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CHEMICAL CHARACTERISTICS AND COMPOSITIONS OF PRICKLY PEAR SEEDS OILS EXTRACTED BY TWO DIFFERENT METHODS

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ABSTRACT

The present work was undertaken to compare the physico-chemical characteristics, fatty acid and sterol compositions as well as the triglyceride composition of *Opuntia ficus indica* seed oils extracted using two different methods: cold pressing and Soxhlet extraction. The results showed that the prickly pear seeds (PPS) were (on a dry weight basis) : water 6.63%, ash 1.1%, oil 8.64%, and protein 9.18%. PPS were also a good source of K, and Mg. Solvent extraction had a significantly ($p < 0.05$) higher oil yield compared to cold pressing. The main fatty acids in PPS oils were linoleic (58.04% , 57.90 %) and oleic (26.29 % , 25.96 % in solvent-extracted and cold pressed oil, respectively. Fatty acid and sterol composition were not affected by the extraction method. The peroxide index and free acidity of the solvent-extracted oil was significantly higher ($p < 0.05$) than that of the pressed oil.

1.Introduction

The prickly pear (*Opuntia ficus-indica L.*) belongs to the Cactaceae family and has spread widely throughout the world. It was introduced into North Africa around the end of the 16 th century (Arba, 2009). About 1500 species of cacti belong to the genus Opuntia and are distributed mainly in South America, Australia, Africa, including the Mediterranean area (Ammar *et al.*, 2014). Prickly pear cactus (*Opuntia ficus-indica*) is extensively cultivated in Tunisia. Actually, about 100.000 ha have been devoted to prickly pear cultivation in Tunisia, spread over Sahel, Kasserine and Kairouan regions (Motri*et al.*, 2022). Currently, the prickly pear is of great interest not only for its ecological roles but also for its nutrtional, pharmaceutical and cosmetic potential (Barba *et al.*, 2017).The literature reports promising information concerning biology, chemical composition, cultivation and applications of the different parts of this plant (Moudene*t al.*, 2016; Paiva *et al.*, 2019; El Azizi *et al.*, 2019; Jouini*et al.*, 2021). The main studies on Opuntia fruits have been the chemical analysis of pulp, skin and seeds and use of the pulp in juice production (Albergamo*et al.*, 2022). The seeds make up about 10-15% of edible pulp and are usually discarded as waste after pulp extraction. Prickly pear seeds showed to contain polysaccharides, cellulose and hemicelluloses (Al-Naqeb*et al.*, 2021). The extracted oil constitutes 0.5-15.5 % of the total seed weight (Regalado et al., 2018). The composition of prickly pear seed oil and its chemical characteristics have been studied (Albergamo*et al.*, 2022;Al-Naqeb*et al.*, 2021). Cactus pear seed oil has been viewed as an important vegetable oil, it is rich in polyunsaturated fatty acids, linoleic acid was established as a major fatty acid in seed oils. Significant levels of carotenoids and γ-tocopherols, which can be used as antioxidants

(Loizzo et al., 2019) and sterols were also found in this oil.

Two different methods are commonly used for the production of oils : pressing and solvent (Soxhlet method). The Soxhlet method generally gives the highest oil yield but can have adverse effects on oil quality (Mechqoqa et al., 2021). The cold pressing procedure is desired because it does not use heat or chemical treatments to obtain natural edible petroleum products. However, Regalado-Rentería et al. (2018) reported that this approach has some disadvantages, such as low productivity and difficulty generating a consistent quality result. There is a scarcity of information on comparative studies of *Opuntia ficus-indica* oils extracted using both cold pressing and solvent extraction methods. The aim of this study was to examine the physicochemical properties of *Opuntia ficus-indica* seeds extracted using two different methods: cold pressing and the Soxhlet extraction system. The physicochemical characteristics assessed included oil yield, fatty acid composition, refractive index, density, peroxide value, free fatty acids, triglyceride composition, as well as sterols.

2.Methods

2.1.Plant material

The fruits of *Opuntia ficus indica*species were harvested at maturity in the region of Knais, (Sousse, Tunisia) at 35.68 m altitude. The fruits are washed under running water and air-dried in the dark and peeled by hand. The seeds are separated from the pulp using a manual cooking grinder then they are washed several times with distilled water and dried in an oven in the dark at 35°C.

2.2.Weight and size

The weight of the seeds was evaluated, randomly, on one hundred seeds. Ten differents samples were used for the size determination of PPS. With the Vernier, the length and diameter were measured and expressed in centimeters (Lassoued et al., 2021).

2.3.Proximate chemical analysis

The moisture content of prickly pear seeds was evaluated using AOAC (2000) techniques. The ash content was determined according to the AOAC(1990) method. Crude protein content was estimated by Kjeldahl (N × 6.25).

2.4.Mineral contents

Ca, K, Mg, and Na were quantified using atomic absorption spectrophotometry. Sample were incinerated and then dissolved with two milliliters of H_2O_2. Concentration standards of 0.5 – 25.0 μgmL^{-1} were used to determine the minerals quantitatively (AOAC, 1984).

