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# Influence of extraction methods on fatty acid composition, total phenolic content and antioxidant capacity of Citrus seed oils from the Atacama Desert, Chile

[Influencia de los métodos de extracción en la composición de ácidos grasos, el contenido fenólico total y la capacidad antioxidante de los aceites de semillas de cítricos del desierto de Atacama, Chile]

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**Abstract**

*Context*: In the north of Chile, the Atacama Desert and the town of Pica are located, where numerous fruits grow, including citrus ecotypes that generate waste such as the seeds of these fruits.

*Aims*: To evaluate the influence of extraction methods on fatty acid composition, nutritional quality indexes of lipids, total phenolic content and antioxidant capacity of acid lime (AL) and sweet orange (SO) seed oils from Pica oasis.

*Methods*: Extraction of these oils was performed using Soxhlet (SE) and direct (D-UAE) or indirect (I-UAE) ultrasound-assisted extraction techniques.

*Results*: The highest oil yields were obtained using SE (AL = 31.90%, SO = 33.32%). These oils were found to be a rich source of unsaturated fatty acids. Linoleic (36.69%, SE), palmitic (21.90%, SE), oleic (18.07%, UAE-D), α-linolenic (11.45%, D-UAE), and myristoleic (5.91%, D-UAE) acids were the highest concentrations found in AL, while in SO they were oleic and α-linolenic acids (22.54 and 4.53%, respectively) in SE. Total phenolic contents were found without statistical differences between extraction methods (AL =0.719 – 0.787 mg GAE/g oil and SO = 0.653 – 0.915 mg GAE/g oil), except D-UAE SO (0.653 mg GAE/g oil). These oils had similar radical scavenging capacity and reducing power, except in ORAC method (41.99 vs. 96.39 µmol TE/g oil for AL and SO, respectively both in SE). The indexes of nutritional quality were similar among the different methods for the same species, but some of them presented statistically significant differences between the species.

*Conclusions*: An influence of extraction methods on fatty acid composition, total phenolic content and antioxidant capacity of AL and SO seed oils with better results achieved using SE followed by I-UAE was demonstrated. This work establishes the potential source of nutritional compounds of these seeds that grow in the Atacama Desert, Chile.

*Keywords*: acid lime seed oil; antioxidant capacity; fatty acids; sweet orange seed oil; total phenolics.

**Resumen**

*Contexto*: En el norte de Chile se localiza el desierto de Atacama y la localidad de Pica donde crecen numerosos frutos que incluyen ecotipos de cítricos que generan residuos como las semillas de estos frutos.

*Objetivos*: Evaluar la influencia de los métodos de extracción en la composición de ácidos grasos, los índices de calidad nutricional de los lípidos, los fenoles totales y la capacidad antioxidante de los aceites de semillas de lima ácida (AL) y naranja dulce (SO) del oasis Pica.

*Métodos:* La extracción de estos aceites se realizó utilizando técnicas de extracción Soxhlet (SE) y asistida por ultrasonido directa (D-UAE) o indirecta (I-UAE).

*Resultados*: Los mayores rendimientos de aceite se obtuvieron utilizando SE (AL = 31,90%, SO = 33,32%). Se descubrió que estos aceites son una rica fuente de ácidos grasos insaturados. Las concentraciones de los ácidos linoleico (36,69%, SE), palmítico (21,90%, SE), oleico (18,07%, UAE-D), α-linolénico (11,45%, D-UAE) y miristoleico (5,91%, D-UAE) fueron las más altas encontradas en AL, mientras que en SO fueron ácidos oleico y α-linolénico (22,54 y 4,53%, respectivamente) en SE. El contenido fenólico total se encontró sin diferencias estadísticas entre los métodos de extracción (AL = 0,719 – 0,787 mg GAE/g de aceite y SO = 0,653 – 0,915 mg GAE/g de aceite), excepto D-UAE SO (0,653 mg GAE/g de aceite). Estos aceites tuvieron una capacidad similar de eliminación de radicales y poder reductor, excepto en el método ORAC (41,99 *vs.* 96,39 µmol de aceite TE/g para AL y SO, respectivamente ambos en SE). Los índices de calidad nutricional fueron similares entre los diferentes métodos para la misma especie, pero algunos de ellos presentaron diferencias estadísticamente significativas entre las especies.

*Conclusiones*: Se demostró una influencia de los métodos de extracción en la composición de ácidos grasos, el contenido fenólico total y la capacidad antioxidante de los aceites de semillas de AL y SO con mejores resultados en SE seguido de I-UAE. Este trabajo establece la fuente potencial de compuestos nutricionales de estas semillas que crecen en el desierto de Atacama, Chile.

*Palabras Clave*: aceite de semilla de lima ácida; aceite de semilla de naranja dulce; ácidos grasos; capacidad antioxidante; fenoles totales.

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## INTRODUCTION

Nowadays, *Citrus* fruits are the most important fruit crops cultivated around the world, due to their nutritive value (Iglesias et al., 2008). Indeed, the world production of these fruits has reached up to 386.9 million tons during the 2014 - 2016 period (Food and Agriculture Organization, 2017).

Sweet oranges (*C. sinensis* L. Osbeck), lemons (*C. limon*) and acid limes (*C. aurantifolia* (Christm.) Swingle) are the most cultivated among *Citrus* fruits (during 2014 - 2016 were produced 205.9 and 43.5 million tons of oranges and lemons/limes, respectively). Chile is one of the largest producing *Citrus* fruits countries in the Southern hemisphere (282.2 thousand tons in 2014 - 2016, including 140.7 and 141.5 thousand tons of orange and lemons/limes, respectively). In this country, the Northern town of Pica is known to have an important production of these fruits. Furthermore, the most popular *Citrus* fruit produced in this town is a local variety of acid lime, called "*limón de Pica*" (in English, lemon of Pica). In 2010, this fruit was awarded with the protected designation of origin or geographical indication by the Chilean National Institute of Intellectual Property (Instituto Nacional de la Propiedad Intelectual, 2010). Pica is located in the Atacama's desert at 1,800 km North of Santiago, Chile. Hydrometeorological data show the predominance of a desert and arid climate, with low humidity, high daily temperature oscillation and a precipitation rate between 10 - 150 mm per year (Bustamante et al., 2012). A significant part of the local production is destined for the domestic supply, juice making stores, and jam production industries. These local factories generate tons of waste, which are composed of peels, seeds, and pulps, increasing the environmental pollution. However, at the same time, these wastes constitute an abundant but underutilized source of valuable components, which may find application as ingredients in the food, feed, cosmetics, and pharmaceutical industries (Yu et al., 2014).

Seeds are considered as raw material for obtaining edible oil, where several phytochemicals can be detected, such as unsaturated fatty acids, phenolic compounds, and tocopherols (Matthaus and Özcan, 2012). In fact, some *Citrus* seed oils are recognized for their fragrance, moisture, and skin and hair conditioning properties (Bergfeld et al., 2014). Furthermore, sweet orange, lime, and grapefruit (*C. paradise* Macf.) seed oils obtained from fruits of Nigeria have demonstrated antioxidant capacity in DPPH assay (Atolani et al., 2012). In a recent study, sweet orange seed oil has been proven to have positive effects on blood glucose, lipid profile, and liver enzymes activity in diabetic rats induced with alloxan monohydrate (Chilaka et al., 2015).

The extraction from citrus seed oils has been little studied. Basically, it has been carried out by conventional methods such as extraction by Soxhlet (Ajewole and Adeyeye, 1993; Atolani et al., 2012; Malacrida et al., 2012; Anwar et al., 2008), while little studies have been reported using unconventional methods such as ultrasound-assisted extraction (UAE). This method has the potential to be shorter in time and could be used at lower temperatures, allowing a more targeted extraction of the desired plant constituents. The release of cavitation energy induced by UAE on solid surfaces of the plant material promotes collapse due to the asymmetric bubble, which generates a very efficient mixture due to the solvent jets that penetrate or break up the plant cells. In addition, the jets formed during the asymmetric collapse of the bubbles give a better mass transfer that arises from the collapse of the cavitation bubbles on or near the walls or interfaces, which creates a larger available surface for the solvent to remove the desired chemical components. The ultrasonic energy arising from asymmetric bubble collapse can also produce the fractured particles, leading to size reduction, which produces a higher plant surface area accessible to the solvent (Mason et al., 2011).

Searching in the scientific literature, UAE of phenolic compounds from acid lime and sweet orange seed oils did not yield any earlier reports. However, no detailed scientific investigations have been reported on the non-conventional obtention and characterization of fatty acids contained in acid lime and sweet orange seed oils cultivated in

Pica and their antioxidant capacity. The aim of this study was to evaluate the influence of extraction methods on fatty acid composition, nutritional quality indexes of lipids, total phenolic content and antioxidant capacity of acid lime and sweet orange seed oils from Pica oasis using Soxhlet extraction compared with direct and indirect ultrasound-assisted extraction. Therefore, the data obtained from the current study could be used to contribute to the potential application of these oils in food, cosmetics, and pharmaceutical industries and even reduce the environmental pollution generated by these wastes.

