)$$

2.4.11. Anisidine value

Anisidine value (AV) is an indication of the aldehyde contents in oil. To evaluate the AV, as described by Karaman et al. (2014), approximately 0.2 g of the oil or AQO sample was dissolved in 25 mL of isooctane. The absorbance of this solution (Ab) was then recorded at 350 nm using a UV-Vis spectrophotometer (VIS-7220G/UV9200). Afterward, 5 mL of resulting mixture was mixed with 1 mL of anisidine reagent prepared by dissolving 0.25 g of anisidine in 100 mL of glacial acetic acid and shaking the mixture vigorously. After 10 min, the absorbance of the mixture (As) was recorded at 350 nm. The anisidine values were calculated using the Eq. (5):

$$\text{Anisidine value (AV)} = \frac{25 \times (1.2\text{As-Ab})}{\text{Sample weight}} \quad (5)$$

2.5. Statistical analysis

Analyses were carried out in triplicates of each setup. Results are expressed as Mean ± STD. Statistical significant difference ($p < 0.05$) was determined using Duncan's multiple range test after analyzing the variance (ANOVA) in a completely randomized design using SAS version 9.4.

3. Results and Discussion

3.1. Seed characteristics

Table 2 summarizes the seeds and inner core physical characteristics. The weight of inner core was on average about 0.08 g which was almost 80% of the weight of whole seed. The inner core has an average length of about 8.5 mm while the length of whole seed was around 14 mm. In some cases, the seeds are slightly longer and even heavier (Habib et al., 1986). The bulk, tapped, and true densities for the inner core were 0.275±.007, 0.367 ± 0.014 and 1.83 ± 0.577 g/mL, respectively. The true density of whole seed was on the other hand 0.973±0.093 g/cm^3. These values of density were strongly moisture dependent. These values were evaluated at moisture content of about 5% (dry basis). The porosity of the whole seed was about 0.75 which was almost the same as inner core. Seeds 1000-grain weight was about 113 g which was higher than that of the lemon seeds. The same relationship was observed regarding the true density and porosity (Fathollahi et al., 2021). Seeds were fairly light after washing and shade drying and these are reflected in color profile. Values of L*, a* and b* for the inner core were 68.666 ± 4.163, 7.333 ± 1.155 and 1.333 ± 0.577, respectively.

Table 3. Inner core approximate analysis.

Component	Value (%)
Ash	2.430 ± 0.061
Moisture (WB)	4.290 ± 0.216
Protein	17.950 ± 0.427
Oil	48.190 ± 0.181
Carbohydrate	27.130 ± 0.759

3.1.1. Inner core approximate analysis

Protein, oil, ash, moisture and carbohydrate content of inner core were listed in Table 3. The main component of inner core is oil, almost 50%. The article published by Zayed et al. (2021) indicates that the amount of seeds oil extracted by other citrus than sour orange depending on the variety as well as the country of origin my vary between 30-75%, which appears to be promising. The analysis of free fatty acid indicates that the oil extracted from the seeds contains both saturated and unsaturated fatty acid (Gorji et al., 2016). The content of palmitic acid was the highest. The inner core also is a good source of protein and carbohydrate. Other investigators also reported almost similar values for these macro nutrients in seeds of some citrus fruits (Habib et al., 1986). As the edible oil is getting scarce, seeds from citrus fruits may have a good potential to replace common oil bearing seeds (Zayed et al., 2021). As sour orange seeds contain almost 20% protein, they could also make good sources of protein if their extraction is optimized. As

pH is an important parameter in most processes, pH values of inner core and out shell were assessed. The outer shell exhibited a lower pH than the inner core. Interestingly, the value of pH for solution containing out shell was almost constant after macerating it in distilled water.

This trend however was not observed for the inner core and its pH value decreased by almost 2 units over 24 hour soaking. This difference in behavior of inner and outer part of the seeds could be attributed to lower porosity of inner core as compared to the outer shell. Table 4 sums up the change in values of pH as well as inner and outer parts of seed's soluble dry matter.