2.5.Extraction techniques of prickly pear seed oils

The oil was extracted with two extraction methods:

- Solvent extraction : The seeds were ground in a grinder (Fritsch International, Germany). The oil was extracted in a Soxhlet with hexane at 68°C for 8 hours. A vacuum rotary evaporator was used to remove the solvent with little heating (40°C). The recovered oil was kept at a temperature of - 20°C.. The recovered oil was stored at -20°C.
- Extraction by cold pressing : a SMIR screw press was used. The recovered oil was decanted and then stored at -20°C.

2.6.Oil yield

The oil yield of each extraction method was calculated as follows :

$$Yield(\%) = \frac{Mass\ of\ oil\ extracted\ (g)}{Mass\ of\ PPS\ (g)} \times 100 (1)$$

2.7.Physicochemical parameters

An Abbe refractometer with a temperature adjustment was used to determine the refractive index of oil samples (model G manufactured by Carl-Zeiss, Germany). A densimeter PAAR DMA 45 was used to measure density at 20 °C (Chempro, Paar, Graz, Austria). Official procedures were used to establish the value of peroxide and to quantify free fatty acids (AOAC, 1997).

2.8.Fatty acid composition

The total fatty acid composition of the PPSoils, determined by conversion oil to fatty acid methyl esters (FAME), was achieved according to the NF T60-233, and NF T60-234 method. 30 mg of lipids were diluted in 3 ml of sodium methoxide (0.05 M) containing three or four drops of phenolphthalein. After a 10-minute reflux, 3 mL of methanol/acetyl chloride (1:1, v/v) were added to the mixture. The mixture was then cooled at room temperature after another 10 minutes of refluxing.10 ml of ultrapure water and 6 ml of hexane were added. The upper organic phase containing FAMEs was finally recovered. The methyl esters were then analyzed by gas chromatography (Agilent, HP 6890 series) with a flame ionization detector equipped with a capillary column packed with a stationary phase: Agilent CP-Sil 8850, 50 m × 0.25 mm internal diameter × 0.2 mm film thickness). Operating conditions were as follows: helium as the carrier gas was at a flow rate of 1.5 mL/min and injection volume was 1 mL.The injector temperature was kept at 250°C, while the oven temperature was kept at 165°C and gradually increased to 210°C at a rate of 2°C/min. By comparing the peaks area and retention times of the fatty acid methyl esters to those of the pure standard FAME, the peaks were classified and quantified. The percentage of total detected fatty acids (g/100 g total fatty acid) was used to calculate the results.

2.9.Triglyceride Composition

The triacylglycerol (TAG) profile was determined using reverse-phase high-performance liquid chromatography equipped with a refractive index detector. The separation of triglycerides was carried out using a hypersil ODS column (250 × 4 mm) with a particle size of 5 μm and were eluted from the column with an acetonitrile-acetone mixture (65/35, v/v) at a flow rate of 0.8 mL/min. The peaks were identified by comparison with standard TAG peak retention times and retention times observed in chromatographs of other vegetable oils such as rapeseed oil, soybean oil, and olive oil. The peak areas produced by the data integrator were used for quantification.

2.10.Sterols analysis

Separation of sterol was performed according to the NF ISO 12228 method. After adding 1 mg of FLUKA cholesterol as an internal standard and a few antibumping granules, 250 mg of PPS oil was refluxed for 15 minutes with 5 mL ethanolic KOH solution (3 %, w/v). 5 mL ethanol was added to the mixture right away. At a flow rate of 2 mL/min, the unsaponifiable component was eluted through a glass column packed with a slurry of 260 aluminum oxide (Scharlau) in ethanol (1:2, w/v) with 5 mL ethanol and 30 mL diethyl ether. The extract was evaporated at 40°C under reduced pressure in a rotary evaporator, and the ether was entirely evaporated under nitrogen steam. A FLUKA silica gel F254 plate was created in the solvent solution n-hexane–diethyl ether (1:1, v/v) for sterol characterisation. The thin-layer plate was sprayed with methanol to detect sterols; the sterol bands were scraped off the plate and recovered using diethyl ether extraction. After that, the extract was evaporated in a rotary evaporator and nitrogen was added. The trimethylsilyl ether sterols (TMS) derivatives were prepared by adding 100 μl of a silylant reagent N-methyl-N-(trimethylsilyl) rifluoroacetamide/pyridine (1:10, v/v) in a capped glass vial and heated to 105 °C for 15 min. Standard solution preparation: derivatization was used to create a combination of sterol standard solutions (cholesterol, sitosterol, stigmasterol, betulin, ergosterol, and campesterol). A Hewlett Packard 6890 (Agilent) gas chromatograph with a FID detector and a capillary column HP5 (5 percent phenylmethylsiloxane, 30 m 0.32 mm; internal diameter, film thickness 0.25 m) was used to analyze sterols by gas chromatography. Injector temperature was 320°C, column temperature was a 4°/min gradient from 240°C to 255°C (65 min), and the carrier gas was helium at a flow rate of 1 mL/min. The ISO 12228 reference method was used to identify the sterol peak, which was then validated by mass spectrometry using the NIST 2002 database.

2.11.Statistical analysis

The values of different parameters were expressed as the mean ± standard deviation ($\bar{x}$ ± SD) of three independent experiments. one-way analysis of variance (ANOVA) was used for statistical analysis where the method of extraction is the only factor taken into account. The comparison between the means was carried out with the Student–Newman–Keuls test and the differences between individual means and each single used mean were deemed to be significant at $p < 0.05$.