## MATERIAL AND METHODS

### Plant materials and sample preparation

Acid limes and sweet oranges used for this study were freshly harvested and purchased from Empresa Torres Contreras Ltd. (Pica, Chile) on August 15th, 2014. Fruits were washed, and their seeds were removed from the peel and pulp. Both types of seeds were dried in an oven (Labtech, model LDO-150N, Equilab, Santiago, Chile) at 45°C until constant weight. Dried seeds were milled into a fine powder with a particle size less than 0.5 mm, by using a commercial kitchen processor (model DP700, Moulinex, Lyon, France). The powdered seed samples were stored separately in glass bottles and put them in a desiccator, before analysis.

### Chemicals and reagents

37-FAME mix standard, 10% w/w boron trifluoride ($BF_3$) in methanolic solution, 2,2-dimethoxypropane, anhydrous sodium sulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ), sodium fluorescein, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), and ferrous sulfate heptahydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteau reagent, HPLC grade water, GC grade *n*-hexane, hydrochloric acid fuming 37% (HCl), dimethyl sulfoxide (DMSO), and PureGrade 96-well microplates (350 µL transparent 'F' bottom) were supplied by Merck (Frankfurt am Main, Germany). Sodium carbonate, sodium acetate, ferric chloride hexahydrate, methanol, ethanol, *n*-hexane, and glacial acetic acid were used in analytical grade and purchased from Winkler (Santiago, Chile).

### Extraction of seed oil

Seed oils were extracted from the finely grounded samples using three different extraction methods:

*Soxhlet extraction (SE)*

This extraction was carried out following a slightly modified protocol of the method described by Rashid et al. (2013). Twelve grams of each ground sample were accurately placed with 150 mL of *n*-hexane at 70°C, for 6 h in a Soxhlet extractor.

*Direct ultrasound-assisted extraction (D-UAE)*

This technique was carried out following a modified protocol of the method used for the extraction of oil from soybeans, described by Li et al. (2004). Three grams of each ground powder were accurately placed in a glass beaker with 50 mL of *n*-hexane. The beaker and its content were immersed into a large beaker containing ice-bath monitoring the temperature at 25°C. This suspension was constantly agitated and sonicated for 90 min using a 30 kHz frequency and 100 W ultrasonic generator (model UP100H, Hielscher, Teltow, Germany).

*Indirect ultrasound-assisted extraction (I-UAE)*

This extraction was carried out following a modified protocol of the method used for the extraction of oil from tobacco seeds, described by Stanisavljević et al. (2009). Three grams of each ground powder were accurately placed in a glass beaker with 50 mL of *n*-hexane. The beaker was immersed in a 40 kHz frequency and 100 W ultrasonic water bath sonicator (model 2510E-DTH, Bransonic, Danbury, CT, USA) and sonicated for 90 min at 25°C.

In the three extraction processes, the mixed suspension was filtered under vacuum using grade 1 Whatman® filter paper, and the excess of extraction solvent was removed under reduced pressure using a rotary evaporator (model RE-100 Pro, Dragon Laboratory Instruments Ltd, Beijing, China) at 40°C. Recovered oils were deposited in vials coated with aluminum foil to protect them from light and stored at 4 - 8°C until use. Three different samples were extracted in triplicate.

### Fatty acid analysis by gas chromatography

The fatty acid composition of each extracted oil was analyzed following the slightly modified method described by Sigma Aldrich (Sigma-Aldrich, 2018). Fatty acid methyl esters (FAME) were prepared using an acid-catalyzed reaction. Twenty milligrams of oil were accurately placed into a 10 mL micro reaction vessel with 2 mL of 10% w/w methanolic solution of $BF_3$. Two milliliters of 2,2-dimethoxypropane were added and heated in a temperature control water bath (Model Excellent $WPE_{45}$, Memmert, Schwabach, Germany) at 60°C for 10 min. After cooling to room temperature, 1 mL of HPLC grade water and 1 mL of GC grade *n*-hexane were added and shaken for 30 s. The mixture was allowed to sit until observing phase separation; the supernatant was collected and, a pinch of anhydrous sodium sulfate was added. FAME was analyzed by gas chromatography (Model GC-2010, Shimadzu, Kyoto, Japan) coupled with a flame ionization detector (FID), using an RTX-wax fused silica column (30 m length × 0.25 mm ID × 0.25 µm film thickness; Restek, State College, PA, USA). Conditions sustained for this analysis included an initial oven temperature of 170°C, which was increased up to 220°C at a rate of 1°C/min. The injector temperature was maintained at 250°C and the FID detector temperature being at 260°C. Nitrogen was used as carrier gas with a total flow of 5.4 mL/min. The sample injection volume was 1 µL, and injection mode was split (100:1). Based on retention times of 37-FAME mix standard, fatty acid components present in every sample were identified and quantified by normalization. Each analysis was performed in triplicate.

### Indexes of lipid nutritional quality

Lipid fraction of samples of citrus seed oils were evaluated for nutritional quality by examining the fatty acid profile and taking into consideration of four indexes (Poerschmann et al., 2004; Hulbert et al., 2007; Lopes et al., 2014; Uysal et al., 2015): atherogenicity (AI), thrombogenicity (TI), unsaturation (UI), peroxidation (PI), the ratio between hypocholesterolemic and hypercholesterolemic fatty acids (HH) and the calculated oxidability (Cox) (Fatemi and Hammond, 1980). The following calculations were employed:

$$AI = (C14:0*2 + C16:0)/(MUFA + PUFA\omega3 + PUFA\omega6) \quad [1]$$

$$TI = (C14:0 + C16:0 + C18:0)/(MUFA*0.5 + PUFA\omega6*0.5 + PUFA\omega3*3 + PUFA\omega3/PUFA\omega6) \quad [2]$$

$$UI = 1\,(\%monoenoics\ FA) + 2\,(\%dienoics\ FA) + 3\,(\%trienoics\ FA) + 4\,(\%tetraenoics\ FA) + 5\,(\%pentaenoics\ FA) + 6\,(\%hexaenoics\ FA) \quad [3]$$

$$PI = 0.025 \times (\%monoenoics\ FA) + 1 \times (\%dienoics\ FA) + 2 \times (\%trienoics\ FA) + 4 \times (\%tetraenoics\ FA) + 6 \times (\%pentaenoics\ FA) + 8 \times (\%hexaenoics\ FA) \quad [4]$$

$$HH = (C18:1 + C18:2\omega6 + C18:3\omega3 + C18:3\omega6 + C20:4\omega6 + C20:5\omega3 + C22:6\omega3)/(C14:0 + C16:0) \quad [5]$$

$$Cox = [C18:1 + 10.3\ C18:2 + 21.6\ C18:3]/100 \quad [6]$$

### Total phenolics content determination

Total phenolics in oils were determined using the slightly modified method described by Malacrida et al. (2012) to determine the phenolic compounds from Brazilian citrus seed oils. For the preparation of the samples, 100 mg of each oil were accurately placed into a 1.5 mL test tube with 300 µL of methanol. The test tube was agitated with a vortex mixer (model Vortex Mixer, Velp Scientifica, Usmate Velate, Italy) for 30 s at 2,400 rpm. Then, the solution was centrifuged in a test tube microcentrifuge (model Force 7, Denver Instrument, Bohemia, NY, USA) for 10 min at 1,000 x*g* and the supernatant was collected. This procedure was repeated twice more. All three extracted supernatants were combined, and the final volume was brought to 1 mL with methanol. Fifteen µL of extract was added to 225 µL of 1 N Folin-Ciocalteu solution and mixed thoroughly. The mixture was

allowed to stand for 3 min at room temperature. After that, 225 µL of 20% w/v sodium carbonate solution and 1,035 µL of bidistilled water were added. The mixture was mixed thoroughly and allowed to stand for 2 h protected from light, at room temperature. Measurement of the absorbance was carried out at 765 nm using a UV-visible spectrophotometer (Model UV-16, MRC, Beijing, China). A calibration curve was prepared, using a solution of gallic acid as a standard (0.020, 0.040, 0.060, 0.080, 0.100, 0.120, 0.140, 0.160, 0.180 and 0.200 mg/mL, $r^2$ = 0.99913). Each analysis was performed in triplicate on three different days. Results were expressed as mg gallic acid equivalents per 1 g of oil (mg GAE/g oil).

