Sustainable lipid extraction from Mexican *Opuntia ficus-indica* seeds

Gloria Itzel Peña-Vázquez[1,2]; Sayra Nayely Serrano-Sandoval[1,2]; José Rodríguez-Rodríguez[3]; Marilena Antunes-Ricardo[1,2]; Daniel Guajardo-Flores[1]

[1]Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Centro de Biotecnología FEMSA, Av. Eugenio Garza Sada 2501 Sur, Monterrey, NL 64849, Mexico
[2]Tecnologico de Monterrey. Institute for Obesity Research, Monterrey, Av. Eugenio Garza Sada 2501 Sur, 64849 Monterrey, NL, Mexico, *A00839639@tec.mx, sayra.serrano@tec.mx, marilena.antunes@tec.mx*
[3]Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Av. Eugenio Garza Sada 2501 Sur, Monterrey, NL 64849, Mexi*co, jrr@tec.mx, danielgdo@tec.mx*

Abstract*– Opuntia ficus-indica (OFI) seeds are a promising source of bioactive lipids. Subcritical and supercritical fluid extraction using CO_2 (SCE-CO_2 and SFE-CO_2) as an extraction solvent offer a greener alternative by reducing volatile organic solvents (e.g. n-hexane) without compromising efficiency and quality of extraction compared to conventional method. Lipid extraction of two different OFI cultivars (Villanueva and Rojo Vigor) was performed with conventional and sustainable methods. Yield, fatty acid profile, and phytosterol content were analyzed through GC-MS to assess the efficiency of SCE-CO_2 and SFE-CO_2 against the maceration method. The results showed SCE-CO_2 y SFE-CO_2 improved fatty acid purity, particularly in Villanueva cultivar, where oleic and linoleic acids, indicating that CO_2-based extraction methods, especially SCE in Villanueva provide the most refined lipid profile. These findings highlight the potential of CO_2-based extraction processes as an environmentally friendly alternative to extract high-quality OFI lipids.

Keywords*– Opuntia ficus-indica, subcritical CO_2 extraction, supercritical fluid extraction, sitosterol, fatty acids.

I. Introduction

Opuntia ficus-indica (OFI) seeds are often discarded despite being a valuable source of bioactive lipids with potential functional applications [1]. Conventional extraction methods, such as hexane maceration, have been widely used to obtain oils but pose challenges in efficiency and environmental impact due to the toxic volatile organic solvent use [2]. In contrast, subcritical and supercritical fluid extraction with CO_2 (SCE-CO_2 and SFE-CO_2) offer a sustainable and economically feasible alternative by reducing toxic solvent use and improving selectivity and lipid quality [3], [4]. Moreover, applying enzymatic pretreatments with cellulolytic enzymes can also enhance seed oil extraction by degradation of the cell wall [5], [6]

This study aimed to compare conventional extraction with SCE-CO_2 and SFE-CO_2 in terms of oil yield, purity of fatty acids, and lipid composition (fatty acids and phytosterols) of oils extracted from OFI seeds, including the effect of enzymatic pretreatment to promote greener extraction techniques.

II. Methods

A. Plant material

Villanueva and Rojo Vigor OFI cultivars were purchased from agro-producers "La Flor de Villanueva, Tuna y Nopal" (Puebla, Mexico) from July to September 2023. Fruits were washed with tap water and peeled. Seeds were extracted with a conventional strainer to remove pulp, then seeds were freeze-dried for 10 days at 0.40 mBar at -49°C. The dried sample was ground and passed through a sieve to obtain flour with a particle size of 400 µm.

B. Conventional oil extraction

Maceration was conducted as a conventional method using *n*-hexane as an extraction solvent [7]. In this process, OFI seed powder was mixed with *n*-hexane (50 g/L) and stirred (250 rpm) for 24 hours at 40°C in an orbital shaker. After maceration, the samples were centrifuged at 10,000×*g* for 10 minutes. The remaining solvent was evaporated under nitrogen to obtain OFI seed oils. Samples were stored at -80°C until further evaluations.