3.1.2. Inner core ascorbic acid and total phenolic content

Ascorbic acid content of inner core and outer shell measured by HPLC were 102.279 and 27.723 ppm, respectively. The values were in a good range with other citrus fruits seeds. Sir Elkhatim et al. (2018) reported these values between about 50 to 100 ppm for several citrus wastes. The value of ascorbic acid in the inner core is about 27% of the ascorbic acid content of sour orange juice as reported by Abbasi and Niakousari (2008). The TPC of the inner core was 0.650 ± 0.035 mg GAE/g of inner core. The TPC of grape seeds are estimated to be 11 mg GAE/g seeds (Castro-Lopez et al., 2019). The value for the edible arils of pomegranate on the other hand was reported as 1.1 mg GAE/g aril (Derakhshan et al., 2018) which was close to that reported for seeds in this study.

3.2. Oil extraction and property assessment

Table 5 sums up some of the important parameters in oil extraction process by various methods. It also includes an important qualitative feature of oil, the anisidine value. As stated in the previous sections, oil was extracted from the inner core using various conventional and novel techniques. The highest yield of almost 50% was obtained when extraction was carried out using Soxhlet system ($p < 0.05$). Interestingly, some oil or better said "aqueous oil" was also obtained by simply macerating inner core in distilled water overnight. This value did not exceed 3%, but it had some interesting physicochemical characteristics. The data was in good agreement with those reported by Latif et al. (2011). The oil yield in the case of ultrasound-assisted system was slightly lower at about 41%. However, despite expectation, the yield for ohmic-assisted extractor was much lower at about 27%. The results indicate that ohmic-assisted extractor as stand may not really be a suitable mean to extract oil from oil seeds by a non-polar solvent such as hexane which has almost no reason conductivity. Furthermore, it seems there should be an ideal voltage or electric field for extraction to ensure suitable yield; i.e. an increment within the voltage higher than the ideal value will not increment the extraction yield (Sofi'i et al., 2022). Another reason for lower yield could be shorter extraction time in the case of ohmic-assisted extractor. This needs to be verified. It is noteworthy that in the hydro distillation of herbal plants to extract essential oil, the ohmic-assisted extractor enhances the process extensively (Seidi Damyeh et al., 2016).

Table 4. pH and soluble dry matter of seeds.

Sample	$pH_{(water)}$	$pH_{(initial)}$	$pH_{(after\ 24h)}$	Dry matter (%)
Inner core	7.060 ± 0.205	6.470 ± 0.257	4.830 ± 0.14	0.770 ± 0.15
Outer shell	7.060 ± 0.205	3.690 ± 0.010	3.640 ± 0.030	0.960 ± 0.23

Table 5. Physicochemical properties of extracted oil.

Parameter	Extraction method			
	Soxhlet	AQO	UAE	OAE
Yield (%)	48.240 ± 0.208^{a}	2.330 ± 0.144^{d}	40.570 ± 2.009^{b}	26.930 ± 0.424^{c}
ΔE	0.000^{c}	13.090 ± 1.822^{a}	7.717 ± 1.759^{b}	9.390 ± 0.574^{b}
Refractive Index	1.470 ± 0.000^{b}	1.430 ± 0.000^{c}	1.470 ± 0.000^{b}	1.472 ± 0.000^{a}
Anisidine value	-0.193 ± 0.075^{c}	117.900 ± 0.035^{a}	0.517 ± 0.057^{b}	-40.000 ± 0.044^{d}
SEC ($kWhkg^{-1}$)	13.870 ± 0.230^{b}	0.000^{d*}	18.000 ± 0.440^{a}	4.800 ± 0.150^{c}

Different letters within a row represent significant difference at $p < 0.05$.AQO= aqueous oil UAE = ultrasound-assisted extraction; OAE = Ohmic-assisted extraction; *No measurement was made as the process on the stirrer at room temperature was made. Color differences are made in comparison to oil extracted by Soxhlet. SEC = Specific energy consumption.

Table 6. Fatty acid composition (%).