**Antioxidant capacity assay**

Antioxidant capacity of oils extracted by SE was determinate using three different methods:

*Oxygen Radical Absorbance Capacity (ORAC)*

The lipid ORAC assay was performed as described by Huang et al. (2002). Analyses were conducted in phosphate buffer 75 mM, pH 7.4 at 37°C. Fluorescein fresh solution (8.16 x $10^{-5}$ mM) was used as the substrate and peroxyl radical was generated using 2, 2'-azobis (2-amidino-propane) dihydrochloride (153 mM) which was also prepared fresh for each run. Each oil was used to 0.00152, 0.00312, 0.00625 and 0.0125 µg/mL stock solutions using DMSO as a solvent. The fluorescence was monitored kinetically with data taken every minute using a microplate reader Synergy HT (Bio-Tek Instruments, USA) at 485 nm (excitation) and 520 (emission). The net AUC of the standards and samples was calculated as followed. The standard curve was obtained by plotting Trolox concentrations (5.0 - 50.0 µM) against the average net AUC of the three measurements for each concentration in three different days. ORAC was expressed as micromoles (µmol) of Trolox equivalents (TE) per gram of oil using an external calibration curve of Trolox. The final ORAC values were calculated using the regression equation between Trolox concentration and the net AUC and were expressed as micromole Trolox equivalent per gram of oil. The AUC was calculated as:

$$AUC = 0.5 + f_1/f_0 + \dots f_i/f_0 + \dots + f_{80}/f_0 + 0.5 \times (f_{80}/f_0) \quad [7]$$

where $f_0$ = initial fluorescence reading at 0 min and $f_i$ = fluorescence reading at time i.

The data were analyzed by a Microsoft Excel macro program (Microsoft, USA) to apply Equation [7] to calculate the AUC. The net AUC was obtained by subtracting the AUC of the blank from that of a sample.

*DPPH radical scavenging assay*

This assay was described by Fukumoto and Mazza (2000), and the protocol was followed with some modifications. To determinate the effective concentration 50 ($EC_{50}$) of the samples, defined as the concentration of oil necessary to produce a 50% decrease of the initial coloration (maximal absorbance) of DPPH, each oil was diluted to 5, 10, 25, 50, 75 and 100 mg/mL stock solutions using DMSO as a solvent,. For testing, 20 µL of each solution was added to 180 µL of 150 µM DPPH solution, using 80% v/v methanol solution as a solvent, into a test tube. The mixture was allowed to stand for 40 min protected from light at room temperature. Measurement of the absorbance was carried out at 515 nm using a multilabel microplate reader and Gen5 software (Synergy HT, Bio-Tek, Winooski, VT, USA). A control assay was necessary replacing the volume of oil solution with the same amount of DMSO. A calibration curve was prepared using different concentrations of an 80% v/v methanolic DPPH solution (10, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µM, $r^2$ = 0.99802). The starting absorption of the DPPH solution was 0.670 ± 0.01. Results were expressed as g of oil per 1 g of DPPH. Furthermore, the percentage of inhibition of the radical was elucidated; each oil was diluted to 100 mg/mL stock solution in DMSO and was conducted with the same assay protocol described above. Results were expressed using the following formula:

$$\%\ inhibition\ of\ DPPH = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100 \quad [8]$$

where, Abs control was the absorbance of the control assay, and Abs sample was the absorbance

of the sample with the DPPH solution. Each assay was performed in triplicate on three different days.

The antiradical efficiency (AE) of oils was determined according to the following formula:

$$AE = 1/EC_{50} \quad [9]$$

*Ferric reducing antioxidant power (FRAP)*

The protocol followed by this assay was determined by Bolanos de la Torre et al. (2015), with slight modifications. FRAP solutions were prepared at the moment by mixing 10 mL of 300 mM acetate buffer solution (pH = 3.60) with 1 mL of 10 mM TPTZ solution (reagent dissolved in 40 mM HCl) and 1 mL of 20 mM ferric chloride solution and warmed at 37°C. Each oil was diluted to 1 mg/mL using DMSO as solvent. Twenty µL of each solution was added to 180 µL of FRAP solution, and the mixture was allowed to stand for 30 min at 37°C. Measurement of the absorbance was carried out at 593 nm using a multilabel microplate reader and Gen5 software. A control assay was necessary, replacing the volume of oil solution with the same amount of solvent. Two calibration curves were elaborated using different concentrations of a 50% v/v ethanolic Trolox solution (25, 50, 75, 100, 125, 150, 175 and 200 µM, $r^2$ = 0.99081) and ferrous sulfate solution (100, 200, 400, 600, 800 and 1000 µM, $r^2$ = 0.99920). Each assay was performed in triplicate on three different days. Results were expressed as mmol of Trolox (TE) or iron equivalents (IT) per 1 mg of oil.

### Statistical analysis

The analytical data were expressed as mean ± standard error of mean (SEM). Statistical significance ($p \leq 0.05$) of the results were analyzed by one-way analysis of variance (ANOVA) and Tukey's test at 5% significance level, using the Minitab software, version 17.0 (Minitab Inc, State College, PA, USA). GraphPad Prism software, version 6 (GraphPad Software, Inc, La Jolla, CA, USA) was used to elucidate the $EC_{50}$ results. In all cases, the $EC_{50}$ was defined as the necessary oil concentration to produce 50% of the tested activity. Multivariate principal component analysis (PCA) was applied to detect the structure in the relationships among variables and to classify the objects (the relationship among acid lime and sweet orange seed oils and fatty acid composition as extraction methods) using Minitab. Agglomerative hierarchic cluster analysis (HCA) was carried out using XLSTAT 2018 software. The dendrogram method shows correlations through clusters diagrams. Pearson correlation was used to evaluate the association between total phenolics and ORAC values of the different extraction methods.

## RESULTS AND DISCUSSION

### Yield of oil

Oil yields obtained from both *Citrus* seeds using different extraction techniques are shown in Table 1.

Among the various extraction methods, SE achieved the highest oil yield. In the case of acid lime seed oil, the yield of extraction was 31.90 ± 1.09%. These results show that the seeds of this ecotype contain more oil than those from Lagos, Nigeria, using similar extraction conditions (Atolani et al., 2012). Also, these exhibit a similar amount compared with acid lime seeds from Vietnam, using petroleum ether as a solvent in a Twisselmann apparatus (Matthaus and Özcan, 2012). Also, the yield of extraction of oil from sweet orange seeds was 33.32 ± 0.13%, which is higher than reported from the same species from Pakistan and Nigeria, using similar extraction conditions (Anwar et al., 2008; Atolani et al., 2012). These differences could be attributed to the variety of solvents, fruits and the geographical origins. Moreover, the soil and climate conditions like the Pica oasis could cause variations in the production of metabolites in plants (Maranz and Wiesman, 2004).

As shown in Table 1, oils obtained by SE have significant differences between both D-UAE (22.07 ± 0.68%, acid lime, and 22.84 ± 0.69%, sweet orange) and I-UAE (22.28 ± 0.76%, acid lime, and 23.11 ± 0.52%, sweet orange).

**Table 1.** Oil content and fatty acids composition from Pica acid lime and sweet orange seeds using the different extraction techniques.