C. Sustainable oil extractions

SCE-CO_2 and SFE-CO_2 were performed according to previous studies [8], [9]. For these extractions, OFI seed flours (50g) were added to a 1L extraction vessel with a 500 mL cyclone heater in an SFE Bio-Botanical Extraction System (Waters Corporation, MA, USA).

The extraction conditions included a pressure of 300 bar and a flow rate of 80 g CO_2/minute for 120 minutes at two different temperatures: 30 °C for the SCE-CO_2 (SCE) and 60°C for the SCE-CO_2 method (SFE). The processes were repeated with pretreated samples for 24 hours (200rpm, 40°C) with a combination of cellulases and xylanases (300 and 50 U/g, respectively) using Cellic CTec 2 by Novozymes (CPH, Denmark), denoting the treatments as PT-SCE and PT-SFE. Finally, extracted OFI oils were concentrated under nitrogen. The yield (%) and density (mg/mL) were measured, and samples were stored at -80°C until further evaluations.

D. Fatty acid methyl esters obtention

The transesterification process was carried out on the OFI oils to produce fatty acid methyl esters (FAMEs) [10]. In brief, 40 mg of each OFI seed oil was mixed with 1 mL of glyceryl triundecanoate (4000 mg/L toluene), which served as an internal standard. Next, 2 mL of a 7% sulfuric acid solution was added to the samples and incubated at 80°C for 60 minutes. After incubation, the samples were allowed to cool at room temperature (25°C) for 10 minutes. To isolate the organic phase, 4 mL of n-hexane was added and thoroughly mixed in each sample. The organic phase was then transferred to a new vial, and this step was repeated. The final total volume was adjusted to 10 mL with *n*-hexane.

E. Fatty acid profile determination by GC-MS

The identification and quantification of the FAMEs [10] from the OFI seed oil samples were performed using a gas chromatograph (Clarus 690 GC, Perkin Elmer, Massachusetts, USA) coupled with a Mass Spectrometry detector (GC-MS) (Clarus SQ 8T, Perkin Elmer, MA, USA). A J&W HP-88 GC column (100 m × 0.25 mm × 0.2 µm) (Agilent, CA, USA) was employed for this process. The analysis conditions involved injecting a 1 µL of the sample, setting the GC oven temperature at 90°C for 5 minutes, followed by a temperature increase of 10°C per minute until reaching 250°C for 15 minutes, where it was held for 15 minutes. The GC-MS system featured an electron impact ion source and a quadrupole analyzer operating at 70 eV, with a transfer temperature of 210 °C and a mass range of *m/z* 30-500 Daltons (Da). Data processing was carried out using TurboMass GC/MS software, and compound identification was based on spectral comparison with the library of the National Institute of Standards and Technology (NIST) database, considering the quality match threshold above 90%. The purity of each fatty acid was calculated as follows:

$$\textbf{Purification factor} = \frac{\textbf{Fatty acid } (\frac{mg}{g})}{\textbf{Total yield of the corresponding oil } (\%)} \quad (1)$$

F. Sitosterol determination by GC-MS

OFI oils (20 mg) were diluted in 5 mL of hexane (80%) containing a cholesterol standard at 150 ppm to identify and quantify sitosterol in both cultivars using GC-MS [11]. The sample was analyzed under specific GC oven condition, starting at 190°C for 1 minute, the increasing at a rate of 15°C per minute until reaching 300°C, where it was held for 15 minutes. The detector temperature was set at 170 °C. A HP 5MS column (30 m × 0.25 mm × 0.25 µm) (Agilent, CA, USA) was used for this analysis. Peak integration and data processing were performed using TurboMass GC/MS software. Identification was confirmed through the retention times and mass spectral matching with the NIST database with a quality match above 90%.

G. Statistical analysis

Results are presented as the mean ± standard deviation (SD). Statistical analysis was performed with Minitab Statistical Software version 21.4.2.0 (PA, USA). A two-factor analysis of variance (ANOVA) was conducted, followed by Tukey's *post hoc* comparison test. A significance level of $p<0.05$ was considered statistically significant.