Sample	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
AQO	35.700^{a}	3.080^{a}	31.270^{a}	27.650^{b}	0.929^{b}
Soxhlet	37.390^{a}	2.559^{b}	27.140^{b}	30.770^{a}	2.142^{b}
UAE	34.440^{a}	2.542^{b}	28.420^{b}	32.280^{a}	$2.3180^{a,b}$
OAE	36.560^{a}	2.661^{b}	25.300^{c}	31.530^{a}	3.949^{a}

AQO was extracted with water as solvent. In other method n-hexane was the solvent. Different letters within a column represent significant difference at $p < 0.05$. Due to the high expense of analyzing the fatty acid composition on GC-FID, these set of data has only one reading with no repetition.

The other parameter examined was the color profile. To achieve a meaningful contrast, the oil color parameters were compared with those obtained using Soxhlet. Therefore, ΔE (total color difference) was reported. The AQO showed the highest ΔE ($p < 0.05$). This rather large difference may be attributed to the degree of dissolution of pigments in the solvents (Latif et al., 2011). The values of refractive index for oil obtained in different ways were almost the same. It varies between 1.43 and 1.47. These values were in good agreement with those reported by (Gorji et al., 2016; Lu et al., 2022). An important parameter when discussing oil or fat is Anisidine value. This is a measure of the aldehyde levels in an oil or fat, in particular those that are unsaturated. Depending on the market, the values required vary for fish oils. The anisidine value must be lower than 30, in other sectors is instead required less than 10. Apart from AQO the AV in this work is well below standard. The very high value of AV in aqueous oil is an indication of production of aldehyde substances and existence of secondary oxidation. This might be due to the high content of unsaturated free fatty acid in AQO (Yun & Surh, 2012). An important factor in optimizing any processes is its energy consumption. In the present study electrical energy consumption in each process was measured. As the amount of seeds used in each process may vary, it was decided to present the value of specific energy consumption ($kWhkg^{-1}$). It is noteworthy that the cooling system for condenser was set up as a closed ice water recycle. Hence, no water consumption was evaluated in this study.

Data indicates that the highest specific energy consumption about 18 $kWhkg^{-1}$ corresponds to oil extraction in the UAE system while the SEC value for OAE was below 5 $kWhkg^{-1}$. OAE system of extraction usually considered as a volumetric heating system; i.e. the principle behind the heating of the seeds is their resistance against electrical energy passage which dissipates as heat within the solid. It is therefore the heating process is much faster, in fact it is expected the solid to get heated faster than the liquid. Although despite expectation the yield was not as high as UAE or Soxhlet but it is believed with further examination of the conditions, the yield may be enhanced further. One of the options being longer extraction period or may be addition of higher amount of salt to facilitate better conductivity of solution.

3.2.1. *Fatty acid composition*

Generally, both saturated and unsaturated or omega fatty acids were reported in the lipid part of Citrus seeds (Zayed et al., 2021). It is expected that various extraction as well as storage may alter the fatty acid composition (FAC) of oil. The FAC values for oil extracted using different technique from simple maceration to complicated ohmic-assisted method are tabulated in Table 6. Palmitic, oleic and linoleic acids were dominant fatty acid in oils obtained from inner core of seeds of sour orange fruit. It was interesting to observe that aqueous oil extracted by maceration using water as solvent also contains considerable amount of fatty acids. The data indicates that considerable quantity of unsaturated and saturated fatty acids contained in various oils. There are no critical contrasts in palmitic acid content among assorted methods of oil extraction ($p < 0.05$). The stearic acid was not significantly different among OAE, Soxhlet and UAE treatments. However, it was thought-provoking that this fatty acid was reasonably high (3.08 ± 0.367) in AQO which was obtained by simply macerating inner core in water. Oleic acid content did not show significant differences among UAE and OAE but had the highest value (31.27 ± 2.078) in AQO. Linoleic acid had a significantly lowest value in AQO ($p < 0.05$), however did not show significantly differences for other extraction methods. The average free fatty acid content in this study was almost in agreement with the amount of fatty acids in the Reazai et al. (2014) study. Extraction method, asides from seeds genotype, represents another potential factor affecting fatty acids composition. Hexane solvent extraction of lipids from sweet orange seeds revealed that linoleic acid (36%) and oleic acid (27%) were the most prominent (Atolani et al., 2020).

