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Tocopherol yield and physicochemical parameters in yellow prickly pear seed oil (*Opuntia ficus-indica (L.) Mill.*) extracted by Soxhlet and microwave using different solvents

Rendimiento de tocoferoles y parámetros fisicoquímicos en el aceite de semillas de tuna amarilla (*Opuntia ficus-indica (L.) Mill.*) extraído mediante Soxhlet y microondas utilizando diferentes solventes

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ABSTRACT

In this work, the determination of α- and γ-tocopherol in oil extracted from prickly pear seeds (*Opuntia ficus-indica*) was carried out. The extraction was performed using solvent extraction methods (Soxhlet) and microwave-assisted extraction, with alcohol, hexane, methanol, and water as solvents. The tocopherols amount obtained in micrograms was determined to compare the performance of each extraction method, as well as the solvents used. The amounts of α-tocopherol and γ-tocopherol were determined by the HPLC method. In addition, the saponification index was determined to analyze the purity and quality of the oil, with the best value being 167.08 mg KOH/g. The acidity index was used to verify the shelf life of the oil, where the best values are those below 0.05 %. FTIR was used to determine the functional groups present in each sample. The use of ethanol as a solvent in both methods was found to produce a higher yield of γ-tocopherol (266.93 µg/g dw).

KEY WORDS: *Opuntia ficus-indica*, prickly pear, seed oil, extraction method, physicochemical properties, tocopherols content.

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RESUMEN

En este trabajo se realizó la determinación de α y γ- tocoferol en aceite extraído de semillas de tuna (*Opuntia ficus-indica).* La extracción se llevó a cabo por los métodos de extracción con solventes (Soxhlet) y extracción por microondas, empleando alcohol, hexano, metanol y agua como solventes. Se determinó la cantidad en microgramos de tocoferoles obtenidos para comparar el rendimiento en cada método de extracción, así como de los solventes empleados. La cantidad de α-tocoferol y γ- tocoferol fue determinada por el método HPLC. Además, se determinó el índice de saponificación para analizar la pureza y calidad del aceite en el cual el mejor valor fue de 167.08 mg KOH/g, el índice de acidez para verificar el tiempo de anaquel del aceite en donde mejores valores son aquellos que están por debajo del 0.05 % y FTIR para determinar los grupos funcionales presentes en cada una de las muestras. Se encontró que el uso de etanol como solvente en ambos métodos produce un mayor rendimiento de γ-tocoferol (266.93 µg/g dw).

PALABLAS CLAVE: *Opuntia ficus-indica*, tunas, aceite, método de extracción, propiedades fisicoquímicas, contenido de tocoferoles.

Introduction

Opuntia ficus-indica is a well-defined plant that may grow as a creeping, shrubby, or tree-like form, it features leaves modified into spines, which appear as small structures grouped in large numbers, as well as larger structures considered modified leaves; an attribute regarded as a distinctive trait, its tender fruits bear ephemeral leaves (Torres-Ponce *et al*. 2015). The cactus, commonly known as nopal, has cladodes, pads or modified leaves, equipped with areoles or buds from which spines, flowers, or new plants emerge; it also produces a fruit known as tuna, which is ovoid, has a thick epicarp, a juicy and sweet pulp, and contains a large number of small seeds. The pads form its stem or trunk, and its root system is shallow profundidad (Aquino Bolaños *et al*., 2012). Tuna is cultivated primarily in arid and semi-arid regions. 23 edible tuna types are known, including yellow, red, purple, and white varieties, among others; this classification is based on the epicarp coloration (Salehi *et al*., 2018).

Mexico is the world's leading tuna producer, accounting for 45 % of global production. However, despite the high production volume, tuna growers face several challenges, among which the limited 90-day annual harvest window is notable, this short timeframe results in a market saturation that makes it difficult for producers to distribute their entire crop, leading to significant losses due to a lack of adequate refrigerated storage facilities. For this reason, efforts

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are being made to add value to this fruit by expanding its uses, currently, tuna is primarily used in the production of wine, jam, beverages, pulque curing, bread, fruit paste, sweets, and other products, in which only the pulp is used. The seeds, often discarded, are considered waste for such products. This study focuses on finding productive applications for these seeds (Al-Naqeb *et al*., 2021; Ciriminna *et al*., 2019).