III. Results and Discussion

The density of OFI seed (cv. Rojo Vigor and Villanueva) oil showed no significant difference between conventional and sustainable extraction methods (ranging from 0.88 to 1.17 g/mL, data not shown). Despite the similar densities, OFI Rojo Vigor oil extracted with sustainable methodologies, especially SFE-CO_2, showed the highest oil yield compared to the maceration method, and SCE-CO_2 showed to be the most efficient in OFI Villanueva regarding the yield (Table I), reducing the use of harmful solvents which was observed in other OFI cultivars [8], [12], [13]. Enzymatic pretreatment significantly reduced the extraction efficiency, decreasing over 70% compared to maceration. This could be attributed to the degradation or release of essential compounds (polysaccharides, proteins, sugars) due to enzyme concentration, which may have hindered oil recovery in the extraction process [14], [15].

TABLE I.
Yield (%) of OFI seed oil extracted through conventional and sustainable CO_2-based methodologies.

Extraction method	Yield (%)	
	OFI Villanueva	OFI Rojo Vigor
Maceration	5.38 ± 0.34^{b}	8.00 ± 0.31^{b}
SCE	8.38 ± 0.45^{a}	$9.50 \pm 0.19^{a,b}$
PT-SCE	$3.78 \pm 0.24^{b,*}$	2.11 ± 0.31^{c}
SFE	8.44 ± 1.47^{a}	10.76 ± 0.80^{a}
PT-SCE	0.85 ± 0.04^{c}	1.07 ± 0.13^{c}

Results are expressed as mean percentage ± SD. Different superscript lowercase letters (^{abc}) represent significant differences between oil extraction yield among extraction methods within the same cultivar (OFI Villanueva or OFI Rojo Vigor). OFI, *Opuntia ficus-indica*; SCE, Subcritical Extraction; SFE, Supercritical Fluid Extraction; PT, pretreated.

The fatty acid composition of OFI seed oils from Villanueva and Rojo Vigor is detailed in Table II. Sustainable CO_2-based methodologies significantly improved the purity and yield of fatty acids. Five fatty acids were identified in all samples, being stearic acid and oleic acid *trans*-isomer the minor components (3.78 to 5.07 %). However, the enzymatic pretreatment significantly influenced the fatty acid profile, where a 91.60 - 93.63% reduction was observed in both cultivars compared to non-pretreated samples. A saturated fatty acid, palmitic acid, was higher in SCE and SFE samples than in

macerated samples from both cultivars. In contrast, pretreated samples led to a significant reduction, showing approximately an 85% decrease compared to non-treated samples. These results are in accordance with other American, Asian, and European cultivars using different extraction solvents and methodologies [8], [12], [16], [17], [18].

Oleic acid, a monounsaturated fatty acid, was identified from 13.80-14.18% in all oils extracted with CO_2-based methodologies and over 11.75% in macerated samples. Conversely, other cultivars showed lower oleic acid content after supercritical conditions [18]. A higher purification factor was also observed in non-pretreated samples from OFI Villanueva. However, over a 90% reduction was found in all pretreated samples. Lastly, linoleic acid (LA), a polyunsaturated fatty acid, was the most abundant component, ranging from 62.03 – 67.46% of the total extracted lipids. LA showed the highest purification factor (5.47) in SCE OFI Villanueva samples, indicating an efficient recovery. Comparable contents have also been reported in Turkish and Mexican cultivars extracted with petroleum ether, hexane, and ethyl acetate [12], [19], [20]. Likewise, similar concentrations of these fatty acids have also been reported in Tunisian and Yemen, and Italian cultivars when extracted with SFE-CO_2 [21], [22]. These findings suggest that sustainable CO_2-based extraction methods outperform conventional maceration by proving to be efficient in obtaining seed oils with enhanced fatty acid yield and purity. This highlights the effectiveness of green extraction techniques in preserving lipid quality while minimizing environmental impact.

TABLE II.
Fatty acid composition (mg/g of OFI seed) and purification factor (PF) of OFI seed oil extracted through conventional and sustainable CO_2-based methodologies.