3.Results

3.1.Size and weight

Few studies have examined the size and morphological description of prickly pear seeds. Morphological characterization showed that *Opuntia ficus indica* seeds are very hard, flat in shape, more or less lenticular. The seed size was 0.52 cm for the length and 0.28 cm for the width (Table 1).

Table 1. Size and weight of PPS

Length (cm)	width (cm)	weigh of 100 seeds (g)
0.52 ± 0.05	0.28 ± 0.04	1.65 ± 0.08

Values are means ± Standard Deviations (SD) of three determinations.

These values are in agreement with those reported by Barabraet al. (1995). The mass of 100 seeds was 1.65 g. This result is higher than that obtained by Ennouriet al. (2005) (1.38 g), and Tliliet al. (2011) (0.89 g to 1.42 g). The variation in prickly pear seed mass and size certainly attributed to botanical origin; it is influenced by differences in growing conditions (agronomic and climatic).

3.2.Proximate chemical analysis

The chemical composition of prickly pear seeds is shown in Table 2. The moisture content was low (8.71%), which was beneficial in extending the shelf life of prickly pear seeds because higher moisture content may cause decomposition by micro organism. The oil yield value (8.64%) obtained for PPSwas lower than that of Nassar (2008) containing 10.43% oil. But they are close to those reported by Habibi (2004) and Tliliet al. (2011) who estimate this content at 9%, and 6.85% respectively. Crude protein, and total ash were 9.18%, and 1.1%, respectively, for prickly pear seeds on a dry weight basis. The protein content was higher than that found by Nebbache et al. (2009) while it is close to the result obtained by Tlili et al. (2011), (8.10%). The ash content of PPSharvested in Tunisia was consistent with those reported by Ennouriet al. (2005) and Habibi (2004) who estimate this content at: 1.1% and 1.3%.respectively. Differences in chemical composition of prickly pear seeds could be attributed to growing climates, ripening stages, extraction methods used and plant varieties (Ettalibi et al., 2021).

Table 2. Chemical composition (% dry matter) of PPS

Moisture	8.71±0.2
Ash	1.1±0.13
Protein	9.18±0.2
Crude oil	8.64±0.62

Values are means ± Standard Deviations (SD) of three determinations.

3.3.Mineral contents

The mineral content of prickly pear seeds is shown in Fig. 1. The most abundant mineral in prickly pear seeds was potassium (167 mg/100 g), while the calcium content was the lowest (18.2 mg/100 g). These results are in agreement with those of Mostafaet al. (2014) for the Moroccan PPS. However, The calcium and magnesium contents are lower than those found by Ozcan and Al Juhaimi (2011) for the turkishPPS. Prickly pear seeds are a good source of minerals, especially potassium, from a nutritional aspect. This mineral contributes to alkalinization and helps to maintain the electrolyte balance of body fluids.

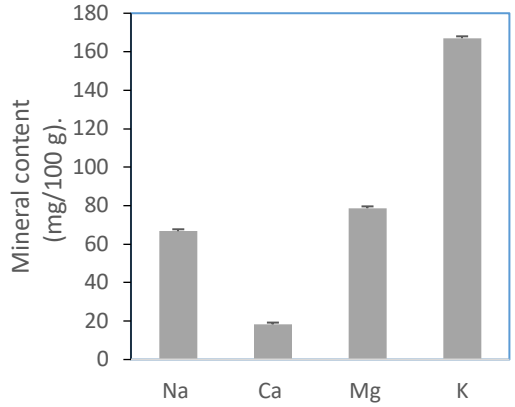

Figure 1. Mineral content of PPS (mg/kg).

3.4.Oil yield

The oil yields of PPSfrom cold pressing extraction are significantly lower ($p < 0.05$) than that obtained by solvent extraction (Fig. 2). The oil yield of *Opuntia ficus indica* seeds was 4.68 % and 8.64% for cold pressing extraction and solvent extraction, respectively. It was also found by Regalado-Rentería et al. (2018), Loizzo et al. (2019)and De Wit et al. (2021) that the oil yield of prickly pear seeds was affected by the extraction technique. Karabagiaset al. (2020) reported that the most important factors on the oil yield of PPS are the variety and the extraction process. Generally, the majority of scientific work is on solvent-extracted oils which give better oil yield (Al Naqeb et al., 2021).

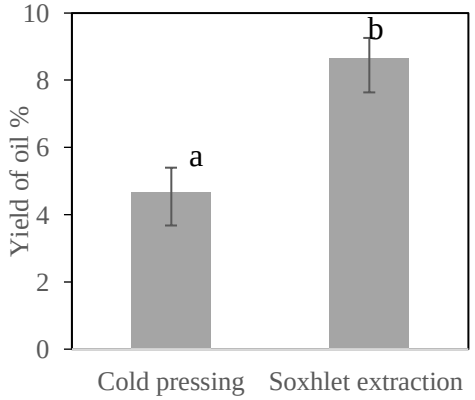

Figure 2. Oil yields obtained by different recovery techniques (Values with different letters are significantly different $p < 0.05$)