The tuna fruit consists of a thick peel (epicarp), a juicy pulp (mesocarp), and hard seeds (Ortega-Ortega *et al*., 2017). Its chemical composition includes approximately 85 % water, 14 % sugars, and 1 % protein; the pulp contains vitamins C and E, polyphenols, essential fatty acids (notably linoleic acid or omega-6), and certain amino acids; meanwhile, the seeds are a source of bioactive compounds such as flavonoids, betalains, essential fatty acids (especially linoleic acid), and most importantly, oil, which, it is widely used in the cosmetic and pharmaceutical industries due to its content of tocopherols (vitamin E) (Ghazi *et al*., 2013; Ramadan & Mörsel, 2003). There are four naturally occurring types of tocopherols: alpha (α), beta (β), gamma (γ), and delta (δ) (Abraham, 2018). α-tocopherol is the most prominent antioxidant, known for its ability to inhibit oxidative reactions by directly neutralizing peroxyl radicals, whereas γ-tocopherol eliminates reactive nitrogen species. Due to these properties, tocopherol-rich oil is widely used in the cosmetic, pharmaceutical, and food industries. Consequently, tuna is recognized as a nutritionally valuable food due to its content of minerals such as calcium, potassium, magnesium, silicon, and iron, among others; additionally, its medicinal properties includes antioxidant, antiviral, anticancer, and cholesterol-lowering effects, support its frequent use in traditional medicine(Torres-Ponce *et al*. 2015).

The extraction of chemical components existing in tuna seeds, as well as the quantity of each compound, is highly dependent on the extraction method employed. Among the available techniques are mechanical extraction, supercritical fluid extraction, maceration, autoclave extraction, ultrasound-assisted extraction, solvent extraction, and microwave-assisted extraction (Al-Naqeb *et al*., 2021; Matthäus & Özcan, 2011).

Solvent extraction is typically performed using a Soxhlet apparatus and involves three main stages, in the first stage, the seeds are separated from the pulp and sun-dried for three days, followed by additional drying in an oven to remove any residual moisture, in the second stage, the oil is extracted under reflux conditions, with the temperature determined by the type of solvent used, in the final stage, the solvent(s) are evaporated (Al-Naqeb *et al*., 2021; Matthäus & Özcan, 2011), this entire process may take between 4 and 6 hours.

In microwave-assisted extraction, water molecules within the seed cells are excited by electromagnetic waves, leading to internal heating and subsequent rupture of the cell membranes. This disruption facilitates the leaching of oils from the seeds into the solvent. This method is notable for significantly reducing extraction time compared to more conventional approaches, such as Soxhlet extraction. However, microwave-assisted extraction presents certain limitations, such as the requirement for thorough mixing of both seeds and solvent. The time and power settings depend on the specific equipment used, and in subsequent stages, separation of the extracted compounds is necessary through techniques such as decantation, filtration, and centrifugation.

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These steps are essential to remove solid residues and to aid in solvent evaporation (Coutiño Laguna *et al*., 2022).

Material and Methods

Sample collection

The seeds were obtained from yellow tunas collected in Otumba town, State of Mexico. Initially, the seeds were separated from the pulp by washing with water. They were then air-dried at room temperature (25 °C) for three days and stored in sealed plastic bags.

Seed quality

The weight of 1,000 to 100 seeds determines physical quality and is used as an agronomic indicator of seed vigor, which contributes to germination, development, growth, and seedling establishment (Rosales-Serna *et al*., 2019).

To determine seed quality, 100 seeds were selected and weighed using a VELAB analytical balance, model UVE-300. Equation 1 was used to calculate the thousand-seed weight (Ávila-Serrano *et al*., 2010).

$$Thousand-seed\ weight: \frac{Sample\ weight}{number\ of\ seeds\ in\ sample} x1000 \quad Equation\ 1$$

Moisture content determination

Moisture content was determined in duplicate. For this purpose, crucibles containing 5 g of seeds each were placed in a Memmert U55 oven at 120°C for 24 hours (Nielsen, 2017) to remove all residual moisture. After drying, the crucibles were transferred to a desiccator to cool to room temperature before being weighed.