Cultivar	Extraction	Fatty acids (mg/g of OFI seed) **(PF)**					
		Stearic acid	*Trans*-oleic acid	Palmitic acid	Oleic acid	Linoleic acid	Total FA
OFI Villanueva	Maceration	0.31 ± 0.01^{c} **(0.06)**	0.41 ± 0.01^{c} **(0.08)**	1.12 ± 0.02^{c} **(0.21)**	$1.04 \pm 0.03^{c,d}$ **(0.19)**	$5.97 \pm 0.12^{c,d}$ **(1.11)**	$8.85 \pm 0.18^{c,d,e}$ **(1.64)**
	SCE	2.76 ± 0.18^{a} **(0.33)**	3.61 ± 0.14^{a} **(0.43)**	9.38 ± 0.23^{a} **(1.12)**	9.86 ± 0.24^{a} **(1.18)**	45.86 ± 1.29^{a} **(5.47)**	71.47 ± 2.08^{a} **(8.53)**
	PT-SCE	1.06 ± 0.13^{b} **(0.28)**	1.45 ± 0.24^{b} **(0.38)**	3.78 ± 0.40^{b} **(1.00)**	4.04 ± 0.38^{b} **(1.07)**	18.12 ± 1.69^{b} **(4.79)**	28.45 ± 2.84^{b} **(7.53)**
	SFE	2.70 ± 0.10^{a} **(0.32)**	3.44 ± 0.10^{a} **(0.41)**	9.22 ± 0.24^{a} **(1.09)**	9.76 ± 0.26^{a} **(1.16)**	44.66 ± 0.94^{a} **(5.29)**	69.79 ± 1.65^{a} **(8.27)**
	PT-SFE	0.18 ± 0.01^{c} **(0.21)**	0.23 ± 0.01^{c} **(0.27)**	0.66 ± 0.02^{c} **(0.78)**	0.67 ± 0.02^{d} **(0.79)**	3.04 ± 0.08^{d} **(3.58)**	4.78 ± 0.14^{e} **(5.62)**
OFI Rojo Vigor	Maceration	$0.59 \pm 0.02^{b,c}$ **(0.07)**	$0.82 \pm 0.03^{b,c}$ **(0.10)**	2.11 ± 0.04^{c} **(0.26)**	2.38 ± 0.05^{c} **(0.30)**	11.23 ± 0.56^{c} **(1.40)**	17.13 ± 0.67^{c} **(2.14)**
	SCE	2.50 ± 0.03^{a} **(0.26)**	3.46 ± 0.03^{a} **(0.36)**	9.73 ± 0.13^{a} **(1.02)**	9.58 ± 0.18^{a} **(1.01)**	42.27 ± 0.82^{a} **(4.45)**	42.27 ± 0.82^{a} **(7.11)**
	PT-SCE	0.54 ± 0.03^{c} **(0.26)**	$0.83 \pm 0.07^{b,c}$ **(0.39)**	2.06 ± 0.21^{c} **(0.98)**	$2.17 \pm 0.12^{c,d}$ **(1.03)**	9.78 ± 0.60^{c} **(4.64)**	$15.39 \pm 0.61^{c,d}$ **(7.29)**
	SFE	2.72 ± 0.31^{a} **(0.25)**	3.83 ± 0.47^{a} **(0.36)**	10.60 ± 1.07^{a} **(0.99)**	10.38 ± 1.09^{a} **(0.96)**	46.19 ± 4.20^{a} **(4.29)**	73.72 ± 7.13^{a} **(6.85)**
	PT-SFE	0.21 ± 0.01^{c} **(0.20)**	0.28 ± 0.02^{c} **(0.26)**	0.86 ± 0.06^{c} **(0.80)**	0.79 ± 0.06^{d} **(0.74)**	3.48 ± 0.22^{d} **(3.25)**	$5.61 \pm 0.36^{d,e}$ **(5.24)**

Results are presented as mean percentage ± SD. Different superscript lowercase letters ($^{a-e}$) indicate statistical differences between cultivars and each fatty acid identified using different extraction methods (OFI Villanueva versus OFI Rojo Vigor). The purification factor is indicated in parenthesis below each fatty acid. OFI, *Opuntia ficus-indica*; SCE, Subcritical Extraction; SFE, Supercritical Fluid Extraction; PT, pretreated; PF, purification factor; FA, fatty acids.