3.5.Physicochemical parameters

The different physico-chemical indices of PPSoil are presented in Table 3. The peroxide index is a useful measure for predicting the onset of oxidation since it quantifies the amount of hydroperoxide in the oil (Martin-Polvillo et al., 2004). The peroxide index of the solvent-extracted oil was significantly higher ($p<0.05$) than that of the pressed oil (Table 3), 5.13 meqO_2 kg^{-1} and 2.14 meq O_2 kg^{-1} for solvent-extracted oil and pressed oil, respectively. These peroxide index values are lower than the 10 meq O_2/kg oil values found in most conventional oils(Codex, 2009). Peroxide index values of chemically extracted prickly pear seed oils were above the recommended limit, De wit et al.(2017) reported that it ranged between 9.5 and 23.30 meq O_2 kg^{-1}. A peroxide index value of 12 meq O_2/kg has been reported for Algerian *Opuntia ficus-indica* cold-pressed seed oils (Koshak et al., 2020). Peroxide value is affected by oil extraction method, cultivars, growth conditions, soil geography, harvesting routines, as well as handling and storage, according to Koubaa et al. (2016).

Lipids are one of the most alterable compounds, the presence of water or air can respectively lead to hydrolysis and oxidation phenomena (Ollé, 2000). Free fatty acids is an important indicator of oil quality by measuring the level of free fatty acids in oils. Lipid hydrolysis is affected by chemical or enzymatic processes, resulting in the presence of free fatty acids (Sharma and Jain, 2015). As shown in Table 3, free fatty acids content of oil cold pressed (4.16%) was significantly ($p < 0.05$) lower than these obtained by soxhlet method (9.09 %). This is due to highly polar solvents induce triglycerides hydrolysis and saponification processes in vegetable oils, allowing for the generation of free fatty acids (Gharby et al., 2015). These results were compared to previous study obtained by De Wit et al. (2017) where free fatty acids ranged between 2.49 % and 5.08% in chemically extracted oil and less than 2 % in cold-pressed oil. The free fatty acid concentration of cold-pressed PPS oil was lower than that found by Brahmi et al. (2020) (21.2% for Algerian cold-pressed PPS oil) but higher than those found by Ozcan and Al Juhaimi (2011) (1.41% for Turkish cold-pressed PPS oil). A combination

of catalysts, such as enzymes, and residues generated after extraction may be responsible for the high levels of free fatty acids. Furthermore, free fatty acids could be present since the oil was not refined.

As indicated in Table 3, there was no significant difference in refraction index, extracts by solvent and by oil press ($p < 0.05$). According to the Table 3 refraction index was 1.4721 for solvent extracted oil and pressed oil. This result is similar to those reported by Gharbyet al.(2011) (1.464) and De Wit et al. (2017) (1.4658 and 1.4676), but it was lower than that reported by Ennouriet al. (2005) (1.4831).

The density of the PPS oil at 20°C was established as 0.901 and 0.907 (Table 3) for cold pressing extraction and solvent extraction, respectively. The density of both samples was generally determined within the range of published cactus seed oil density values(Ennouri, 2005; Zine et al., 2013)]. The results of the one-way ANOVA study revealed no significant differences between PPS oil extracted by pressing and PPS oil extracted by solvent.

Table 3. Comparison of physic-chemical properties of Soxhelt extracted and cold pressed PPS oils

Parameter	**Soxhlet extracted**	**Cold pressed**
Refractive Index	$1,4721^{a}\pm 0.01$	$1,4712^{a}\pm 0.0$
Density	$0,901^{a}\pm 0.03$	$0,907^{a}\pm 0.01$
Free acidity (%)	$9,09^{b}\pm 0.20$	$4,16^{a}\pm 0.05$
Peroxide value (meq O_2 kg^{-1})	$5,13^{b} \pm 0.2$	$2,14^{a} \pm 0.1$

Values with different letters in the same row are significantly different $p < 0.05$. Values are means ± Standard Deviations of three determinations.

3.6.Fatty acid composition

Fatty acid composition of PPS oils is shown in Table 4. Eight kinds of fatty acids were detected in PPS oils samples. Analysis of the fatty acid composition of the lipid fractions obtained with differents extraction methods (cold-pressing and soxhlet) did not reveal significant difference ($p < 0.05$) in the fatty acid profile. These results were similar to those previously reported for red pepper seed oils, which found no significant variations in fatty acid composition between cold pressing and soxhlet extracted seed oils (Chouaibi et al., 2019). By comparing the results of the two extraction methods, it can be seen that the PPS oil produced by cold pressing is also rich in unsaturated fatty acids (UFA) (C18:1 =25.96% and C18:2 = 57.90 %) than that extracted by the Soxhlet method (C18:1 = 26.29 and C18:1 = 58.04%). Polyunsaturated fatty acids (PUFAs) were the most prominent fatty acid with rate, of total fatty acids, greater than 58%. They are mainly represented by linoleic acid with a higher content than in sesame oil (42.10%) (Kurt, 2018) and a much higher content than in *Pistacia lentiscus* seeds oil (<24%) (Ait Mouhamed et al., 2020). This fatty acid may have nutritional and physiological benefits in the prevention of coronary heart disease and cancer (Oomah et al., 2000). Adulteration of cactus oil with other oils rich in linolenic acid, such as rapeseed oil and soybean oil, can be detected by measuring the modest amount of linolenic acid (0.4 and 0.65 percent) (Taoufik et al., 2015) PUFAs are followed by monounsaturated fatty acids (MUFA) making up more than 25% of total fatty acids. In addition of his nutritional value, the presence of a significant amount of oleic acid ensures the oil's stability and suitability for industrial use.Total saturated fatty acid (SFA) contents were 14.51% and 14,97% for PPS oil extracted by solvent and pressing, respectively. Palmitic acid is the most abundant SFA which offers the appropriate amount of plasticity for cosmetics applications. Furthermore, it should be noted that PPS oil is highly unsaturated with unsaturation rate UFA/SFA greater than 4.5%. The high unsaturated/saturated ratio may give PPS oil its nutritional, dietetic virtues and curative properties. On the other hand, high ratio signifies high potential for oxidation reactions of the oils with relation to the presence of double bonds. The richness of omega-6 of this oil makes it as an alternative source of this essential fatty acid. Results