Moisture content was calculated using Equation 2 (Nielsen, 2017):

$$\%Moisture = \frac{(Pc+PM)-PF}{PM} X100 \qquad Equation\ 2$$

Where:

PM = Weight of the sample

PF = Constant weight

Pc = Weight of the crucible

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Ash content determination

Ash content was quantified according to the NMX-F-607-NORMEX-2020 standard. The analysis was performed in duplicate, using 5 g of previously ground tuna seeds per crucible. The crucibles were initially placed over a Bunsen burner until the samples were fully carbonized.

The crucibles containing the carbonized samples were then placed in a muffle furnace (TERLAB model TE-M12D) at 550 °C for 2 hours. After the heating period, they were transferred to a desiccator and cooled to room temperature (25 °C). Ash content was calculated using Equation 3:

$$\%Ash = \frac{(P-p)}{M} x100 \qquad Equation\ 3$$

Where:

P= Mass of crucible with ash (g)

p= Mass of empty crucible (g)

M= Mass of the sample (g)

Oil extraction

The seeds were dried at 80 °C for 25 minutes in a Memmert U55 oven, then ground until a homogeneous powder was obtained.

Soxhlet extraction

For oil Soxhlet extraction, four samples of 20 g of ground seed were packed into cartridges. Each extraction used 130 mL of a different solvent: ethanol (E-S), methanol (Met-S), hexane (H-S), or distilled water (A-S). Each extraction is labeled according to the solvent and extraction method used.

The system was run under reflux for 5 hours. After cooling to room temperature, a simple distillation was performed for each extract.

Microwave-assisted extraction

Microwave extractions were performed using a WINIA microwave oven, model KOR-667DG. Unlike the Soxhlet method, the ground seeds and solvent were mixed in a round-bottom flask. The same solvent types and volumes were used as in the previous method. Each extraction

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was performed in a separate flask for 1 hour at 60 W of power. To minimize solvent loss, Liebig condensers were attached to reduce the system temperature.

Once the time had elapsed, the equipment was disassembled, and the solid particles were filtered from the sample. It was then centrifuged in a VELAB VE-4000 centrifuge at 500 rpm for 30 min to settle any residual particles that might have remained in the sample.

Finally, the excess was placed in a round-bottom flask, and a simple distillation was performed. As with the previous method, each extraction is differentiated by the abbreviation of the solvent and the extraction method.

Water degumming

Degumming was performed to remove any remaining organic particles and solvent residues from the oil samples. Each sample was placed in a beaker, heated with agitation at 75 °C and 500 rpm, and 2 % distilled water (relative to the oil volume) was added. This condition was maintained for 1 hour. Afterward, the samples were centrifuged in a Velab VE-4000 centrifuge at 1000 rpm for 30 minutes (Stroppiano & Comba, 2016).

After degumming, the samples were stored in 10 mL amber glass vials, wrapped in aluminum foil to prevent degradation, and kept refrigerated until analysis.

Oil yield

Oil yield was determined using Equation 4 (Condori & Palomino, 2022), where P_p is the weight (g) of the degummed oil extract, and P_m is the weight (g) of the dry plant material used.

$$Yield = \frac{P_p}{P_m} x100 \qquad Equation\ 4$$

Relative density

To determine the oil's relative density, a 10 mL graduated cylinder was used, and the mass was measured on a VELAB VE-300 analytical balance. The cylinder was first filled with distilled water, avoiding bubble formation, and submerged in a water bath at 20 °C for 30 minutes, with temperature monitored using a thermometer. After the specified time, the mass was recorded.

The same procedure was repeated using the oil extract, with the cylinder previously washed with ethanol. After adding the oil, also avoiding bubbles, the sample was kept at 20 °C in a water bath for 30 minutes, and its mass was recorded.

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Relative density was calculated according to the NMX-F-075-SCFI-2012 standard using Equation 5:

$$\rho = \frac{G_1}{G_2} \quad Equation\ 5$$

Where:

$G_1 = M_1 - M$

$G_2 = M_2 - M$

M_1= Mass of cylinder with oil

M_2= Mass of cylinder with distilled water

M= Mass of empty cylinder

Determination of the saponification index

A round-bottom flask containing 0.2 g of oil was mixed with 2.5 mL of 0.5 M alcoholic KOH solution. The mixture was refluxed gently for 1 hour, then cooled. Subsequently, 100 µL of phenolphthalein solution (1 %) was added, and the mixture was titrated with 0.5 N HCl (Nielsen, 2017).

