Chemical characteristics of seed oil from wild prickly pear (*Opuntia ficus indica*) in eastern Morocco

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Abstract. Prickly pear cactus (*Opuntia ficus indica*), is a Cactaceae plant with a great economic relevance in the world. Nowadays, nopal cactus industry is active and rapidly expanding. In fact, the seeds oil sector is the best exploitation due to the high price of this oil. Moisture content, oxidative stability, quality index, total phenol, flavonoids, chlorophyll and carotenoid content of prickly pear seed oil were analyzed. The seeds used in this study were provided by a cooperative DAR ATABIAA located in Chouihia (Eastern region of Morocco) during the harvesting season 2020. The samples were obtained from wild trees grown in this region. Results showed that moisture content of prickly pear seeds was 9.12%, whereas the acidity and peroxide index value was 1.11% and 4.44 meq O₂/kg respectively, and an oxidative stability of 17.31 hours. The total phenolic and the flavonoid content values for these seed oils are 260.07 mg/kg and 65.99 mg/kg, respectively. Furthermore, the results show a carotenoid content of 0.61 mg/kg and total chlorophyll of 1.52 mg/kg.

1 Introduction

Prickly pear (*Opuntia ficus indica*), a member of the Cactaceae family, originally from American continent more exactly from the south of this continent. Grows abundantly in arid and semi-arid regions around the world, including Mediterranean countries, South Africa, Middle East, India, Australia and other areas [1] and grows everywhere in Morocco [2]. Prickly pear species are well-adapted to arid lands and to a variety of climates, they grow wild in areas with restricted access to water, limiting the development of a lot of succulent food plants [2]. On one side, this plant is cultivated in dry regions as an important nutrient and food source, and it can also be used as natural wind break barrier, helping to protect the soil from the spread of the desert and controlling erosion [2]. Moreover, Prickly pears are considered to be a versatile plant that can be cultivated and grown for use in several fields, including food, cosmetic and pharmaceutical industries [2]. On the other side, the cultivation of this plant requires little investment. All these characteristics have contributed to an unceasing extension of this culture in several areas all over the world, and the plant is becoming increasingly economically important [1]. The fruit is the most consumed part of the plant, it contains around 300 seeds (2% to 10% of the fruit) [1], while the pericarp and the pulp of the fruits accounts for 33 to 55% and 45 to 67% respectively [3]. However, until now, this part of the fruit (seeds) remains poorly exploited. In fact, Millions of pounds of seeds are thrown away each year as waste [4], although proper utilization of these by-products leads to a production of a very important and a costly oil. Prickly pear seed oils (PPSO) are of great interest because they are edible oils with high level of unsaturation and antioxidant radical scavenging properties [5]. This oil has been shown to have considerable amounts of antimicrobial, biological activity, cardio protective, anti-thrombotic, anti-inflammatory, anti-arrhythmic, hypolipidemic and anti-hyperglycemic [4]. The seeds are composed mainly by 45.1 wt% of cellulose, followed by lipids (23%) [6]. Thus, they are rich in albumin protein with 6.5 kDa molecular weight, and proanthocyanidins with strong antioxidant activity [7], they are composed by 49.6% fiber, 17 fat, 16.6% protein and 3.0% ash. Aspartic acid, glutamic acid, arginine and glycine make up the most abundant part

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of the amino acids [8]. According to literature data, *Opuntia ficus indica* seed oil is characterized by a fairly low acidity (1.27 %) [9] and Peroxide values ranged between 9.50 and 33.67 meq O₂/kg, another study conducted by De Wit, Hugo [10] revealed that these oils have a relatively low oxidative stability ranging between 2.16 and 4.15 h. Thus, this previous research has shown that chemical properties such as peroxide number and oil content correlate with oxidative stability. According to Khemiri and Bitri [11], PPSO has a total phenolic and the flavonoid content of 26.5 Gallic acid eq/g and 3.1 mg Quercetin eq/g respectively, carotenoid content (10.52 mg/kg) oil and a total chlorophyll content of 4.57 mg/kg.

The oil content and composition are influenced by the environmental and genetic factors such as soil, cultivar and geographical area [12]. Moisture content, oxidative stability, quality index, and pigments content of *Opuntia ficus indica* seed oil from Chouihia region of eastern Morocco were investigated in this study. These chemical properties will provide further information on the valorization of seeds in various fields, such as the food, cosmetic, and pharmaceutical industries. The results were compared with other studies to evaluate the quality of this vegetable oil from Eastern Morocco.

2 Material and Methods

2.1 Samples and plant material

Prickly pear seeds used in this study were extracted from the fruits provided by the cooperative DAR ATABIA obtained from wild trees located in rural zone of Chouihia (Berkane Province) eastern Morocco. The fruits were peeled by hand, and the pulps were scraped from the seeds with multiple washes of water. The seeds were then dried externally in the sun. The oil was extracted via mechanical pressing which is the most prevalent method for obtaining oils in the world [13].