revealed that fatty acid compositions of the studied PPS oils were consistent with the results obtained by Ozcan and Al Juhaimi (2011) where content of palmitic acid (12.23%), oleic acid (25.52%) and linoleic acid (61.01%). Nonetheless, other studies have reported lower levels of oleic acid (18.88%) and higher levels of linoleic acid (63.46%) (Ettalibi et al., 2021). Some investigations observed differences in linoleic acid content, which could be attributed to fruit genotype, growth environment, or maturation stage. PPS oil, based on our results, could be a valuable supply of essential fatty acids, which could be beneficial to the cosmeceutical and pharmaceutical industries.

Table 4. Fatty Acid Composition (g/100 g) of Soxhelt extracted and cold pressed PPS oils

	Soxhletextracted	**Cold pressed**
Palmitic C16 :0	11.30 ± 0.30 [a]	11.40 ± 0.10 [a]
PalmitoléicC16 :1	0.40 ± 0.20 [a]	0.55 ± 0.05 [a]
Stearic C18 :0	2.94 ± 0.30 [a]	3.30 ± 0.01 [a]
Oleic C18 :1n9	26.29 ± 1.05 [a]	25.96 ± 1.50 [a]
Linoleic C18 :2	58.04 ± 2.10 [a]	57.90 ± 2.20 [a]
LinolénicC18 :3n3	0.40 ± 0.05 [a]	0.65 ± 0.01 [a]
Arachidic C20 :1	0.13 ± 0.01 [a]	0.14 ± 0.01 [a]
Arachidic C20 :0	0.27 ± 0.02 [a]	0.27 ± 0.05 [a]
SFA	14.51 ± 0.62 [a]	14.97 ± 0.16 [a]
PUFA	58.44 ± 2.15 [a]	58.55 ± 2.21 [a]
MUFA	26.82 ± 1.26 [a]	26.65 ± 1.56 [a]
UFA/SFA	4.58	4.55

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids;
Values with different letters in the same row are significantly different $p < 0.05$.
Values are means ± Standard Deviations (SD) of three determinations.

3.7.Triglyceride Composition

Determining the type and amounts of TAG species present in oil can be used to understand their physical and functional properties. According to the Table 5, prickly pear seed oils contain 12 TAGs, the predominant component is LLL (22.79% – 21.99%), followed by OLL (21.36%–21.56%) for the oil extracted by solvent and cold press, respectively. PLL and PLLn triglycerides are found in trace amounts. This result is in agreement with that reported by Ettalibiet al. (2021).

Table 5. Triacylglycerol (TAG) composition ofSoxhelt extracted and cold pressed PPS oils

	Relative composition (%)			**Relative composition (%)**	
	Soxhlet extracted	**Cold pressed**		**Soxhlet extracted**	**Cold pressed**
LLLn	22.79^{b} ±0.02	21.99^{a} ±0.01	**OOO**	1.86^{a} ±0.00	2.32^{b} ±0.01
OLL	21.36^{a} ±0.02	21.56^{b} ±0.03	**PLLn**	0.29^{a} ±0.02	1.13^{b} ±0.04
POL	14.03^{a} ± 0.01	15.06^{b} ± 0.01	**POP**	2.65^{b} ±0.05	2.21^{a} ±0.02
LOO	13.22^{a} ±0.05	13.29^{a} ±0.03	**ALO**	2.41^{a}±0.00	2.99^{b}±0.01
POO	1.99^{a} ±0.02	1.95^{a} ±0.03	**SOO**	9.61^{b}±0.01	6.93^{a}±0.02
PLL	0.31^{a} ±0.01	0.30^{a} ±0.02	**OPS**	3.78^{b}±0.02	2.99^{a}±0.03

Values with different letters in the same row are significantly different $p < 0.05$.
Values are means ± Standard Deviations (SD) of three determinations.

In addition, the triglyceride profile obtained for cold press oil extraction in this study are close to those described by Zineet al. (2013). When the triglyceride content of oils extracted using two different procedures was compared, significant differences (p0.05) were found for LLLn, OLL, POL, OOO, PLLn, POP, ALO, SOO, and OPS. Therefore, the extraction method should affect triglyceride levels.