The saponification index was calculated using Equation 6:

$$SI = \frac{(B - M) * N * 56.1}{g\ of\ sample} \quad Equation\ 6$$

Where:

B= mL of HCl used in the blank

M= mL of HCl used in the sample

N= Normality of HCl solution

Determination of the acidity Index

The acidity index was determined following the NMX-F-101-SCFI-2012 standard and Nielsen (2017). A 0.2 g oil sample was weighed into a 25 mL Erlenmeyer flask, and 4 mL of neutralized 95 % ethanol and 80 µL of 1 % alcoholic phenolphthalein solution were added. The

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mixture was placed on a heating plate at 15 °C for 20 minutes, then gently stirred and titrated with 0.1 N NaOH solution (Nielsen, 2017).

Acidity was calculated using Equation 7:

$$\%AGL = \frac{VxNx0.282}{pm} \quad Equation\ 7$$

Where:

V= Volume of NaOH used (mL)

N= Normality (0.1 N)

pm= Sample mass (g)

Tocopherol determination

Simultaneous quantification of α- and γ-tocopherol was performed using a high-performance liquid chromatography (HPLC) system (Hewlett-Packard HP-1100) equipped with a Waters Spherisorb column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of methanol (19 %), acetonitrile (75 %), and hexane:dichloromethane (1:1, v/v) (6 %), with a flow rate of 1.5 mL min^{-1} for 30 minutes. The diode array detector (DAD, Hewlett-Packard HP-1100) was set at λ = 300 nm.

To identify and quantify α- and γ-tocopherol, calibration curves were constructed using standard solutions of pure (+)-α-tocopherol and (+)-γ-tocopherol (Sigma Co.) at concentrations of 0.0015, 0.003, 0.006, 0.0125, and 0.025 mg mL^{-1} in HPLC-grade hexane:dichloromethane (1:1, v/v). The regression equations are presented in Table 1.

Table 1. Average retention time, linear regression equation, and correlation coefficient for α- and γ-tocopherol contents.

Concept	γ-tocopherol	α-tocopherol
Linear regression	y= 6.6896 x + 0.1817	y= 234.3129 x + 1.6177
Correlation coefficient	0.99	0.99
Retention time (min)	6.7	10.2

y = Area under the curve in milliunit of absorbance for minute (mUA min^{-1}); x = mg of tocopherol for milliliter (mg mL^{-1}).

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Infrared Analysis (FT-IR)

The absorption spectra of the oils in the infrared region were obtained using an FTIR Spectrometer (PerkinElmer), with a wavelength range of 400–4000 cm^{-1} and a resolution of 4 cm^{-1}, using Attenuated Total Reflectance (ATR). The samples were analyzed directly after extraction without any pretreatment.

Results and Discussion

Seed quality

The physical quality of the seeds was determined to be 2.39 g/100 seeds and 23.89 g/1000 seeds, according to the literature, greater seed weight is associated with higher levels of α- and γ-tocopherol, as these measurements reflect seed vigor. Lower values than those obtained in this study would suggest reduced seed quality, which could imply that the crop did not meet optimal growing conditions for a full harvest, this may be attributed to seasonal climate variability, as tuna crops are seasonal (Ávila-Serrano *et al*., 2010; Rosales-Serna *et al*., 2019).

Moisture determination

The moisture content indicates the amount of water the seeds contain. This is important because it indicates the permissible limits of water content required to maintain a healthy seed during storage. Excess moisture content can lead to deterioration through the development of fungi, bacteria, or insect infestations.

According to Willan (1991), the moisture content for small seeds, such as *Picea* or *Alnus*, should be below 1 2 %, with a tolerance margin of ± 0.3 %. As shown in Table 2, the moisture content of the tuna seed samples was 6 %, with a standard deviation of 6.55 ± 0.21 % and a coefficient of variation of 0.03 %. These values fall below the recommended maximum moisture levels.

Given this moisture content, tuna seeds can be stored using various methods. However, it is recommended to keep them in cool, dark places in sealed containers or bags to preserve their quality (Hernández & Carballo, 2017).

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Table 2. Moisture determination.

	Initial weight (g)	Sample weight (g)	Total, weight (g)	Weight 1 (g)	Weight 2 (g)	Final weight 24h (g)	Moisture %
Sample 1	25.0477	5.0095	30.0572	29.7624	29.7346	29.7359	6.4
Sample 2	24.0591	5.0083	29.0674	28.7568	28.7301	28.7293	6.7

Ash content determination

The ash percentage indicates the mineral content present in the inorganic residue of a carbonized sample, which in turn can be used to assess seed quality and storage potential.