Regarding phytosterol content in OFI seed oils (Table III), an isomer of sitosterol (γ-sitosterol) was identified in all samples. These findings differ from previous studies that have reported a broader variety of phytosterols [23], [24], [25]. This discrepancy may be attributed to sample oxidation due to storage, temperature, and pressure of sustainable extractions or temperature that compromised the stability of these compounds [26], [27], [28].

Sitosterol levels were notably higher in oils extracted through CO_2-based methodologies than in maceration and pretreated samples (p<0.05). SFE from OFI Villanueva showed the highest sitosterol content, nearly threefold, relative to the macerated sample. Similar to yield and fatty acid content, enzymatic pretreatment led to a significant decrease of over 54% in PT-SCE and over 90% in PT-SFE samples, with a similar trend in OFI Rojo Vigor. This behavior has been reported in other food matrices when enzymatic pretreatment was employed, indicating that this process may negatively impact phytosterol concentrations [29].

Nonetheless, the high efficiency of CO_2 based methodologies in sitosterol extraction underscores their potential as a more sustainable method over conventional methods. These methodologies enable higher fatty acid extraction and facilitate the recovery of phytosterols, which could contribute to the obtention of functional oils, supporting the principles of circular economy and sustainability.

TABLE III.
Sitosterol (mg/kg of OFI seed) of OFI seed oil extracted through conventional and sustainable CO_2-based methodologies.

Extraction method	Sitosterol (mg/kg of OFI seed)	
	OFI Villanueva	OFI Rojo Vigor
Maceration	697.90 ± 16.95^{c}	912.95 ± 41.70^{c,*}
SCE	993.34 ± 40.86^{b}	1262.78 ± 3.51^{b}
PT-SCE	456.31 ± 11.21^{d,*}	414.77 ± 6.64^{d}
SFE	1928.19 ± 3.33^{a,*}	1096.55 ± 0.90^{a}
PT-SFE	189.37 ± 0.77^{e,*}	143.98 ± 0.35^{e}

Results are presented as mean percentage ± SD. Different superscript lowercase letters ($^{a-e}$) indicate significant differences in sitosterol among extraction methods within the same cultivar (OFI Villanueva or OFI Rojo Vigor). *Represent the highest sitosterol of the same extraction method between cultivars (OFI Villanueva vs OFI Rojo Vigor). OFI, *Opuntia ficus-indica*; SCE, Subcritical Extraction; SFE, Supercritical Fluid Extraction; PT, pretreated.

IV. Conclusions

Subcritical and supercritical fluid extraction with CO_2 are effective methods for extracting OFI seed oils, providing superior oleic and linoleic acid purity compared to the conventional maceration method. These methodologies showed the highest oil yield, fatty acid, and phytosterol recovery, while enzymatic pretreatment did not improve extraction efficiency. These results contribute to understanding sustainable lipid extraction methodologies, highlighting CO_2-based methods as promising alternatives for obtaining these high-quality oils with bioactive compounds.

Acknowledgment

The authors thank Secretaria de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI) for G. I. P.-V. (CVU 860261) scholarship.

References

[1] M. A. Silva *et al.*, "Opuntia ficus-indica (L.) Mill.: A Multi-Benefit Potential to Be Exploited," *Molecules*, vol. 26, no. 4, p. 951, Feb. 2021, doi: 10.3390/molecules26040951.

[2] C. P. Mungwari, C. K. King'ondu, P. Sigauke, and B. A. Obadele, "Conventional and modern techniques for bioactive compounds recovery from plants: Review," *Sci Afr*, vol. 27, p. e02509, Mar. 2025, doi: 10.1016/j.sciaf.2024.e02509.

[3] P. A. Uwineza and A. Waśkiewicz, "Recent Advances in Supercritical Fluid Extraction of Natural Bioactive Compounds from Natural Plant Materials," *Molecules*, vol. 25, no. 17, p. 3847, Aug. 2020, doi: 10.3390/molecules25173847.