| **Seed oil material** | **Acid lime seed oil** | | | **Sweet orange seed oil** | | |
|---|---|---|---|---|---|---|
| **Extraction method** | **SE** | **D-UAE** | **I-UAE** | **SE** | **D-UAE** | **I-UAE** |
| Total lipid content (%) | 31.90 ± 1.09$^{a}$ | 22.07 ± 0.68$^{b}$ | 22.28 ± 0.76$^{b}$ | 33.32 ± 0.13$^{a}$ | 22.84 ± 0.69$^{b}$ | 23.11 ± 0.52$^{b}$ |
| **Fatty acid (% of total FAME)** | | | | | | |
| **ΣSFA** | **28.65 ± 0.77$^{b,c}$** | **26.75 ± 0.32$^{c}$** | **28.59 ± 0.90$^{b,c}$** | **28.80 ± 0.89$^{a,b,c}$** | **31.87 ± 0.21$^{a}$** | **30.70 ± 0.24$^{a,b}$** |
| Myristic acid (C14:0) | 3.53 ± 0.29$^{a,b}$ | 3.17 ± 0.15$^{a,b}$ | 3.85 ± 0.27$^{a}$ | 2.99 ± 0.09$^{a,b}$ | 3.16 ± 0.22$^{a,b}$ | 2.85 ± 0.10$^{b}$ |
| Palmitic acid (C16:0) | 21.90 ± 0.84$^{a,b}$ | 19.83 ± 0.19$^{b}$ | 20.79 ± 0.61$^{b}$ | 21.33 ± 0.56$^{a,b}$ | 23.56 ± 0.32$^{a}$ | 23.75 ± 0.50$^{a}$ |
| Stearic acid (C18:0) | 2.95 ± 0.39$^{b}$ | 3.43 ± 0.10$^{b}$ | 3.54 ± 0.09$^{b}$ | 4.21 ± 0.35$^{a,b}$ | 4.92 ± 0.34$^{a}$ | 3.93 ± 0.26$^{a,b}$ |
| Arachidic acid (C20:0) | 0.26 ± 0.06$^{a}$ | 0.32 ± 0.02$^{a}$ | 0.41 ± 0.10$^{a}$ | 0.28 ± 0.02$^{a}$ | 0.22 ± 0.01$^{a}$ | 0.17 ± 0.02$^{a}$ |
| **ΣMUFA** | **22.01 ± 0.59$^{c}$** | **26.14 ± 0.51$^{a,b}$** | **24.29 ± 0.23$^{b}$** | **27.65 ± 0.18$^{a}$** | **26.38 ± 0.44$^{a}$** | **27.99 ± 0.52$^{a}$** |
| Myristoleic acid (C14:1) | 3.38 ± 0.57$^{b}$ | 5.91 ± 0.28$^{a}$ | 3.64 ± 0.36$^{b}$ | 3.21 ± 0.11$^{b}$ | 4.18 ± 0.15$^{b}$ | 4.85 ± 0.38$^{a,b}$ |
| Cis-7-hexadecenoic acid (C16:1) | 3.17 ± 0.37$^{a,b}$ | 2.15 ± 0.37$^{a,b,c}$ | 3.38 ± 0.32$^{a}$ | 1.90 ± 0.19$^{b,c}$ | 1.24 ± 0.14$^{c}$ | 1.56 ± 0.18$^{c}$ |
| Oleic acid (C18:1 n-9) | 15.46 ± 0.35$^{d}$ | 18.07 ± 0.14$^{c}$ | 17.27 ± 0.27$^{c}$ | 22.54 ± 0.35$^{a}$ | 20.95 ± 0.20$^{b}$ | 21.58 ± 0.48$^{ab}$ |
| **ΣPUFA** | **49.34 ± 1.04$^{a}$** | **47.11 ± 0.80$^{a,b}$** | **47.13 ± 0.73$^{a,b}$** | **43.54 ± 1.07$^{b,c}$** | **41.75 ± 0.23$^{c}$** | **41.31 ± 0.40$^{c}$** |
| Linoleic acid (C18:2 n-6) | 36.69 ± 0.66$^{a}$ | 34.07 ± 0.51$^{b}$ | 34.25 ± 0.38$^{a,b}$ | 36.70 ± 0.85$^{a}$ | 35.74 ± 0.38$^{a,b}$ | 36.32 ± 0.34$^{a,b}$ |
| γ-linolenic acid (C18:3 n-6) | 0.69 ± 0.03$^{a}$ | 0.52 ± 0.13$^{a}$ | 0.56 ± 0.02$^{a}$ | 0.61 ± 0.06$^{a}$ | 0.59 ± 0.11$^{a}$ | 0.42 ± 0.07$^{a}$ |
| α-linolenic acid (C18:3 n-3) | 10.16 ± 0.15$^{b}$ | 11.45 ± 0.11$^{a}$ | 10.96 ± 0.25$^{a,b}$ | 4.53 ± 0.12$^{c}$ | 3.98 ± 0.24$^{c}$ | 3.67 ± 0.16$^{c}$ |
| Docosadienoic acid (C22:2) | 1.80 ± 0.34$^{a}$ | 1.06 ± 0.13$^{a}$ | 1.36 ± 0.28$^{a}$ | 1.71 ± 0.26$^{a}$ | 1.44 ± 0.31$^{a}$ | 0.91 ± 0.23$^{a}$ |
| Total UFA | 71.35 ± 0.92$^{a,b}$ | 73.25 ± 0.32$^{a}$ | 71.41 ± 0.90$^{a,b}$ | 71.20 ± 0.89$^{a,b,c}$ | 68.13 ± 0.21$^{c}$ | 69.30 ± 0.24$^{b,c}$ |
| Total EFA | 47.54 ± 10.77$^{a}$ | 46.04 ± 9.88$^{a}$ | 45.77 ± 9.96$^{a}$ | 41.84 ± 11.43$^{a}$ | 40.31 ± 11.19$^{a}$ | 40.41 ± 11.46$^{a}$ |
| Total n-3 | 10.16 ± 0.15$^{b}$ | 11.45 ± 0.11$^{a}$ | 10.96 ± 0.25$^{a,b}$ | 4.53 ± 0.12$^{c}$ | 3.98 ± 0.24$^{c}$ | 3.67 ± 0.16$^{c}$ |
| Total n-6 | 37.38 ± 0.70$^{a}$ | 34.60 ± 0.64$^{a}$ | 34.81 ± 0.40$^{a}$ | 37.31 ± 0.91$^{a}$ | 36.33 ± 0.49$^{a}$ | 36.74 ± 0.42$^{a}$ |
| Unsaturated/saturated | 2.49 | 2.74 | 2.50 | 2.47 | 2.14 | 2.26 |
| n-6/n-3 | 3.68 | 3.02 | 3.18 | 8.24 | 9.13 | 10.01 |
| **Nutritional quality** | | | | | | |
| UI | 131.35$^{a}$ | 132.32$^{a}$ | 130.07$^{a}$ | 119.89$^{b}$ | 114.46$^{b}$ | 114.72$^{b}$ |
| PI | 60.55$^{a}$ | 59.72$^{a}$ | 59.26$^{a}$ | 49.38$^{b}$ | 46.98$^{b}$ | 46.11$^{b}$ |
| AI | 0.42$^{a}$ | 0.36$^{b}$ | 0.41$^{a}$ | 0.39$^{a,b}$ | 0.45$^{a}$ | 0.43$^{a}$ |
| TI | 0.36$^{a}$ | 0.32$^{a}$ | 0.35$^{a}$ | 0.44$^{a,b}$ | 0.51$^{b}$ | 0.49$^{b}$ |
| HH | 2.48$^{a}$ | 2.79$^{a}$ | 2.56$^{a}$ | 2.65$^{a}$ | 2.29$^{a}$ | 2.33$^{a}$ |
| Cox | 6.28$^{a}$ | 6.28$^{a}$ | 6.19$^{a}$ | 5.12$^{a,b}$ | 4.88$^{b}$ | 4.84$^{b}$ |

Data from three independent determinations are expressed as mean ± SEM. Treatments not sharing the same letters in the same row are significantly different by ANOVA followed by a Tukey's test ($p < 0.05$). SE: Soxhlet extraction; D-UAE: direct ultrasound-assisted extraction; I-UAE: indirect ultrasound-assisted extraction; SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EFA: essential fatty acids; UI: Unsaturation index; PI: Peroxidation index; AI: Atherogenicity index; Thrombogenicity index; HH: hypocholesterolemic/hypercholesterolemic ratio; Cox: calculated oxidability.

These results are comparable to yields of oil extracted from tobacco and rose-hip seeds (Szentmihályi et al., 2002; Stanisavljević et al., 2009). It could be due to extraction conditions such as the temperature of the medium and time of extraction. Compounds present in samples can acquire greater kinetic energy using temperature, which allows a higher diffusion and transfer of these substances from samples to the medium. Furthermore, a higher temperature used during SE compared to the other extraction techniques has always allowed sampling to be in contact with the fresh solvent, giving a greater extraction ability of substances, a fact that did not exist during UAE. Moreover, it has been found that the extraction time influences the yield of the extraction; as long as the contact

time between sample and solvent, a higher yield was obtained (Li et al., 2004).

On the other hand, no significant differences were observed between oil yields of both types of *Citrus* seeds, according to each extraction technique (Table 1). Although D-UAE contacted with the samples directly without any barrier, which was expected to have higher oil yield compared to I-UAE, no statistically significant differences were found between the yields of the oils obtained by D-UAE and I- UAE for both species.

**Fatty acids composition**

Fatty acids composition of oils extracted from acid lime and sweet orange seeds using the different extraction techniques are showed in Table 1.

The acid lime seed oils mainly contain unsaturated fatty acids (UFA) (71.35 – 73.25%), including monounsaturated fatty acids (MUFA) (22.01 – 26.14%) and polyunsaturated fatty acids (PUFA) (47.11 – 49.34%). Independent of the extraction techniques, linoleic (C18:2 n-6), oleic (C18:1 n-9) and α-linolenic (C18:3 n-3) acids were the principal UFAs, ranging from 34.07 – 36.69%, 15.46 – 18.07%, and 10.16 – 11.45%, respectively, which were comparable with other varieties (Ajewole and Adeyeye, 1993; Matthaus and Özcan, 2012), and essential fatty acids (EFA) ranging (45.77 – 47.54%). However, palmitic acid (C16:0) was the dominant saturated fatty acid (SFA), ranging from 19.83 – 21.90%. These concentrations were lower than those reported by Ajewole and Adeyeye (1993) and Matthaus and Özcan (2012) for other varieties of acid lime seeds. Interestingly, higher percentages of fatty acids were obtained using direct ultrasound-assisted extraction for myristoleic and α-linolenic acids, with statistically significant differences with respect to Soxhlet extraction, principally (Table 1).