The samples showed ash values ranging from 14 % to 15 %, with a standard deviation of 15.20 ± 0.43 % and a coefficient of variation of 0.02 %, exceeding the values reported by Moreno (2008), which ranged from 2.16 % to 0.15 %.

Based on these results, it can be inferred that tuna cultivated in the Otumba municipality, State of Mexico, contains a higher amount of organic matter compared to the levels reported by Moreno Álvarez *et al*. (2008). This elevated organic content may suggest that the oil extracted from these seeds is more prone to oxidation, potentially shortening its shelf life (Moreno Álvarez *et al*., 2008).

Table 3. Ash content determination.

	Constant weignt of the crucible (g)	**Sample weight (g)**	**Crucible weight with carbonized simple (g)**	**Carbonized simple weight (g)**	**Final weight for the crucible with sample(g)**	**Results (%)**
Crucible 1	38.583	5g	41.061	2.478	39.3599	15.51
Crucible 2	45.523	5g	48.530	3.007	46.2686	14.90

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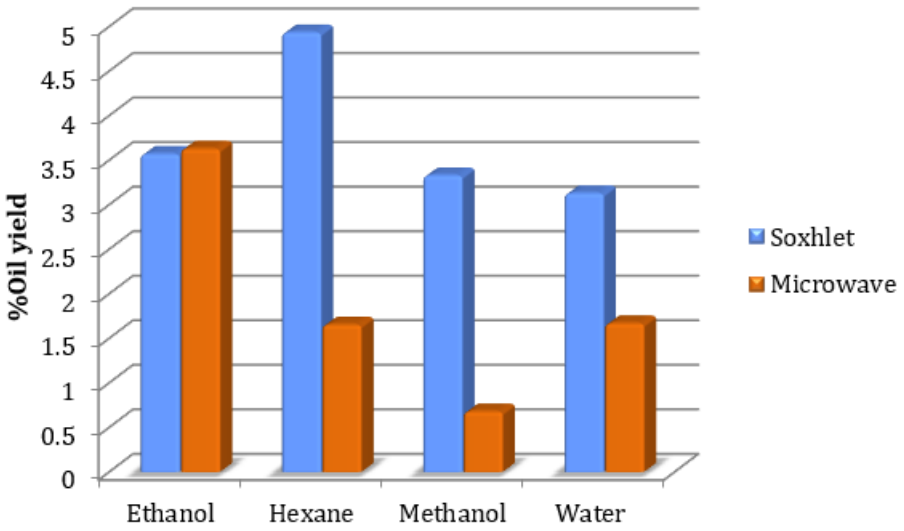

Figure 1. Oil yield obtained by the Microwave and Soxhlet extraction methods.

Figure 1 presents the oil extraction yields from tuna seeds using Soxhlet and microwave-assisted methods. The highest yield was obtained using hexane as the solvent in the Soxhlet extraction process, reaching 4.93 %, while the lowest yield was recorded with methanol, at 0.68 %. The standard deviation was 2.82 ± 1.37 %, with a coefficient of variation of 0.4 %.

In the case of methanol combined with microwave-assisted extraction, the observed yield may be attributed to the release of intracellular lipids into the solvent, as polar compounds align with the direction of the electric field and rotate rapidly, generating heat and causing cellular rupture (Kumar *et al*., 2021).

The yield achieved with hexane in Soxhlet extraction was significantly lower than that reported by Al-Naqeb *et al*. (2021), who obtained yields ranging from 11.75 % to 5.4 % in seeds from both cultivated and wild plants. These authors suggest that oil yield is mainly influenced by two factors: the cultivation conditions of the tuna plant and the extraction method used. Agronomic factors such as geographic origin, harvest timing, and fruit ripeness directly impact the quantity and quality of oil in the seeds. Additionally, the extraction method, reflected in the variability of yields across solvents and techniques, plays a critical role in process efficiency (Al-Naqeb *et al*., 2021; Mena-Rodríguez *et al*., 2017).

Microwave-assisted extraction efficiency largely depends on the dielectric susceptibility of both the solvent and the solid sample matrix. Consequently, polar solvents (such as water, methanol, or dimethyl sulfoxide) are more suitable for this method than non-polar solvents like chloroform or hexane (Khot *et al*., 2020).