Table 7. Total phenol content of various oils.

Sample	mg GAE/g oil
Soxhlet	0.398 ± 0.020^{b}
UAE	0.300 ± 0.008^{c}
OAE	0.383 ± 0.098^{b}
AQO	0.880 ± 0.017^{a}

Different letters within a column represent significant difference at $p < 0.05$.

3.2.2. *Oil Total phenolic content (OTPC)*

Phenolic compounds are secondary metabolites in plants, which contribute to the sensorial (taste, flavor, color, etc.) and functional (antioxidant activity, antidiabetic, anticancer activity, etc.) characteristics of food products. The OTPC values are tabulated in Table 7. The values are not so high. The highest OTPC of about 0.9 mg GAE/g sample was for those samples obtained from the inner core by water as solvent, the aqueous oil ($p < 0.05$). For the rest of samples, the OTPC are almost half of those reported for the inner core. The value for Soxhlet was almost the same as that obtained when using OAE. This indicates that there was no adverse effect on the bioactivity of oil when it was extracted by OAE. The higher value of OTPC for AQO may be attributed to low extraction temperature. Data reported by Lu et al. (2022) gives the same indication, too. The results of this study were in general agreement with these reported in study of Falcinelli et al. (2020).

3.2.3. *Free radical scavenging activity*

DPPH radical scavenging activity method is widely used to determine the ability of antioxidants in a sample to quench free radicals of DPPH by donating hydrogen. DPPH radicals have a purple color, which undergoes a color change upon neutralization when it receives hydrogen. The result of evaluating the DPPH radical scavenging activity of oil obtained by various methods at different concentrations is reported in Table 8. The scavenging effect of oil on DPPH exhibited dose dependent manner. The values ranged from about 51 to 90 % DPPH inhibition ($p < 0.05$). The highest value of about 90% was for AQO at the highest concentration of 100 µg/ml ($p < 0.05$). The DPPH radical scavenging mechanism may happen through the oil giving electrons or hydrogen molecules to DPPH. The contrasts in oil items in their bioactive compounds such as phenolic compounds and unsaturated fatty acids influence their hydrogen-donating capacities (Lu et al., 2022).

Table 8. Free radical scavenging activity (%).

Sample	Concentration of extract (µg/ml)			
	25	50	75	100
Soxhlet	50.630 ± 0.069^{d}	55.770 ± 0.051^{c}	56.620 ± 0.080^{c}	58.610 ± 0.069^{d}
UAE	52.770 ± 0.069^{c}	53.770 ± 0.069^{d}	56.860 ± 0.091^{c}	62.250 ± 0.183^{c}
OAE	65.410 ± 0.266^{b}	69.710 ± 1.264^{b}	73.710 ± 0.069^{b}	78.047 ± 0.372^{b}
AQO	67.960 ± 0.069^{a}	73.750 ± 0.043^{a}	79.830 ± 0.300^{a}	89.180 ± 0.450^{a}

Different letters within a column represent significant difference at $p < 0.05$.

4. Conclusion

As the food resources are getting scares worldwide, the need to discover new sources of protein, carbohydrate and oil is becoming more crucial. Seeds from numerous fruits particularly from citrus fruits which presently considered as wastes from fruit processing plants are becoming more important as a new source of macro nutrients. To realize this opportunity, it is essential to optimize extraction of these components. In the present study, the seeds from sour orange processing plant were used as a potential source of these components. By exploring various conventional and novel techniques, these components were extracted. Prior to oil extraction physicochemical properties and bioactive characteristics of seeds were assessed. Interestingly, seeds are reasonably high in ascorbic acid. These helped us to set up an appropriate approach in extraction procedure. Oil was extracted by three methods; yield and specific power consumption were estimated. Fatty acid compositions as well as total phenol content and antioxidant activity of oil extracted by different methods were also assessed. Data was promising in terms of quantitative and qualitative traits. It seems seeds extracted from sour orange while processing the fruit for juice or other products have the potential to be as a new source of food components, food packaging and even antioxidant substance.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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