Like acid lime seed oils, those extracted from sweet orange seeds also have shown a high percentage of UFA (68.13 – 71.20%), specifically, MUFA (26.38 – 27.99%) and PUFA (41.31 – 43.54%), as shown in Table 1. Linoleic acid was the most abundant fatty acid, ranging from 35.74 – 36.70%, similar to other varieties of sweet oranges cultivated around the world (Ajewole and Adeyeye, 1993; Anwar et al., 2008; Malacrida et al., 2012; Matthaus and Özcan, 2012). Oleic acid was the principal MUFA (20.95 – 22.54%), and palmitic acid was the most important SFA (21.33 – 23.75%). It is noteworthy, the composition of these fatty acids in other varieties of sweet oranges from Turkey, Vietnam, Brazil, Pakistan or Nigeria has shown a higher content than the current study using petroleum ether or n-hexane, and Twisselmann or Soxhlet apparatus during 6 - 8 h (Ajewole and Adeyeye, 1993; Anwar et al., 2008; Malacrida et al., 2012; Matthaus and Özcan, 2012).

In general, the few significant variations observed in the composition of fatty acids of each type of oil, as shown in Table 1, could be due to the degradation of these compounds by hydrolysis or oxidation mechanisms, leading to the generation of volatile compounds, such as limonene, hexanal and *Z*-hept-2-enal, responsible to contribute the rancid odor and off-flavor of oils (Chemat et al., 2004a, 2004b). This degradation process could be attributed to extraction factors, including temperature and ultrasound. Sufficient energy is produced using temperature as a parameter during SE, which may cause covalent bond ruptures, such as C–C and C–H bonds in the acyl portion of fatty acids to form a variety of lipid alkyl radicals, generating the activation of oxidation process (Pingret et al., 2013). On the other hand, UAE is based on cavitation phenomenon, which causes bubble implosions and facilitates the cellular disruption, leading to solvent penetration and, therefore, intensifying the transfer of compounds to the medium (Vinatoru et al., 2001). Hot points above 5,000°C and high-pressure areas near to 500 megapascals (MPa) are generated due to the cavitation process, which favors the oxidation of fatty acids (Chemat et al., 2004b).

On the other hand, in humans, the intake of UFA compounds must be needed. Benefits of MUFA and PUFA consumption, comprising EFA, include prevention of cardiovascular diseases, modulation of immune activity in inflammatory health problems and regulation of mental health and maturation of the central nervous system during fetal development (Ruxton et al., 2004; Rustan

and Drevon, 2005). The high levels of UFA of these oils make them suitable sources for nutritional applications.

The composition of major UFA of oils extracted by the different extraction techniques from both samples is shown in Table 1. The amount of oleic acid, an UFA, in sweet orange seed oil was significantly higher than from acid lime seed oil, independent of the extraction technique, which was consistent with the value of the total amount of UFA. Otherwise, the composition of α-linolenic acid, an essential omega-3 PUFA, was 2.5 times higher in acid lime seed oil than the oil extracted from sweet orange seeds, which leads to a 1.1 times significantly higher amount of total PUFA, although the amount of linoleic acid, an essential omega-6 PUFA, did not differ significantly between both samples.

Corresponding to the above results, unsaturated/saturated ratio was highest in acid lime oils ranging 2.49 – 2.74, with the highest value in D-UAE, while sweet orange seed oil showed values between 2.14 – 2.47, highest in SE. These values were similar to those reported in different studies using the Soxhlet extraction, both for acid lime and sweet orange seed oils (Ajewole and Adeyeye, 1993; Anwar et al., 2008; Malacrida et al., 2012).

The n-6/n-3 ratio was lowest in acid lime oils (3.02 – 3.68) with the lowest value in D-UAE and highest in SE, while sweet orange oils showed ratio between 8.24 – 10.01 with the lowest in SE and the highest in I-UAE. These values were lower than those described in other recent studies (Ajewole and Adeyeye, 1993; Anwar et al., 2008) and like those to Malacrida et al. (2012), all reported in extractions by Soxhlet. Some studies report that cardiovascular, cancer, inflammatory and autoimmune diseases are promoted by a high n-6/n-3 ratio, whereas a low ratio exerts suppressive effects (Simopoulos, 2002). For example, a ratio 4/1 was associated with a 70% reduction in total mortality in cardiovascular disease, while in patients with rheumatoid arthritis, the inflammation was blocked by a ratio of 2 – 3/1. That study considers that the optimal ratio of n-6/n-3 fluctuates from 1/1 to 4/1 depending on the disease under consideration. This variation could be due to that many of the chronic diseases prevalent nowadays are multigenic and multifactorial (Simopoulos, 2002). The n-6/n-3 ratio in acid lime oils, from the present study, could be considered for future studies in any of those pathologies.

According to the composition of fatty acids, and the low variation in the percentage of these metabolites with the use of different extraction methods, both Soxhlet and I-UAE could be used to obtain these compounds. With the use of I-UAE, extraction time, energy and solvents will be saved.

*Fatty acids of principal component analysis (PCA)*

To better discriminate among the extraction methods and citrus seed oils under investigation, PCAs were performed on the combined extraction method (SE, D-UAE, I-UAE), 11 fatty acids detected, and saturation nature of fatty acids (total SFA, UFA, MUFA, PUFA, n3, n6, and n6/n3 ratio) with a total of 21 variables for each citrus seed oil (Fig. 1A). For acid lime seed oils, the first two principal components (PCs) accounted the 100% of the total variance, with PC1 (79.0%) explaining 3.76 times as much as PC2 (21.0%). In the same way, for sweet orange seed oils, the first two principal components (PCs) also accounted the 100% of the total variance, with PC1 (66.4%) explaining 1.98 times as much as PC2 (33.6%).

In Fig. 1B we can observe the SE method for acid lime seed oils was very close on the first axe with linoleic, γ-linolenic, docosadienoic, palmitic acids, total PUFA, total n-6, and n-6/n-3 ratio. On the other hand, D-UAE method was very close on the second axe with myristoleic, α-linolenic, oleic acids, total UFA, total MUFA, and total n-3. I-UAE was distant from all other methods on the third axe surrounded by two variables (stearic and arachidic acids). Myristic, cis-7-hexadecenoic acids and total SFA were distant from other fatty acids on the fourth axe.

The eigenvectors indicating an association between variables and PCs are presented in Table 2. The bigger the eigenvectors, the correlations between variables and PCs. For acid lime seed oil, docosadienoic, palmitic, γ-linolenic acids, total n-6,

and n-6/n-3 ratio were positively associated with PC1, whereas oleic, α-linolenic acids, total MUFA, and total n-3 were negatively associated with PC1. Myristoleic acid and total UFA were positively associated with PC2. However, for sweet orange seed oil, arachidic, oleic, α-linolenic acids, total UFA, total PUFA, total n-3, and total n-6 were positively associated with PC1, whereas myristoleic, palmitic acids, total SFA, and n-6/n-3 ratio were negatively associated with PC1. Myristic, stearic, and γ-linolenic acids were positively associated with PC2.

The fatty acid composition of the seed citrus oils was also useful for the realization of dendrograms (Fig. 2). For acid lime fatty acids (Fig. 2A), cluster analysis enabled the formation of four groups. The first group comprised linoleic acid; the second group consisted of docosadienoic, arachidic and γ-linolenic acids; the third group involved of myristoleic, cis-7-hexadecenoic, myristic and stearic acids; while the fourth one included α-linolenic, oleic and palmitic acids with dissimilarity of 12.5.

On the other hand, for sweet orange fatty acids (Fig. 2B), cluster analysis enabled the formation of three groups. The first group comprised arachidic, γ-linolenic, cis-7-hexadecenoic and docosadienoic acids; the second group consisted of myristic, myristoleic, stearic and α-linolenic acids; while the third one included linoleic, oleic and palmitic acids with dissimilarity of 16.2.

**Nutritional quality index**

The fatty acid profiles also allowed to evaluate the indexes of nutritional quality of the lipid fraction. Therefore, the atherogenicity (AI) and thrombogenicity (TI) indexes and the hypocholesterolemic and hypercholesterolemic (HH) fatty acid ratios could be determined. According to Turan et al. (2007), low AI and TI values indicate high quantities of anti-atherogenic fatty acids in oil or fat and healthier food. However, a high ratio of hypocholesterolemic (UFA) and hypercholesterolemic fatty acids (SFA) indicates a nutritionally suitable oil (Souza Bentes et al., 2009). Table 1 shows the evaluation of the nutritional quality of the lipid fractions of the acid lime and sweet orange seed oils from conventional (SE) and non-conventional methods (I-EUA and D-UEA), as determined by the AI and TI indexes and the HH ratios.

The AI and TI indexes in acid lime had values ranging from 0.36 – 0.42 and 0.32 – 0.36, respectively, whereas the HH ratios ranged 2.48 – 2.79 without statistically significant differences between the three extraction methods. The AI (0.39 – 0.45) and TI values (0.44 – 0.51) for sweet orange seed oils were similar to those registered for acid lime, whereas the HH ratios (2.29 – 2.65) were also similar to acid lime.