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Therefore, the low yields observed in this study may be explained by a combination of agronomic and technical factors. Crop management practices, including fertilization, irrigation, and pest and disease control, could have influenced the quality and quantity of the oils in the tuna seeds used. Moreover, variability inherent to the extraction process, such as solvent type and operational conditions, may have also contributed to the reduced yields, suggesting that both plant genetics and processing conditions must be optimized to improve extraction efficiency.

Relative density

Table 4 shows the density values of the oil samples, all of which were lower than the density of water (0.998 g/cm^3). According to the CODEX STAN 33-1981, the relative density for edible oils should range between 0.910 and 0.916 g/cm^3; all measured values in this study fell below this range, indicating that the oils are insoluble in water. The standard deviation was 0.05 ± 0.02 g/cm^3, with a coefficient of variation of 0.49 %.

Density values can serve as indicators of oil quality, as reported by Paucar-Menacho *et al*. (2015), in the case of Sacha Inchi oil, which contains components similar to those found in tuna oil, lower density is associated with better cold storage stability and higher overall quality. Less dense oils tend to be lighter and have a lower melting point compared to other vegetable oils (Paucar-Menacho *et al*., 2015).

Table 4. Sample density.

Empty graduated cylinder (g)	Graduated cylinder with sample (g)	Graduated cylinder with water (g)	ρ (g/cm^3)
26.727	27.441	36.659	0.0718
26.227	27.214	36.021	0.1007
26.893	27.559	36.617	0.0684
27.563	28.189	37.756	0.0614
27.661	28.387	37.704	0.0722
26.310	26.641	36.303	0.0331
26.893	27.029	36.782	0.0137
26.225	26.559	35.913	0.0344

Saponification index

The saponification index refers to the number of milligrams of KOH required to saponify one gram of fat, including the presence of triglycerides, diglycerides, free fatty acids, and any other lipids present in the sample. This index serves as an indicator of oil purity.

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According to the NMX-F-475-SCFI-2017 standard, the minimum acceptable value is 182 mg KOH/g. As shown in Table 4, only the samples labeled H-M and Met-M exhibited the highest saponification values, although they still did not reach the threshold set by the NMX-F-475-SCFI-2017 standard. The standard deviation was 103.28 ± 57.84 mg KOH/g, with a coefficient of variation of 0.56 %. These results suggest that the oils analyzed had lower purity, potentially due to the temperature and reaction time during processing, as well as the presence of impurities.

The Codex Alimentarius standard for olive and olive pomace oils establishes an acceptable range for the saponification index between 182 and 196 mg KOH/g (Comisión del Codex Alimentarius, 2024), thus, values below this range may indicate a higher proportion of long-chain fatty acids in the oil, which is inversely related to its purity (Chatterjea & Shinde, 2012).

Table 5. Saponification value.

Sample	Weight (g)	Titration volumen HCl (ml)	**Saponification value (mgKOH/g)**
E-S	0.2	2.0	140.25
H-S	0.2	2.0	140.25
Met-S	0.2	2.5	70.125
W-S	0.2	3.4	56.1
E-M	0.2	2.9	14.025
H-M	0.2	1.8	168.30
Met-M	0.2	2.2	167.08
W-M	0.2	2.5	70.125

Acidity index

The presence of free fatty acids in oils can lead to undesirable changes, commonly referred to as rancidity, this chemical feature is a critical parameter for assessing oil quality. As shown in Table 6, higher acidity index values indicate lower oil quality, as such oils are more susceptible to oxidative rancidity over time.

According to the NMX-F-808-SCFI-2018 standard, the acidity value should not exceed 0.05 %. Of the eight oil samples analyzed, the highest quality was observed in those with free fatty acid values ranging between 0.005 % and 0.008 %. Lower levels of free fatty acids correspond to higher oil quality. The standard deviation of the results was 0.014 ± 0.008 %, with a coefficient of variation of 0.5 %. Based on the values reported in Table 6, the samples labeled E-M, H-M, and Met-M exhibited the lowest levels of free fatty acids. This suggests that microwave-assisted

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extraction better preserves the triglyceride integrity in the oil, resulting in higher-quality samples with greater resistance to rancidity.

Table 6. Free Fatty Acid Value.