3.8.Sterols analysis

The sterol composition of PPS oil is presented in Table 6. Six kinds of sterols were identified in PPS oils and quantified by corresponding sterol standards. Sterolic fraction was composed by β-sitosterol: 71.56% and 70.26%, campesterol: 11.26% and 10.68%, Δ5-avenastérol: 8.68% and 9.45%, Δ7- avenastérol: 3.75% and 3.84%, stigmasterol: 3.44% and 3.71% and cholestérol: 1.12% and 1.05% for PPS oil extracted by pressing and solvent methode, respectively. These results are in good agreement with prior studie' finding (Taoufik et al., 2015). The one-way ANOVA analysis did not show a significant ($p < 0.05$) difference between PPS oil extracted by pressing and solvent methode. β-sitosterol was reported as the main sterol identified in seed oils with about 70% of total sterols. Furthermore, campesteroland Δ5-avenasterol were found in concentrations that exceeded 10% and 9% of the total sterol content, respectively. The presence of low levels of stigmasterol, cholesterol, and 7-avenasterol was also discovered.El Mannoubi*et al.* (2009) and Zine et al. (2013) were published similar results where β-sitosterol was the sterol marker with 72% and 75.3% respectively.The obtained results for β-sitosterol were lower than those reported for PPS oil (80.82% and ≥ 77%) from Morocco (Taoufik et al., 2015; Gharby et al., 2020). However, in our study, the cholesterol values were higher than those found in literature. The mature stage of seeds may be responsible for differences in sterol content. In addition to their antioxidant activity, sterols are interesting because they affect the human body by preventing cholesterol absorption from the intestine and lowering blood levels of the low density lipoprotein cholesterol component. Phytosterols have been proven to have a variety of nutritional impacts (Tapieroet al., 2003).

Table 6. Sterol composition (Weight Percentages) of Soxhelt extracted and cold pressed PPS oils

	Soxhlet extracted	**Cold pressed**
Cholesterol	1.12 ± 0.01[a]	1.05 ± 0.10 [a]
Campesterol	11.26 ± 0.05 [a]	10.68 ± 0.60 [a]
Stigmasterol	3.44 ± 0.02 [a]	3.71 ± 0.01 [a]
β-Sitosterol	71.56 ± 1.05 [a]	70.26 ± 2.00 [a]
Δ5-avenasterol	8.68 ± 0.50 [a]	9.45 ± 0.01 [a]
Δ7- avenasterol	3.75 ± 0.01 [a]	3.84 ± 0.01 [a]

Values with different letters in the same row are significantly different $p < 0.05$. Values are means ± SD of three determinations.

4.Conclusion

Comparative study of physicochemical, fatty acid, triglyceride and sterols compositions of PPS oil according to its extraction method had been examined. Refractive index and density did not change significantly with the used technique, while free acidity and peroxide value increased significantly when organic solvent extractionwas used. This study revealed that fatty acid and sterols composition were not affected by the extraction technique, however extraction with hexane greatly affect triglyceride levels.

Highlighting, the good chemical composition and the richness in PUFA of PPS oil permitted to establish its potential applications in nutritional or pharmaceutical industry. PPS oil is also a suitable ingredient in the fields of cosmetic and phytotherapy industry.Other biological activities should be evaluated, as well as the active components responsible for the oil's antioxidant activity.

5.References

Ait Mouhamed, B., El Antari, A., Benkhalti, F. (2020). Chemical Composition of Pistacia lentiscus Seeds' Oil from Moroccan High Atlas Mountain. Hindawi*Journal of Food Quality* DOI.org/10.1155/2020/5190491

Albergamo, A., Potortí, A.G., Di Bella, G., Amor, N.B., Lo Vecchio, G., Nava, V. Rando R, Ben Mansour H, Lo Turco V (2022). Chemical Characterization of Different Products from the Tunisian Opuntia ficus-indica (L.) Mill. *Foods*, 11, 155. DOI.org/10.3390/

Al-Naqeb, G., Fiori, L., Ciolli, M., Aprea, E. (2021). Prickly Pear Seed Oil Extraction, Chemical Characterization and Potential Health Benefits. *Molecules*, 26, 5018.

Ammar, I., Ennouri, M., Bali, O., Attia, H. (2014). Characterization of two prickly pear species flowers growing in Tunisia at four flowering stages. LWT. *Food Science andTechnology,* 59, 448-454.

AOAC (1984) Official Methods of Analysis. Association of Official Analytical Chemists. 14th Edition, AOAC, Arlington.,

AOAC (1990) Official Methods of Analysis. 15th Edition, Association of Official Analytical Chemist, Washington DC.

AOAC (2000) Oils and fats. In AOAC International Official Methods of Analysis, 17th Edition Association of Official Analytical Chemists, Gaithersburg.

AOCS (1997). Official Methods and Recommended Practices of the American Oil Chemist's Society, 5th ed. AOCS Press, Champaign, USA.

Arba, M. (2009). "Le cactus Opuntia une espèce fruitière et fourragère pour une agriculture durable au Maroc" in Proceedings of the Actes du Symposium International—Durabilité des systèmes de culture en zone méditerranéenne et gestion des ressources en eau et en sol (AGDUMED), Rabat Morocco. 215–223.

Barba, F.J., Putnik, P., Kovačević, D.B., Poojary, M.M., Lorenzo, J., Koubaa, M. (2017). Impact of conventional and non-conventional processing on prickly pear (Opuntia spp.) and their derived products: From preservation of beverages to valorization of by-products.*Trends Food Science and Technology*, 67, 260-270 DOI: 10.1016/j.tifs.2017.07.012

Brahmi, F., Haddad, S., Bouamara, K., Yalaoui-Guellal, D., Prost-Camus, E., Pais de Barros, J.P., Prost, M., Atanasov, A.G., Madani, K., Boulekbache-Makhlouf, L., Lizard, G. (2020). Comparison of chemical composition and biological activities of Algerian seed oils of Pistacia lentiscus L., Opuntia ficus-indica (L.) mill. and Argania spinosa L. skeels. *Industrial Crops and Products*, 151, 1-12.