All these values were lower than walnut (AI = 0.61, TI = 0.80), carob (AI = 2.62, TI = 2.13), and lemon (AI = 2.24, TI = 0.97) recently reported (Uysal et al., 2015) using Soxhlet and petroleum ether as solvent during 6 h. Also, in the analysis of *Syagrus oleracea* (Nozaki et al., 2012) were reported higher AI and TI values than in the present study. These researchers registered AI (0.69 and 11.53) and TI (1.32 and 4.82) for pulp and kernel oils, respectively, and HH ratio for pulp oil (1.39) and kernel oil (0.40). However, these values were similar to mulberry (AI = 0.44, TI = 0.19), and pomegranate (AI = 0.43, TI = 0.26). These low ratios reported in the present work could indicate a nutritional acceptability for the all analyzed seed oils.

UI was superior in acid lime seed oil extractions (ranged 130.07 – 132.32) without statistical differences between samples; but with statistically significant differences with respect to sweet orange (ranged 114.46 – 119.89). These values were similar than those described (115 – 135) by Kowalczyk-Pecka et al. (2018) and much higher than those reported by Poerschmann et al. (2004) in which they found values between 4.63 – 5.46 in *Chlamydomonas* sp. Given the relative susceptibility of different membrane lipid- forming fatty acids to oxidative stress, the PI, which is a measure of susceptibility of each membrane to peroxidation, could be calculated. The peroxidation index was highest in acid lime (59.26 – 60.55) than sweet orange fatty acids (46.11 – 49.38) manifested, mainly, in the high significant percentage of α-linolenic

acid possessed by the acid lime oil. Hulbert et al. (2007) have shown that the greater the degree of polyunsaturation of PUFA, the more prone it is to peroxidative damage. These values were lower than those described (65 – 90) in other recent studies (Kowalczyk-Pecka et al., 2018).

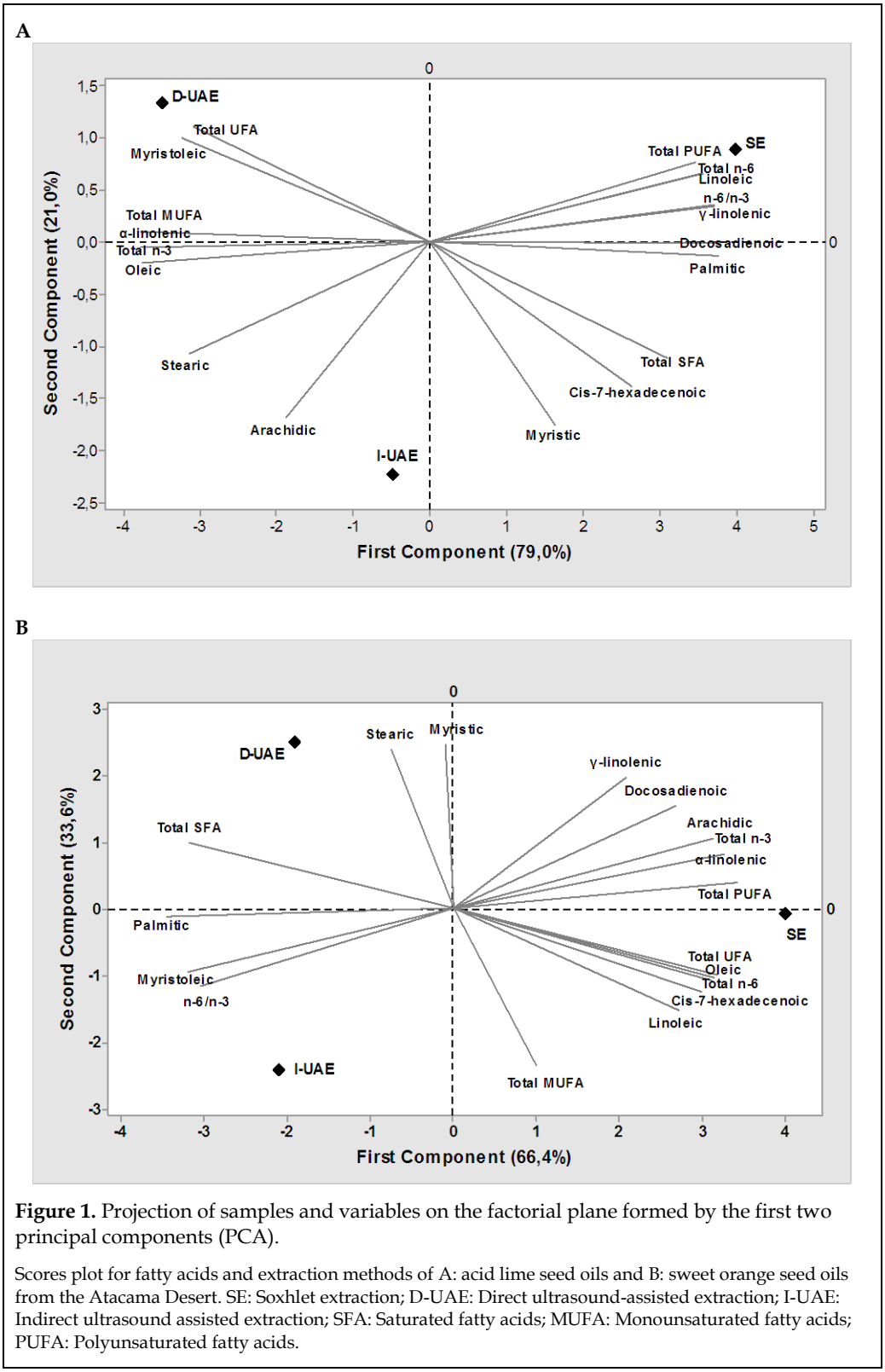


**Figure 1.** Projection of samples and variables on the factorial plane formed by the first two principal components (PCA).

Scores plot for fatty acids and extraction methods of A: acid lime seed oils and B: sweet orange seed oils from the Atacama Desert. SE: Soxhlet extraction; D-UAE: Direct ultrasound-assisted extraction; I-UAE: Indirect ultrasound assisted extraction; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

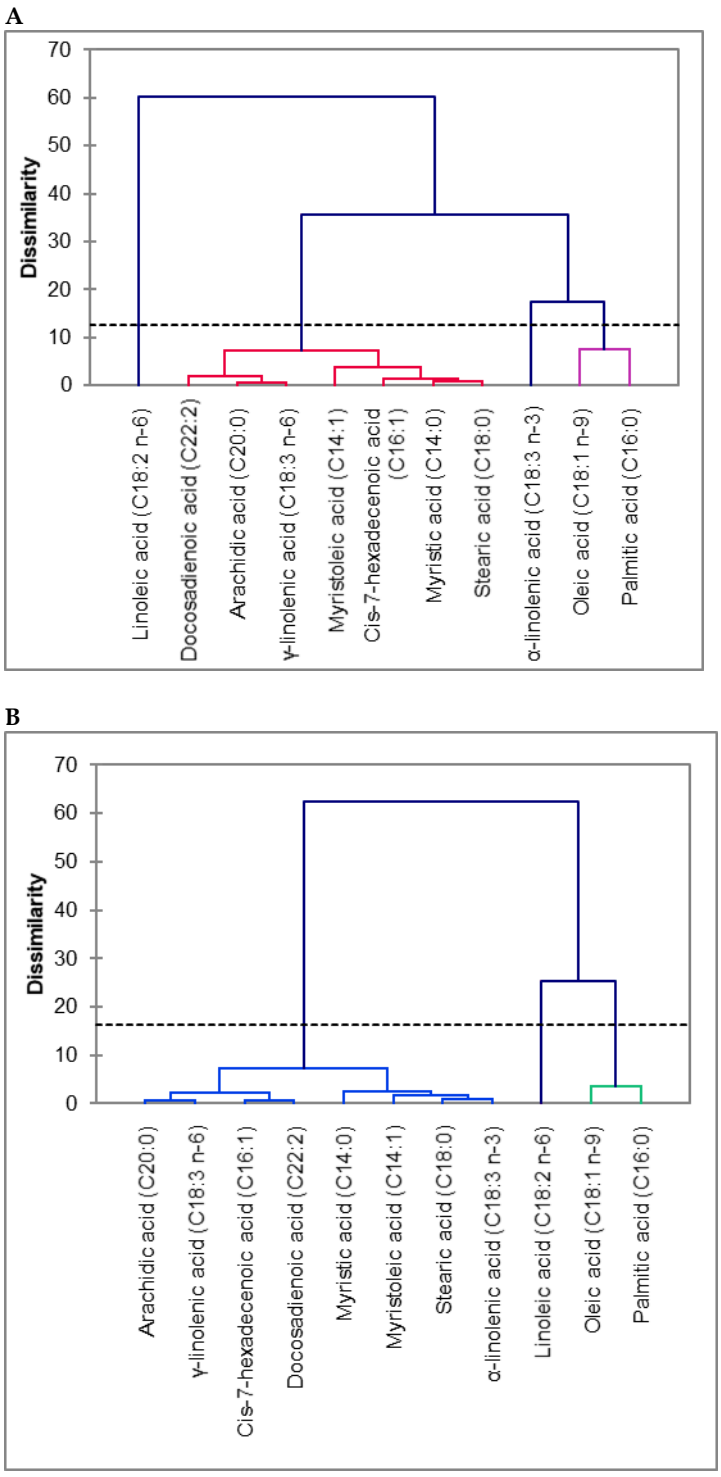


**Figure 2.** Dendrogram obtained by algorithmic hierarchical clustering of 11 fatty acids (Ward Linkage with Squared Euclidean Distance) detected in seed oils of acid lime **(A)** and sweet orange **(B)**.