Sample	Weight (g)	Titration volumen NaOH (mL)	Free fatty acid value (%)
E-S	0.2	1.4	0.019
H-S	0.2	1.2	0.016
Met-S	0.2	1.8	0.025
W-S	0.2	0.6	0.008
E-M	0.2	0.5	0.007
H-M	0.2	0.4	0.005
Met-M	0.2	1.7	0.023
W-M	0.2	0.4	0.005

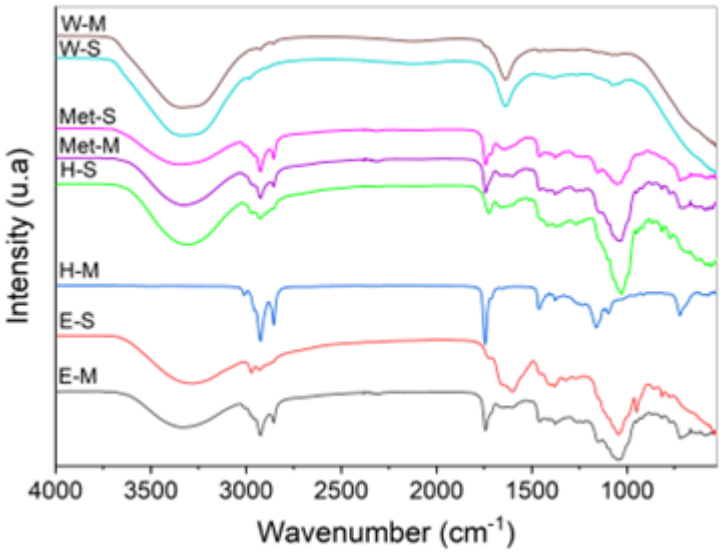

Figure 2. Spectrum FT-IR for the samples.

In Figure 2, characteristic absorption bands between 3200 and 3000 cm^{-1} correspond to O-H stretching vibrations, indicative of phenolic compounds such as tocopherols. The signals at 1750 cm^{-1} and 1725 cm^{-1} suggest the presence of carbonyl (C=O) functional groups, typically associated with ester bonds in triglycerides. Peaks observed between 1400 and 1000 cm^{-1} are

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attributed to C-O stretching, while the region from 3000 to 2800 cm^{-1} corresponds to C-H stretching and fatty acid unsaturation (Mondragón, 2020), a relevant feature of vegetable oils rich in bioactive compounds. The intensity of these bands, particularly in the samples extracted using microwave-assisted methods with hexane and ethanol, suggests a higher efficiency in recovering lipophilic compounds, including tocopherols. The pronounced presence of γ-tocopherol may therefore be attributed both to the biosynthetic profile of the seed and to the effectiveness of the extraction method in preserving phenolic compounds that are sensitive to thermal conditions.

Additionally, the characteristic absorption band of C=C stretching was observed near 1650 cm^{-1}, indicating the presence of double bonds in unsaturated fatty acids. Signals at 1565 cm^{-1} and 1520 cm^{-1} were also detected, corresponding to amide II vibrations, typical of peptide bonds in proteins (Mondragón, 2020).

Tocopherols

High-performance liquid chromatography (HPLC) equipped with a diode array detector (DAD) is one of the most widely used techniques for separating and analyzing chemical components in complex mixtures (Suarez Ospina & Morales Hernández, 2018).

Tables 7 and 8 present the quantified amounts (in µg) of α- and γ-tocopherol for each sample, based on the solvent used.

The highest concentrations correspond to γ-tocopherol, with the highest value of 266.93 µg found in the oil extracted through microwave using ethanol as the solvent, while the second highest γ-tocopherol concentration was 207.26 µg, obtained through Soxhlet extraction with ethanol. The standard deviation was 63.21 ± 108.59 µg, with a coefficient of variation of 1.71 %. Regarding α-tocopherol, the highest concentration was 0.4106 µg in the sample obtained via Soxhlet extraction with hexane, followed by 0.2966 µg from the microwave extraction using methanol. The standard deviation was 0.10 ± 0.15 µg, with a coefficient of variation of 1.4 %.