2.2 Moisture content

The determination of the moisture percentage of the seeds was carried out according to AOAC [14], 2 g of prickly pear seeds are dried in stove at 103 ° C for 3 hours until weight stability, are then placed in the desiccator for one hour before being weighed.

The loss of mass found after steaming is then equated with the mass of water contained in the product. The moisture content is equal to the average of the results of the grading tests, expressed in g of water / 100 g of seed.

$$\text{Humidity level (\%)} = [(P2 - P3) \div (P2 - P1)] \times 100$$

- P1 : mass in grams of the tare vessel
- P2 : mass in g of (tare vessel + sample) before drying
- P3 : mass in g of (tare vessel + sample) after drying

2.3 Free acidity

The acidity was determined according to the official method of the European Commission (EEC, 2003). Acidity value determines the percentage of free fatty acid expressed by the major fatty acid (linoleic) present in the oil resulting from the hydrolysis of triglycerides. Using the official method of the European Commission: 0.5 g of *Opuntia ficus indica* oil was dissolved in 5 ml of a mixture of absolute ethanol and diethyl ether (2.5 ml, 2.5 ml; v/v), to which eight drops of phenolphthalein (1%) were added. The free fatty acids are titrated under agitation by an alcoholic solution of 0.1 N potassium hydroxide (the pink color of the phenolphthalein persists for at least 10 seconds). The free acidity is calculated according to the following formula [15]:

$$\% \text{ of linoleic acid} = \frac{C(\text{KOH}) \times V(\text{KOH}) \times M(\text{C}_{18}\text{H}_{32}\text{O}_2)}{P.E}$$

- C (KOH) : KOH concentration expressed in mol/l
- V (KOH) : Poured volume of KOH expressed in ml
- P.E : test sample in gram

2.4 Peroxide index

The peroxide indice is used to estimate the content of the primary oxidation products present in the oil (hydroperoxides), which is expressed in meq O₂/kg of oil. This index is determined by the so-called acetic acid and chloroform method according to the AOAC [14] standard. Acetic acid-chloroform mixture (15:20 v/v) was used to dissolve 1 g of tested oil. To this solution, 0.5 ml of a saturated solution of potassium iodide (KI) is added. Finally, 30 ml of distilled water is added to the mixture after one minute of stirring. This solution is titrated with 0.01 N sodium thiosulfate using starch as a color indicator (colored indicator starch paste). The Peroxide values calculated according to the following equations.

$$\text{Peroxide index (meq O}_2\text{ / Kg)} = \frac{(V_t - V_w) \times 0.01 \times 1000}{P.E}$$

V_t : Volume in ml of sodium thiosulphate added to the titration
V_w : Volume in ml of sodium thiosulphate used for the white.
P.E : test sample in grams

2.5 Oxidative stability

The stability oxidative was estimated via the measure of induction time (oil resistance to thermo-oxidative stress) using the Rancimat 743 (Metrohm Co., Basel, Switzerland). In order to achieve this, 3g of oil was weighed and placed in a heating block at 100°C. The principle of this method is to accelerate the aging of the oil by thermo-oxidative deterioration. The degradation products results are transferred to the measuring cell containing distilled water, and a conductivity meter was used in measuring. The operating conditions are as follows: an air flow rate of 20 L/h and a temperature of 100 °C (Delta T: 1.6° C) [16]. The result was expressed in hours.

2.6 Determination of chlorophyll and carotenoids content

The analysis of the chlorophyll and carotenoid content (expressed in mg/kg) was determined by the colorimetric method described by Isabel Mínguez-Mosquera, Rejano-Navarro [17]. 1.5g of oil was dissolved in 5 mL cyclohexane, and the absorption was measured with a UV spectrophotometer (RAYLEIGH UV1800; UV-Visible) at 670nm for chlorophylls and 470 nm for carotenoids. The content of these pigments is calculated by the following equations:

$$\text{Chlorophyll mg/kg} = \frac{A_{670} \times 106}{613 \times 100 \times d}$$

$$\text{Carotenoid mg/kg} = \frac{A_{470} \times 106}{2000 \times 100 \times d}$$

A₄₇₀ : Absorbance at wavelength 470 nm
A₆₇₀ : Absorbance at wavelength 670 nm
L : Tank thickness in cm