[4] P. Pao-la-or, B. Marungsri, K. Posridee, R. Oonsivilai, and A. Oonsivilai, "Supercritical CO2 Extraction of Seed Oil from Psophocarpus tetragonolobus (L.) DC.: Optimization of Operating Conditions through Response Surface Methodology and Probabilistic Neural Network," *Processes*, vol. 11, no. 7, p. 1949, Jun. 2023, doi: 10.3390/pr11071949.

[5] C. P. Passos, R. M. Silva, F. A. Da Silva, M. A. Coimbra, and C. M. Silva, "Enhancement of the supercritical fluid extraction of grape seed oil by using enzymatically pre-treated seed," *J Supercrit Fluids*, vol. 48, no. 3, pp. 225–229, Apr. 2009, doi: 10.1016/j.supflu.2008.11.001.

[6] L. Wang *et al.*, "Effects of different proteases enzymatic extraction on the lipid yield and quality of Antarctic krill oil," *Food Sci Nutr*, vol. 7, no. 7, pp. 2224–2230, Jul. 2019, doi: 10.1002/fsn3.1017.

[7] W.-K. Tan *et al.*, "Supercritical carbon dioxide extraction of pomegranate peel-seed mixture: Yield and modelling," *J Food Eng*, vol. 301, p. 110550, Jul. 2021, doi: 10.1016/j.jfoodeng.2021.110550.

[8] G. Al-Naqeb *et al.*, "Supercritical Fluid Extraction of Oils from Cactus Opuntia ficus-indica L. and Opuntia dillenii Seeds," *Foods*, vol. 12, no. 3, p. 618, Feb. 2023, doi: 10.3390/foods12030618.

[9] L. dos Santos Freitas, J. V. de Oliveira, C. Dariva, R. A. Jacques, and E. B. Caramão, "Extraction of Grape Seed Oil Using Compressed Carbon Dioxide and Propane: Extraction Yields and Characterization of Free Glycerol Compounds," *J Agric Food Chem*, vol. 56, no. 8, pp. 2558–2564, Apr. 2008, doi: 10.1021/jf0732096.

[10] G. Latimer, *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th ed. Gaithersburg: AOAC INTERNATIONAL, 2005.

[11] J. Rodríguez-Rodríguez *et al.*, "Optimización de un método para determinación de fitoesteroles y escualeno en ácidos grasos destilados usando la metodología de superficie de respuesta y su validación," *Revista mexicana de ciencias farmacéuticas*, vol. 45, no. 4, 2014.

[12] E. Ramírez-Moreno *et al.*, "Antioxidant and Antimicrobial Properties of Cactus Pear (*Opuntia*) Seed Oils," *J Food Qual*, vol. 2017, pp. 1–8, 2017, doi: 10.1155/2017/3075907.

[13] M. J. H. Akanda, M. Z. I. Sarker, S. Ferdosh, M. Y. A. Manap, N. N. N. Ab Rahman, and M. O. Ab Kadir, "Applications of Supercritical Fluid Extraction (SFE) of Palm Oil and Oil from Natural Sources," *Molecules*, vol. 17, no. 2, pp. 1764–1794, Feb. 2012, doi: 10.3390/molecules17021764.

[14] S. P. J. Kumar, S. R. Prasad, R. Banerjee, D. K. Agarwal, K. S. Kulkarni, and K. V. Ramesh, "Green solvents and technologies for oil extraction from oilseeds," *Chem Cent J*, vol. 11, no. 1, p. 9, Dec. 2017, doi: 10.1186/s13065-017-0238-8.

[15] S. S. Nadar, P. Rao, and V. K. Rathod, "Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review," *Food Research International*, vol. 108, pp. 309–330, Jun. 2018, doi: 10.1016/j.foodres.2018.03.006.

[16] R. Abdnim *et al.*, "Fatty acids characterisation by GC-MS, antiglycation effect at multiple stages and protection of erythrocytes cells from oxidative damage induced by glycation of albumin of Opuntia ficus-indica (L.) Mill seed oil cultivated in Eastern Morocco: Experimental and computational approaches," *J Ethnopharmacol*, vol. 329, p. 118106, Jul. 2024, doi: 10.1016/j.jep.2024.118106.