**Table 2.** Eigen analysis of the correlation matrix loadings of the significant principal components (PCs) of fatty acids of acid lime and sweet orange seed oils from the Atacama Desert.

| Variable | Acid lime | | Sweet orange | |
|---|---|---|---|---|
| | PC1 | PC2 | PC1 | PC2 |
| Arachidic | -0.132 | **-0.446** | **0.261** | 0.175 |
| Cis-7-hexadecenoic | 0.185 | **-0.368** | 0.249 | -0.207 |
| Docosadienoic | **0.265** | -0.002 | 0.224 | 0.257 |
| Linoleic | 0.249 | 0.176 | 0.227 | **-0.253** |
| Myristic | 0.114 | **-0.465** | -0.008 | **0.407** |
| Myristoleic | -0.227 | **0.266** | **-0.267** | -0.155 |
| Oleic | **-0.264** | -0.055 | **0.263** | -0.170 |
| Palmitic | **0.264** | -0.037 | **-0.289** | -0.018 |
| Stearic | -0.221 | **-0.285** | -0.063 | **0.397** |
| α-linolenic | **-0.265** | -0.013 | **0.273** | 0.135 |
| γ-linolenic | **0.261** | 0.094 | 0.174 | **0.325** |
| Total SFA | 0.217 | **-0.294** | **-0.265** | 0.164 |
| Total UFA | -0.217 | **0.294** | **0.265** | -0.164 |
| Total MUFA | **-0.265** | 0.027 | 0.084 | **-0.389** |
| Total PUFA | 0.244 | 0.202 | **0.285** | 0.065 |
| Total n-3 | **-0.265** | -0.013 | **0.273** | 0.135 |
| Total n-6 | **0.250** | 0.173 | **0.260** | -0.179 |
| n-6/n-3 | **0.261** | 0.090 | **-0.255** | -0.193 |
| Eigenvalue | 14.23 | 3.77 | 11.96 | 6.04 |
| Proportion (%) | 79.0 | 21.0 | 66.4 | 33.6 |
| Cumulative (%) | 79.0 | 100 | 66.4 | 100 |

The Cox values of the analyzed citrus oils ranged from 4.84 to 6.28. For comparison Cox value was on the level of seed oils from beech (5.21), black cumin (6.6), grape (7.3), in tomato (6.5), and in wheat germ (7.8), what confirms relatively high resistance to oxidation of citrus seeds oil (Hassanien et al., 2014; Siger et al., 2017). The calculated oxidizability value of citrus oils was higher than for the apricot kernel oil (3.3) examined by Hassanien et al. (2014), kernel oils of *Amygdalus reuteri* (3.3) and *Amygdalus scoparia* (3.2) and extra-virgin olive oil (2.2) described by Tavakoli et al. (2018), *Prunus pedunculatus* seed oil (3.50) learned by Yan et al. (2017), palm oils (1.37), palm super oleins (1.82), and olive oil (1.89) investigated by Rossi et al. (2007), and olive (1.59), palm (1.73), and fish (1.34) oils detected by Nosratpour et al. (2017).

## Total phenolics content

Total phenolics content (TPC) of oils extracted from both *Citrus* seed samples is shown in Fig. 3A.

As shown in Fig. 3A, no significant differences were obtained from the oil extracted from acid lime seeds using the different extraction techniques, ranging from 0.682 to 0.787 mg GAE/g oil. However, significant differences were observed between the various oils extracted from sweet orange seeds, in which the highest phenolics content

was reached using SE (0.932 ± 0.071 mg GAE/g oil) and I-UAE (0.876 ± 0.087 mg GAE/g oil).

Furthermore, phenolics content in oil extracted by I-UAE was higher than those extracted by D-UAE, with the significant difference between sweet orange seed oils, as shown in (Fig. 3A). These results could be attributed to the possible degradation of phenolic compounds by the application of ultrasound (Entezari and Pétrier, 2005; Bremner et al., 2011; Qiao et al., 2013). The effect of the ultrasound energy during the D-UAE is more powerful than used in I-UAE, as in the first extraction technique, the sample is in direct contact with the source of emission of ultrasound waves and, therefore, the degradation of these compounds could be intensified. Instead, no significant differences were observed between phenolic contents of both types of *Citrus* seeds, according to each extraction technique (Fig. 3A) except for SE in which sweet orange oil had higher TPC (0.932 ± 0.0071 mg GAE/g oil) than acid lime oil (0.724 ± 0.041 mg GAE/g oil).

Phenolic contents obtained in this study for both oils were lower than those reported for Brazilian varieties of sweet orange, lemon (*C. limon*) and tangerine (*C. reticulata* Blanco) using petroleum ether during 6 h by Soxhlet extraction (Malacrida et al., 2012). This difference could be attributed to the type of solvent, the variety and the geographical origin of samples, among others (Maranz and Wiesman, 2004). Phenolic acids may be found among different species of *Citrus* seeds, including *C. sinensis* from Italy and South Africa, such as caffeic, *p*-coumaric, ferulic and sinapinic acids (Bocco et al., 1998) in addition to salicylic acid in a variety of Brazilian orange (da Silva and Jorge, 2017). As well as flavonoids, especially glycosylated flavanones, such as hesperidin, narirutin, naringin, and neohesperidin are reported (Bocco et al., 1998).

**Antioxidant capacity**

All tested seed oils exhibited significant oxygen radical absorbing capacity with ORAC values of 7.07 – 96.39 µmol TE/g at 0.0125 µg/mL for all oils (Fig. 3B). The sweet orange oil extracted by SE had the highest ORAC (96.39 µmol TE/g). The ORAC value of the SE sweet orange oil was about 2.2 times higher than that of the SE acid lime oil (41.99 µmol TE/g) and almost 3.0 times higher than that of the D-UAE sweet orange (32.05 µmol TE/g) and 4.6 times higher than the D-UAE acid lime oil (20.93 µmol TE/g) (Fig. 3B). The current study also determined the ORAC values of I-UAE acid lime and sweet orange to be 33.72 and 7.07 TE µmol/g, respectively (Fig. 3B). These data suggest that these citrus seed oils could become in dietary sources for absorbing oxygen radical components. The ORAC values were correlated to TPC. Only the relationships between TPC of I-UAE for acid lime *versus* TPC values of D-UAE of sweet orange and TPC of I-UAE *versus* ORAC I-UAE for sweet orange seed oils were negatively correlated, while I-UAE of ORAC for acid lime correlated positively for ORAC of D-UAE of sweet orange seed oils (Table 3).

No studies have been found in which the ORAC potential for sweet orange or acid lime seed oils has been determined, so the values presented in this study could be the first reported for the oils of these species.

Results of DPPH scavenging assay of each oil, regarding $EC_{50}$, are shown in Fig. 3C. No significant differences of $EC_{50}$ were obtained with a lower value for acid lime (634.6 g oil/g DPPH) than sweet orange seed oil (844.4 g oil/g DPPH) for SE. $EC_{50}$ results of both types of oils are higher than $EC_{50}$ values obtained from four varieties of Brazilian orange seed oils (Hamlin, Nata, Pera-rio, and Valencia) whose $EC_{50}$ were 37.19, 37.25, 35.08, and 38.31 g oil/g DPPH, respectively (Jorge et al., 2016). Antiradical efficiencies were $1.58 \times 10^{-5}$ for acid lime and $1.18 \times 10^{-5}$ for sweet orange seed oil in SE. These values were lower than those registered for those Brazilian orange seed oils ranged from $2.61 - 2.79 \times 10^{-2}$ (Jorge et al., 2016). Furthermore, a higher percentage of radical inhibition was reached for acid lime (23.06%) than for sweet orange seed oil (16.97%), with no differences for each other. Malacrida et al. (2012) have reported the percentage of inhibition of DPPH displayed by the oil obtained from Brazilian orange (54.20%), lemon (29.25%) and tangerine (25.56%) seeds extracted

with petroleum ether by Soxhlet. These differences can be attributed to the solvent nature, diversity of species and the geographical origin of samples (Maranz and Wiesman, 2004).