2.7 Determination of total phenolic content

The extraction of phenolic compounds from cactus seed oil done using liquid-liquid extraction method described by Khemiri and Bitri [11]. In a centrifuge tube, 2 ml of methanol/water mixture (80/20, V/V) are added to 2 g of cactus oil. After stirring for 10 minutes, the tubes are centrifuged for 15 min at 3000 rpm. The hydro-methanolic phase was recovered by a Pasteur pipette. This extract is then used for the colorimetric determination by the Folin-Ciocalteu reagent in order to determine the total content of phenolic compounds [18]. 2 ml of each polyphenol extract are added to 1 ml of Folin-Ciocalteu reagent diluted 10 times in distilled water, and 1 ml of an aqueous solution of 10% Na₂CO₃ (P/V). After stirring, this solution was put in the obscurity for 90 min at room temperature, the absorbance measurements are carried out using a UV-visible spectrometer (RAYLEIGH UV1800) at 760 nm. Total phenolic were expressed as mg of Gallic acid equivalents per g of oil (G.A. eq/g oil) [1].

Expression of results:

$$T = \frac{C \times V}{M}$$

T : Total polyphenol content
 C : Concentration of extract equivalent to Gallic acid (mg/ml)
 V : Volume of the extract (mL)
 M : Mass of the dry matter (g)

2.8 Total Flavonoid Content

The Bahorun method[19] was employed to determine the total flavonoids in extracts, and aluminumtrichloride (AlCl₃) was utilized to quantify the flavonoids in the various extracts. 1.5 mL of each extract is added to 1.5 ml of an AlCl₃ solution (2% in methanol), after 30 min of incubation in the dark, the absorbance is measured at 430nm[20] using a UV-visible spectrometer (RAYLEIGH UV1800). The flavonoids concentration is deduced from a calibration range established with quercetin at different concentrations.

Expression of results:

$$T = \frac{C \times V}{M}$$

T : Flavonoid content
 C : Equivalent extract concentration in quercetin (mg/ml)
 V : Volume of the extract (ml)
 M : Mass of the dry matter (g)

3 Results and Discussion

3.1 Moisture Content of prickly pear seeds

The amount of water detected in prickly pear seeds in the current study was (9.12 g/100 g), which is generally a low value compared to other species[21, 22]. In fact, this reduction of water content is one of the mechanisms adopted by cactus to reduce respiration, which causes oxidation and stock depletion. Therefore, the seeds of prickly pear resist well to dehydration. In other words, water reduction is a strategy to increase the viability of the seeds, which is why they are classified orthodox seeds. In comparison with the results found in the literature, our finding found slightly higher compared to that of Bahorun, Gressier [19], Karabagias, Karabagias [4], BENATTIA [20], and El Mannoubi, Barrek [9], which are 6.0, 6.0, 6.43, and 6.9 g/100 g respectively. Our study shows a slightly high humidity, which may be due to the climatic conditions of the eastern region, which are more favourable in comparison with the arid climate. Moreover, the high moisture content probably gives information about the water absorption capacity of the wild cultivar and the soil conditions.

The obtained results of acidity, peroxide index, total phenol, flavonoids, chlorophyll and carotenoid content of prickly pear seed oil are presented in the table 1.

Table 1: Chemical characteristics of wild prickly pear (*Opuntia ficus indica*) seed oil in eastern Morocco

Chemical quality parameters	Mean	SD	Min	Max
Acidity %	1.11	0.03	1.09	1.15
Peroxide (meqO ₂ /kg)	4.44	0.44	3.99	4.88
Stability oxidative (h)	17.31	1.79	16.33	20.94
Phenol (mg/kg)	260.07	30.1	201.19	314.9
Flavonoid (mg/kg)	65.99	12.77	46.33	84.95
Chlorophyll (mg/kg)	1.52	0.19	1.12	1.69
Carotenoid (mg/kg)	0.61	0.03	0.55	0.63

SD: standard deviation

3.2 Quality index

The measurement of free acidity is the main parameter used to determine oil quality and therefore provides information on its aging. Over time, the triacylglycerol hydrolyzes to free fatty acids and glycerol, the degraded oil contains more free acids, consequently, higher acidity index. The recorded value of this parameter was about 1.11% of linoleic acid (table 1). This result is lower comparable to that obtained by El Mannoubi, Barrek [9] who found a free acidity value of 1.27 %. In addition our outcome is higher than that recorded by Karoui et al [23] (0.27%), and is slightly lower compared to the value reported by Ozcan et al [24] and by Khemiri and Bitri [11] who found a free acidity about 1.41 % and 1.95% respectively.