Gharby *et al*. (2020) reported that γ-tocopherol is the predominant form in prickly pear seed oil, a finding corroborated in the present study through quantitative tocopherol analysis. The dominance of γ-tocopherol over α-tocopherol may be attributed to genetic traits of the plant species and specific biosynthetic pathways in the seeds. Tocopherols are lipophilic phenolic compounds that differ in the number and position of methyl groups on the chromanol ring: γ-tocopherol contains methyl groups at positions 7 and 8, while α-tocopherol has them at positions 5, 7, and 8. The biosynthetic pathway favoring γ-tocopherol is more common in less-domesticated plant species, whereas α-tocopherol typically predominates in cultivars selected for higher vitamin E activity. Therefore, the higher abundance of γ-tocopherol in prickly pear seed oil may reflect a characteristic metabolic profile of this species, which has not undergone genetic improvement focused on α-tocopherol accumulation.

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Table 7. Quantities in µg of α-tocopherols.

α-tocopherol			
Sample	Retention-Time	Area (mAU*s)	Amount (µg)
E-S	4.335	4.54621	0.01872
H-S	4.607	50.24949	0.41064
Met-S	4.55	1.34916	0.00555
W-S	4.544	4.21013	0.01734
E-M	4.499	13.40111	0.04881
H-M	4.624	16.33055	0.07758
Met-M	4.475	38.64079	0.29665
W-M	4.549	0.52681	0.00217

Table 8. Quantities in µg of γ-tocopherols.

γ-tocopherol			
Sample	Retention-Time	Area (mAU*s)	Amount (µg)
E-S	4.004	851.39191	207.26736
H-S	3.99	12.20809	2.9845
Met-S	4.063	48.47993	11.81417
W-S	4.078	6.24009	1.5317
E-M	4.027	1096.49927	266.93394
H-M	4.024	50.63538	12.33887
Met-M	4.03	4.12517	1.01687
W-M	4.073	7.58088	1.85809

Conclusions

The analyses conducted in this study indicate that moisture and ash content in the seeds significantly influence the quality of the extracted oil, particularly with respect to its chemical characteristics relevant for storage and use. These parameters appear to affect the concentration of tocopherols in the oil extractions. Consequently, it can be concluded that oil yield is closely dependent on crop management practices, which may explain the relatively low yields observed.

The saponification index reflects oil quality and purity; hence, higher values correspond to greater purity, the best results were obtained with hexane (168.3 mg KOH/g) and methanol (167.08 mg KOH/g), both using microwave-assisted extraction. Regarding the acidity index, lower

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values indicate higher oil quality. According to NMX-F-808-SCFI-2018, the maximum acceptable value is 0.05 %, with no specified minimum. All obtained values in this study fell below the established maximum. The most favorable results were observed with ethanol (0.007 %), hexane (0.005 %), and water (0.005 %), all using microwave extraction.

HPLC analysis revealed that all oils contained higher levels of γ-tocopherol, with the highest concentration being 266.93 µg in the sample extracted via microwave using ethanol. The highest α-tocopherol content was 0.29 µg, also obtained through microwave-assisted extraction. Therefore, we suggest that oil extraction using microwaves is better than the Soxhlet method, since considering the values of all the chemical analyses performed on the oil extractions, it can be seen that the best values are obtained from extractions performed with microwaves, which is why we consider this extraction method to be much more effective in obtaining good quality prickly pear seed oil.

FTIR spectra supported these findings: samples with more intense absorption bands at ~2800 cm^{-1}, attributed to lipids, suggest a higher presence of tocopherols, which are lipophilic. This confirms that higher lipid content correlates with greater tocopherol concentration. The best-performing oil samples were obtained using microwave-assisted extraction with hexane and ethanol, consistent with the HPLC results.

In conclusion, among the two extraction methods evaluated, microwave-assisted extraction, particularly using ethanol and hexane as solvents, proved to be the most efficient for producing tocopherol-rich prickly pear seed oil. Moreover, microwave extraction (1 h) is substantially faster than Soxhlet extraction (5 h), offering a more time-effective and potentially scalable method for oil recovery.

Author contributions

Zarazua-Aguilar: Conceptualization, Investigation, Writing, and Review. Vega-Franco: Methodology, Investigation, Writing, and Original Draft. Escobar-Cisneros: Conceptualization, Writing, Review, and Editing, Visualization. San Miguel-Chavez: Conceptualization, Writing, Review and Editing, Visualization. Piña-Victoria: Review and Editing.

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Ethical Statements

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Informed consent statement

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Conflict of interest

The authors declare no conflict of interest.

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