[17] A. Albergamo *et al.*, "Chemical Characterization of Different Products from the Tunisian Opuntia ficus-indica (L.) Mill.," *Foods*, vol. 11, no. 2, p. 155, Jan. 2022, doi: 10.3390/foods11020155.

[18] N. Yeddes, J. K. Chérif, A. Jrad, D. Barth, and M. Trabelsi-Ayadi, "Supercritical SC-CO_2 and Soxhlet *n* -Hexane Extract of Tunisian *Opuntia ficus indica* Seeds and Fatty Acids Analysis," *J Lipids*, vol. 2012, pp. 1–6, 2012, doi: 10.1155/2012/914693.

[19] N. Chougui *et al.*, "Oil composition and characterisation of phenolic compounds of Opuntia ficus-indica seeds," *Food Chem*, vol. 139, no. 1–4, pp. 796–803, Aug. 2013, doi: 10.1016/j.foodchem.2013.01.054.

[20] F. Al Juhaimi *et al.*, "The effect of harvest times on bioactive properties and fatty acid compositions of prickly pear (Opuntia ficus-barbarica A. Berger) fruits," *Food Chem*, vol. 303, p. 125387, Jan. 2020, doi: 10.1016/j.foodchem.2019.125387.

[21] W. Liu *et al.*, "Supercritical carbon dioxide extraction of seed oil from Opuntia dillenii Haw. and its antioxidant activity," *Food Chem*, vol. 114, no. 1, pp. 334–339, May 2009, doi: 10.1016/j.foodchem.2008.09.049.

[22] G. Al-Naqeb, L. Fiori, M. Ciolli, and E. Aprea, "Prickly Pear Seed Oil Extraction, Chemical Characterization and Potential Health

Benefits," *Molecules*, vol. 26, no. 16, p. 5018, Aug. 2021, doi: 10.3390/molecules26165018.

[23] A. S. Alqurashi, L. M. Al Masoudi, H. Hamdi, and A. Abu Zaid, "Chemical Composition and Antioxidant, Antiviral, Antifungal, Antibacterial and Anticancer Potentials of Opuntia ficus-indica Seed Oil," *Molecules*, vol. 27, no. 17, p. 5453, Aug. 2022, doi: 10.3390/molecules27175453.

[24] M. Rehioui *et al.*, "Corrosion inhibiting effect of a green formulation based on Opuntia Dillenii seed oil for iron in acid rain solution," *Heliyon*, vol. 7, no. 4, p. e06674, Apr. 2021, doi: 10.1016/j.heliyon.2021.e06674.

[25] H. Bouchab, A. Ishaq, Y. Limami, G. Saretzki, B. Nasser, and R. El Kebbaj, "Antioxidant Effects of Cactus Seed Oil against Iron-Induced Oxidative Stress in Mouse Liver, Brain and Kidney," *Molecules*, vol. 29, no. 18, p. 4463, Sep. 2024, doi: 10.3390/molecules29184463.

[26] P. Jafarian Asl, R. Niazmand, and F. Yahyavi, "Extraction of phytosterols and tocopherols from rapeseed oil waste by supercritical CO2 plus co-solvent: A comparison with conventional solvent extraction," *Heliyon*, vol. 6, no. 3, p. e03592, Mar. 2020, doi: 10.1016/j.heliyon.2020.e03592.

[27] Md. S. Uddin *et al.*, "Phytosterols and their extraction from various plant matrices using supercritical carbon dioxide: a review," *J Sci Food Agric*, vol. 95, no. 7, pp. 1385–1394, May 2015, doi: 10.1002/jsfa.6833.

[28] C. A. S. Almeida, S. R. Baggio, L. R. B. Mariutti, and N. Bragagnolo, "One-step rapid extraction of phytosterols from vegetable oils," *Food Research International*, vol. 130, p. 108891, Apr. 2020, doi: 10.1016/j.foodres.2019.108891.

[29] T. Kaseke, U. L. Opara, and O. A. Fawole, "Effects of Enzymatic Pretreatment of Seeds on the Physicochemical Properties, Bioactive Compounds, and Antioxidant Activity of Pomegranate Seed Oil," *Molecules*, vol. 26, no. 15, p. 4575, Jul. 2021, doi: 10.3390/molecules26154575.