According to the joint Food and Agriculture Organization/World Health Organization food standards program codex alimentarius commission 2019, the examination of hydroperoxides allows for the determination of the oxidation stage of fatty acid. The acceptable peroxide value must be less than 20 meq O₂/kg for Virgin oils. The obtained value (4.44 meq O₂/kg) is below the standard for virgin oil. This result allows us to conclude that the studied oil has a less oxidized. In comparison with other studies, this value was slightly higher compared to the results reported by Karoui, Ayari [23] (3.71 meq O₂/kg) and much higher than the finding of Ozcan and Al Juhaimi [24], and Khemiri and Bitri [11] who found (1.63 meq O₂/kg) and (2.230 meq O₂/kg), respectively. Low acidity and peroxide values indicate a low degree of deterioration of the tested oil, these quality indices are mainly affected by several factors such as improper harvesting systems, transportation, storage of the fruit, and in particular oil extraction method. In fact, the oil acidity increased during storage of fruit. It was highest when fruit were stored at 7.5°C and decreased with lower storage temperature. Oil stored in a controlled atmosphere had lower acidity than oil stored in air. The increase in acidity was probably the result of fungal lipase activity [25]. Regarding the peroxide value of oil obtained from fruit stored at various temperatures and controlled atmospheres increased during storage, compared to oil from freshly harvested fruit. The increase was significant only for fruit stored at 7.5°C [25].

3.3 Oxidative stability

The induction time in the Rancimat test is used to assess oxidation sensitivity to thermo-oxidative stress. According to the literature, cactus oils have a relatively low oxidative stability index ranging from 1.79 to 4.15 hours [10, 26]. This higher sensitivity is due mainly to its richness in polyunsaturated fatty acids. However, the studied seed oil obtained from wild cultivar of Chouihia region shows higher stability oxidative index (17.31 hours), which confirms the lower recorded value of acidity and peroxide index. This higher SOI recorded could be linked to the phenol and chlorophyll content. Similar results have been demonstrated by Salama, El Harkaoui [16] who found that natural antioxidant richness increases the SOI for vegetable oil.

3.4 Total phenolic and flavonoid content

The phenolic and flavonoids compounds have significant antioxidant activities and are classified among the most main antioxidants in the oils. These secondary metabolites are considered as determining factors in terms of organoleptic quality [27]. The flavonoids are the most important polyphenolic class. These bioactive molecules are able to liberate the hydrogen that allows oxidative stability improvement [26]. The colorimetric evaluation of the total phenolic and flavonoid content shows that the studied seed oil of prickly pear has a value of 260.07 mg/kg and 6.599 mg/kg (table 1), respectively. Concerning the total phenol content, our results are lower than those found by Karabagias, Karabagias [4], which reported a total phenol concentration of 551 mg/kg. The process of oil extraction affects the amount of polyphenols, and therefore decreases the content of these natural antioxidants resulting in low oxidation resistance. Indeed, the total phenolic content was increased with increasing temperature [28]. Regarding the flavonoid, the estimated value is lower than that reported by Chavez-Santoscoy, Gutierrez-Urbe [29] who found values ranging between 95 and 374 mg/kg respectively. This difference could be explained by the difference in the method utilized and the extraction conditions, which have an impact on the total concentration of phenols and flavonoids, and hence on antioxidant activity [30]. The richness in polyphenols increases the interest of the oil, since it reduces glucose level in the blood and lowered level of bad cholesterol. Polyphenols are natural calming and anti-fatigue compounds, and have a sedative action in conditions such as angina, heart spasms, headaches and stomach aches [20].

3.5 Carotenoid and chlorophyll content

These two natural components are the major pigments of vegetable oil, ensuring an orange, red, or yellow color [31]. Green oils are rich in chlorophyll pigments, whilst oils with high levels of carotenoids are more yellowish [32]. Because of their antioxidant nature in the dark and pro-oxidant nature in the light [28-29], chlorophyll play an important role in the oxidative activity of the oil during storage and in the preservation of its quality. On the other hand, carotenoids are the photo-oxidation inhibitors, and are able to neutralize active-singlet oxygen or triplet activator [33]. In the present study, *Opuntia ficus indica* seed oil has the lowest carotenoid and chlorophyll contents with respectively 0.61 and 1.52 mg/kg. In comparison with the results of El Mannoubi et al [9] (8.01 and 2.403 mg/kg) the studied oil is lower in terms of pigments. The presence of these antioxidants has contributed in the remarkable improvement of the oxidative stability of this oil [9].

4 Conclusion

The chemical composition of PPSO has recently attracted the interest of scientists and experts. According to the results of this study, Chouihia PPSO is characterized by better oxidative stability on the one hand, richness in antioxidants on the other hand and could be considered as a good source of bioactive compounds (polyphenols, carotenoids and chlorophyll). This result shows that this Prickly Pear is a versatile plant and a source of bioactive components for industrial application for food and pharmaceutical purposes. Further studies are needed to better characterize this seed oil produced in eastern Morocco by evaluating fatty acid profile, triacylglycerol composition, tocopherols and phytosterols content.

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