Chouaibi, M., Rezig, L., Hamdi, S., Ferrari, G. (2019). Chemical characteristics and compositions of red pepper seed oils extracted by different methods. *Industrial Crops and Products*, 128, 363-370.

Codex Alimentarius Commission , CODEX STAN 210 (2009) 1999.

De Wit, M., Hugo, A., Shongwe, N. (2017). Quality assessment of seed oil from selected cactus pear cultivars (Opuntia ficus-indica and O. robusta). *Journal of Food Process Preservation*, 41 (3).

De Wit, M., Motsamaia, V.K., Hugoa, A. (2021). Cold-pressed cactus pear seed oil: Quality and stability. *Grasas y aceites*. 72 (3).

El Azizi, C., · Hammi, H., · Chaouch, M.A., Majdoub, H., · Mnif, A. (2019). Use of Tunisian Opuntia ficus-indica Cladodes as a Low Cost Renewable Admixture in Cement Mortar Preparations. *Chemistry Africa*, 2, 135–142. DOI.org/10.1007/s42250-019-00040-7

El Mannoubi, I., Barrek, S., Skanji, T., Casabianca, H., Zarrouk, H. (2009). Characterization of Opuntia ficus indica seed oil from Tunisia. *Chemistry of Natural Compounds*, 45(5), 616-620.

Ennouri, M., Bourret, É., Mondolot, L., Attia, H. (2005). Fatty acid composition and rheological behaviour of prickly pear seed oils. *Food Chemistry*, 93, 431-437

Ettalibi, F., El Antari, A., Hamouda, A., Gadhi, C., Harrak, H. (2021). Comparative Assessment of Physical and Chemical

Characteristics of Prickly Pear Seed Oil from Opuntia ficus-indica and Opuntia megacantha Varieties. Hindawi*Journal of Food Quality*, 1-8 pages DOI: 10.1155/2021/3098608

Gharby S, Harhar H, El Monfalouti H, Kartah BE, Maata N, Guillaume D, Charrouf Z (2011) Chemical and oxidative properties of olive and Argan oils sold on the Moroccan market. A comparative study. *Mediterranean Journal of Nutrition and Metabolism*, 44,1-8. DOI: 10.1007/s12349-011-0076-5

Gharby, S., Harhar, H., Guillaume, D. (2015). Chemical investigation of Nigella sativa L. seed oil produced in Morocco. *Journal of the Saudi Society of Agricultural Sciences,*14(2): 172–177. DOI.org/10.1016/j.jssas.2013.12.001

Gharby, S., Ravi, H. K., Guillaume, D., Vian, M. A., Chemat, F., Charrouf, Z. (2020). 2-methyloxolane as alternative solvent for lipid extraction and its effect on the cactus (Opuntia ficus-indica L.) seed oil fractions. *Oilseeds and fats, Crops and Lipids*, 27, 1-7

Habibi, Y. (2004). Contribution à l'étude morphologique, ultra-structurale et chimique de la figue de barbarie les polysaccharides pariétaux : caractérisation et modification chimique, thèse de doctorat.

Jouini, M., · Horchani, M., · Zardi-Bergaoui, A., · Znati, M., · Romdhane, A., · Krisa, S., · Waffo-Téguo, P., · Ben Jannet, H. (2021).Phytochemical analysis, neuroprotective, anticholinesterase, cytotoxic and catalase potentials of Opuntia microdasys var. rufida and Opuntia leptocaulis. *Chemistry Africa*, 4, 285–298. DOI.org/10.1007/s42250-020-00217-5

Karabagias, V. K., Karabagias, I. K., Gatzias, I., Badeka, A.V. (2020). Prickly pear seed oil by shelf-grown cactus fruits: waste or maste? *Process*, 8, 132. DOI.org/10.3390/pr8020132

Koshak, A., E., Abdallah, H. M., Esmat, A., Rateb, M., E. (2020) Anti-inflammatory activity and chemical characterization of Opuntia ficus-indica seed oil cultivatedin SaudiArabia.*ArabianJournal for Science an d Engineering*,45, 4571-4578. DOI.org/10.1007/s13369-020-04555-x

Koubaa, M., Mhemdi, H., Barba, F. J., Roohinejad, S., Greiner, R., Vorobiev, E. (2016). Oilseed treatment by ultrasounds and microwaves to improve oil yield and quality: an overview. *Food Research International*, 85, 59-66.

Kurt, C. (2018). Variation in oil content and fatty acid composition of sesame accessions from different origins. *GrasasAceites*, 69 (1), 241.

Lassoued, R., Abderrabba, M., Mejri. J. (2021). Comparative phytochemical studies and starch extraction of acorn's quercus species growing in Tunisia. *South Africa Journal Botanic*, 146, 71. DOI: 10.1016/j.sajb.2021.10.003

Loizzo, M. R., Bruno, M., Balzano, M., Giardinieri, A., Pacetti, D., Frega, N.G., Sicari, V., Leporini, M., Tundis, R. (2019). Comparative chemical composition and bioactivity of Opuntia ficus-indica Sanguigna and Surfarina seed oils obtained by traditional and ultrasound-assisted extraction procedures. *European Journal of Lipid Science and Technology*,121,1800283.