On the other hand, $EC_{50}$ results in both types of oils are also lower than $EC_{50}$ values obtained from walnut and peanut seed oils obtained with petroleum ether (after shaken for 20 min and then centrifuged at 2500*g* for 10 min), with 1514.3 and 1395.9 g oil/g DPPH, respectively (Arranz et al., 2008). Therefore, acid lime and sweet orange seed oils have a better antioxidant performance than those nut oils.

In this way, the exploitation of these seeds as sources of vegetable oils supports the reduction of agro-industrial waste in an arid zone as the Atacama Desert.

Otherwise, FRAP results are expressed in Fig. 3D. No significant differences were obtained for SE with a lower value for acid lime (0.273 ± 0.075 mmol TE/mg oil or 0.755 ± 0.012 mmol IT/mg oil) than sweet orange seed oil (0.528 ± 0.088 mmol TE/mg oil or 1.338 ± 0.157 mmol IT/mg oil).

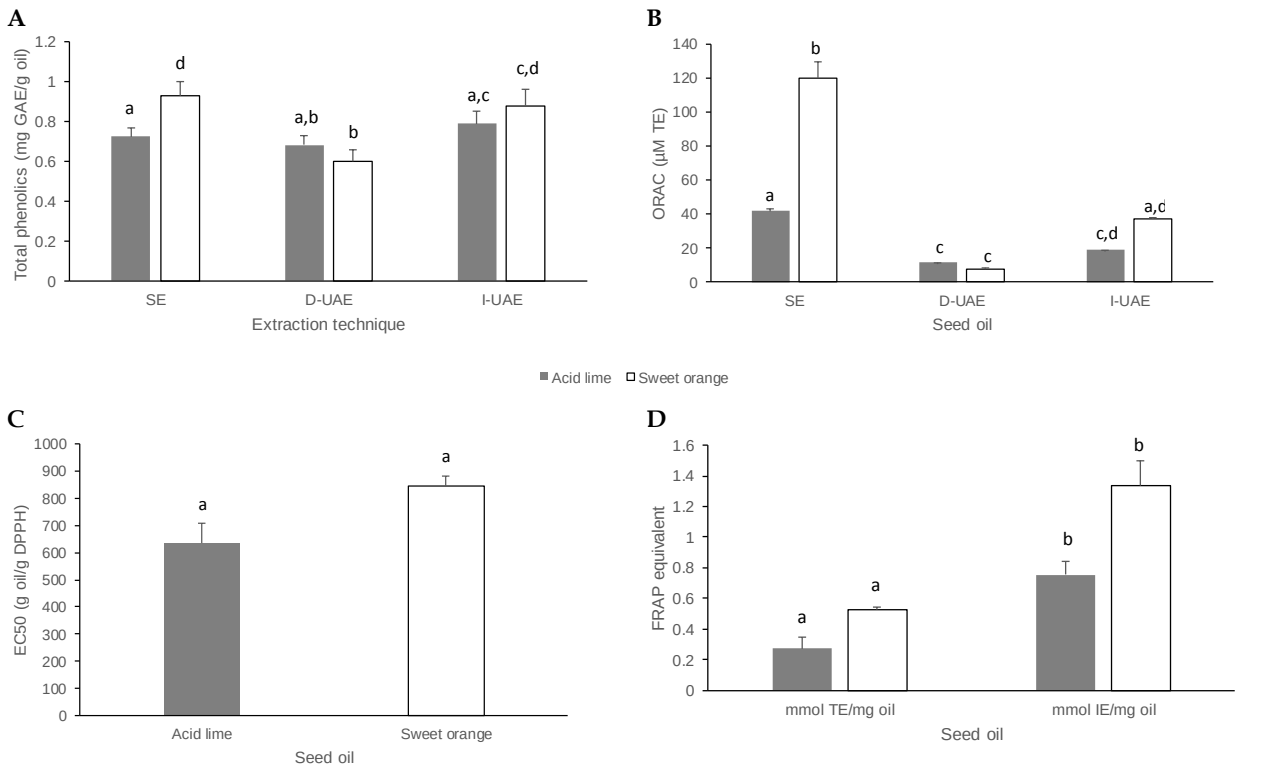


**Figure 3.** Antioxidant properties of the studied seed oils. **(A)** Total phenolics content among the different extractions techniques from Pica acid lime and sweet orange seeds; **(B)** ORAC equivalents in seed oils obtained by Soxhlet and ultrasound-assisted extractions (in ORAC, oil concentrations of 0.0125 µg/mL were tested); **(C)** Antioxidant capacity using DPPH scavenging capacity, in terms of half maximal effective concentration ($EC_{50}$) in Soxhlet extractions; and **(D)** FRAP reaction in seed oils obtained by Soxhlet.

Data from three independent determinations are expressed as mean ± SEM. Results that not sharing the same letters in the same graphic are significantly different by ANOVA followed by a Tukey's test ($p \leq 0.05$). SE: Soxhlet extraction; D-UAE: direct ultrasound-assisted extraction; I-UAE: indirect ultrasound-assisted extraction; TE: Trolox equivalents; IE: iron equivalents; ORAC: Oxygen Radical Absorbance Capacity.

**Table 3.** Pearson correlation used to evaluate the association between total phenolics and ORAC values of the different extraction methods.

| Parameter | L-SE TPC | L-DUAE TPC | L-IUAE TPC | O-SE TPC | O-DUAE TPC | O-IUAE TPC | L-SE ORAC | L-DUAE ORAC | L-IUAE ORAC | O-SE ORAC | O-DUAE ORAC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **L-DUAE TPC** | 0.638 | | | | | | | | | | |
| **L-IUAE TPC** | -0.642 | 0.182 | | | | | | | | | |
| **O-SE TPC** | 0.385 | -0.466 | -0.955 | | | | | | | | |
| **O-DUAE TPC** | 0.595 | -0.240 | **-0.998*** | 0.971 | | | | | | | |
| **O-IUAE TPC** | 0.258 | 0.909 | 0.575 | -0.792 | 0.623 | | | | | | |
| **L-SE ORAC** | 0.891 | 0.219 | -0.920 | 0.762 | 0.895 | -0.208 | | | | | |
| **L-DUAE ORAC** | 0.443 | 0.973 | 0.403 | -0657 | -0.457 | 0.981 | -0.012 | | | | |
| **L-IUAE ORAC** | 0.958 | 0.391 | -0.834 | 0.632 | 0.800 | -0.028 | 0.984 | 0.168 | | | |
| **O-SE ORAC** | 0.027 | 0.787 | 0.750 | -0.913 | -0.788 | 0.973 | -0.430 | 0.908 | -2.60 | | |
| **O-DUAE ORAC** | 0.948 | 0.361 | -0.852 | 0.657 | 0.819 | -0.061 | 0.989 | 0.136 | **0.999*** | -0.292 | |
| **O-IUAE ORAC** | -0.212 | -0.888 | -0.614 | 0.821 | 0.659 | **-0.999*** | 0.255 | -0970 | 0.076 | -0.983 | 0.109 |

L: acid lime; O: sweet orange; SE: Soxhlet extraction; DUAE: direct ultrasound-assisted extraction; IUAE: indirect ultrasound-assisted extraction; TPC: total phenolic compounds; ORAC: Oxygen Radical Absorbance Capacity. *$p < 0.05$.

DPPH scavenging capacity and FRAP results confirm that acid lime and sweet orange seed oils have the ability to capture free radicals and reducing power. This property could be because of the presence of antioxidant compounds, such as phenolics and tocopherols. Tocopherols are lipophilic substances related to vitamin E and are synthesized by many plants (Kamal-Eldin and Appelqvist, 1996). *Citrus* seeds are composed mainly of α-tocopherol (Anwar et al., 2008; Malacrida et al., 2012; Matthaus and Özcan, 2012; da Silva and Jorge, 2017).

## CONCLUSIONS

Results of the current study revealed acid lime and sweet orange seed oils ecotypes Pica to be potential sources of nutrients exhibiting antioxidant capacity. Both types of oil showed detectable levels of unsaturated fatty acids, specifically monounsaturated and polyunsaturated fatty acids (such as oleic, linoleic and α-linolenic acids), with potential benefits to human health. Results for total phenolic content, ORAC values, the percentage of inhibition, $EC_{50}$ and antioxidant efficiency of DPPH values, and FRAP revealed that acid lime and sweet orange seed oils from the Atacama Desert have antioxidant capacity. On the other hand, among the extraction techniques, Soxhlet has had a better result in the oil extraction yield compared to novel methods, such as ultrasound-assisted extraction. Given the little difference found between obtaining individual fatty acids, total phenols and nutritional quality indices, the use of ultrasound-assisted extraction could be suggested as an efficient and energy-saving method for extracting these compounds from citrus seeds. Further other studies are needed to undertake toxicity studies, characterize the phenolic compounds and other antioxidant assays.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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|---|---|---|---|---|---|
| Concepts or ideas | x | x | | x | |
| Design | x | x | | x | |
| Definition of intellectual content | x | x | | | |
| Literature search | x | x | | | x |
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