Martin-Polvillo, M., Márquez-Ruiz, G., Dobarganes, M.C. (2004). Oxidative stability of sunflower oils differing in unsaturation degree during long-term storage at room temperature. *Journal of the American Oil Chemists' Society*, 81, 577-583.

Mechqoqa, H., El Yaagoubia, M., Momchilovab, S., Msandaa, F., El Aouad, N. (2021). Comparative study on yields and quality parameters of argan oils extracted by conventional and green extraction techniques. *Grain & Oil Science and Technology*, 4, 125-130

Mostafa, K., Kharrassi, Y., Badreddine, A., Andreoletti, P., Vamecq, J., Kebbaj, M.S., Latruffe, N., Lizard, G., Nasser, B., Cherkaoui-Malki, M. (2014). Nopal Cactus (Opuntia ficus-indica) as a Source of

Bioactive Compounds for Nutrition, Health and Disease. *Molecules*, 19, 14879-14901.

Motri, S., Touil, A., Zagrouba, F. (2022). Desorption Isotherms and Thermodynamic Properties of Prickly Pear Seeds. In book: Advances in the Modelling of Thermodynamic Systems. Publisher: IGI Global DOI: 10.4018/978-1-7998-8801-7.ch004

Mouden, M., Boujnah, M., Salmaoui, S., Zantar, S., Douira, A. (2016). Effect of two Extraction Methods and Harvest Period and Performance Their Statement of Fatty Oils of Figs Pear Seed. *International Journal of Pure & Applied Bioscience,*4 (1), 1-8. DOI: 10.18782/2320-7051.2192

Nassar, A. G. (2008). Chemical Composition and Functional Properties of Prickly Seeds Flour and Protein Concentrate. *World Journal of Dairy & Food Sciences,*3(1), 11-16. ISSN 1817-308X

Nebbache, S., Chibani, A., Chadli, R., Bouznad, A. (2009). Chemical composition of Opuntia ficus-indica (L.) fruit.*African Journal of Biotechnology,*8 (8),1623-1624.

NF ISO 12228, French norm T60-258, AFNOR Paris 18 (1999).

NF T60-233 Corps gras d'origines animale et végétale - Préparation des esters méthyliques d'acides gras.

NF T60-234 Corps gras d'origine animale ou végétale. Analyse par chromatographie en phase gazeuse des esters méthyliques d'acides gras.

Ollé, M. (2000). Analyse des corps gras. Techniques de l'ingénieur, dossier P3325, 15.

Oomah, B. D., Ladet, S., Godfrey, D.V., Liang, J., Girard, B. (2000). Characteristics of raspberry (Rubus idaeus L.) seed oil. *Food Chemistry*, 69 (2), 187–193.

Ozcan, M. M., AlJuhaimi, F.Y. (2011). Nutritive value and chemical composition of prickly pear seeds (Opuntia ficus indica L.) growing in Turkey. *International Journal of Food Sciences and Nutrition*,62(5), 533. DOI: 10.3109/09637486.2011.552569

Paiva, P.M.G., Costa de Souza, I.F.A., Costa, M.C.V.V., Santos, A.F.S., Napoleão, T.H., Coelho, L.C.B.B. (2019). Biology, Chemical Composition, Cultivation and Applications of Cacti from the Opuntia Genus. *Advances in Applied Science and Technology* Vol. 5. Print ISBN: 978-93-89246-72-8, eBook ISBN: 978-93-89246-73-5. DOI:10.9734/bpi/aast/v5

Regalado Rentería, E., Aguirre Rivera, J.R., González Chávez, M.M., ·Sánchez Sánchez, R., Martínez Gutiérrez, F., Juárez Flore, B.I. (2018). Assessment of Extraction Methods and Biological Value of Seed Oilfrom Eight Variants of Prickly Pear Fruit (Opuntia spp.). *Waste Biomass Valorization*, 11, 1-9. DOI: 10.1007/s12649-018-0409-4

Sharma, S., Jain, V. K. (2015). Recherche J Science et technologie, 7, 109.

Taoufik, F., Zine, S., El Hadeka, M., IdrissiHassanib, L. M., Gharbyc, S., Harharc, H., Matthaus, B. (2015). Oil content and main constituents of cactus seed oils. *Mediterranean Journal of Nutrition and Metabolism,*8, 85–92. DOI: 10.3233/MNM-150036

Tapiero, H., TownsendbK, D. M., Tewb, D. (2003). Phytosterols in the prevention of human pathologies. *Biomedicine &* Pharmacotherapy,57 (8), 321-325. DOI: 10.1016/s0753-3322(03)00104-5

Tlili, N., Bargougui, A., Elfalleh, W., Triki, S., Nasri, N. (2011). Phenolic compounds, protein, lipid content and fatty acids compositions of cactus seeds. *Journal of Medicinal Plants Research,* 5(18), 4519-4524.
http://www.academicjournals.org/JMPR

Zine, S., Gharby, S., El Hadek, M. (2013). Physicochemical characterization of Opuntia ficus-indica seed oil from Morocco.B*iosciences Biotechnology Resear ch Asia*, 10 (1), 1–7