

# Temperature Effects on Yields, Fatty Acids and Tocopherols of Prickly Pear (*Opuntia Ficus Indica* L.) Seed Oil of Oriental Region of Morocco

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## Research Article

**Keywords:** *Opuntia ficus indica*, fatty acids, Tocopherols, oil extraction, prickly pear.

**Posted Date:** February 9th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-163800/v1>

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# Abstract

This study focuses on yields, chemical quality, composition, and the stability of the fatty acids of the oil extracted from *Opuntia ficus indica* seeds, collected from the eastern region of Morocco, regardless of the temperature and the extraction method used. The results of this study reveal that prickly pear is a rich source of oil. The obtained oil yields varied from  $12.49\% \pm 0.09$  for the mechanical extraction,  $11.46 \pm 0.10$  for the chemical extraction, and  $10.52\% \pm 0.09$  for the maceration. The main fatty acids found in *O. Ficus indica* are linoleic acid  $75.80\% \pm 0.10$  (Chemical),  $74.07\% \pm 0.14$  (Maceration) and  $71.59\% \pm 0.14$  (Mechanical), and palmitic acid  $17.32\% \pm 0.02$  (Chemical)  $22.419\% \pm 0.06$  (Maceration) and  $26.58\% \pm 0.00$  (Mechanical). So the oil of prickly pear could be classified as a linoleic.

Among the Tocopherols founded, a high value of b-tocopherol has been detected in the mechanical extraction with  $502.04 \pm 0.76$  mg/kg followed by the chemical and the maceration extraction with ( $430.12 \pm 0.61$  mg/kg,  $315.47 \pm 0.96$  mg/kg) respectively. The findings of the present study reveal that the oil of *O. ficus indica* could be used in cosmetics and pharmacological products.

## Introduction

*Opuntia ficus indica* (L.) is one among the foremost popular species of the Cactaceae family (El Hayek et al. 2015). It's cultivated in dry regions as a crucial source of nutrients (El Mannoubi et al. 2006). Over centuries, Prickly pear may be a medicinal plant endemic to Mexico and currently introduced to several parts of the planet (Rasoul Rasoulpour et al. 2018), including North Africa, Europe, Mediterranean countries and therefore the Middle East (Coskuner et al. 2003). Thanks to its ability to adapt in several environmental conditions, the cactus grows in plains, coastal regions and plateaus (Lahsasni et al. 2004).

*Opuntia ficus-indica* has received greater attention over the past few years and has been extensively investigated for its pharmaceutical properties (Bouaouine et al. 2018). It is a source of food but also the origin of products and by-products for industrial, medical and cosmetic use. (Lee et al 2002).

The species *O. ficus indica*, *Opuntia robusta*, and *Opuntia amyclaea* are extensively used in Mexico as a basic element for the production of cosmetics, pharmaceutical and agricultural products, textiles, additives for construction, coagulants (in wastewater treatment), and other applications (Orozco et al. 2018).

This cactus has multiple edible uses, just like the preparation of the juices and salads, also its medicinal benefits supported antioxidant properties and for treatment of hyperglycemia and hypercholesterolemia. Fruits and young stems are traditionally utilized to treat hypertension, asthma, edema, diabetes, burns, indigestion, and other health disorders (Galati et al. 2003; Chaouch et al. 2016; Barba et al. 2017).

Most existing data describes the chemical composition of the pulp, skin and seeds (El Kossori et al. 1998; Felker et al. 2002; Brahmi et al. 2020), the antioxidant, anti-inflammatory and anti-ulcerogenic properties

of the pulp (Galati 2001 ; Ahmed et al. 2005 ; Livrea and Tesoriere, 2006 ; Maataoui et al. 2006) and, more generally on the nutritional benefits of this species (Stintzing, 1999).

Seeds represent approximately 10–15% of the comestible pulp and are usually discarded as waste after the pulp is removed. The oil obtained from the seeds is approximately 7–15% of the total weight (Moukal, 2004).

The main objective of this work is to study the effect of temperature on yields, fatty acids and tocopherols of *Opuntia ficus indica* seed oil from the eastern region of Morocco using different extraction methods and temperatures.

## **Material And Methods**

### **1. Collection area**

*Opuntia ficus indica* seeds are collected from Guercif (Province of Taourirt). This province belongs to the eastern region of Morocco (Fig. 1).

### **2. Methods of extraction**

Three types of extractions were used:

#### **2.1 Chemical extraction**

The seeds of *O. ficus indica* were crushed until the obtaining of a fine powder. 40g of this powder is subjected to an extraction in a Soxhlet by 200ml of n-hexane (99%) at a temperature of 60°C for 7 hours. The solvent was removed using a steam rotator (HEIDOLPH) at a temperature of 40°C. The obtained oil was conserved in a refrigerator at a temperature of 4°C.

#### **2.2 Maceration extraction**

This technique consists of letting a solid mass remain in a cold liquid to extract soluble compounds. The extracts are prepared from 400g of the seeds powder using 400ml of n-hexane 99% under stirring at room temperature for 2 hours. The extracts are then filtered by a Büchner type filter and the filtrate is then filtered through a crucible with a porosity equal to 4µm. The solvent was removed from the filtrate by a steam rotator to recover the oils which are then conserved at 4°C until their use.

#### **2.3 Mechanical extraction: Hot press (120°C)**

The mechanical extraction is a biological method used to extract oil from the seeds. The seeds are placed between permeable barriers by increasing the mechanical pressure thus reducing the volume available for the seeds. In general, regardless of the seeds used, the higher the pressure, the higher the oil extraction efficiency.

### **3. The physicochemical analysis**

### **3.1 Acid index: Method ISO 660: 1996 (AFNOR 2000)**

1 g of fatty substance is stirred in an Erlenmeyer flask, then 5 ml of ethanol with a concentration of 99% and 5 drops of phenolphthalein (PP) are added. Neutralization is done by gradual addition using a burette containing the ethanoic solution of KOH (0.1 mol/l) until a persistent pink colour is obtained.

### **3.2 Saponification index: Method ISO 3657: 2002 (AFNOR 2000)**

In a 250ml round-bottomed flask, 1g of fatty substance is placed with 3ml of solvent (ethanol-ether (v: v)), and 25ml of alcoholic potassium hydroxide with a concentration of 0.5 mol/l. the flask is then placed in a water bath for 45 to 60 minutes. After cooling the flask to room temperature, 2 to 3 drops of phenolphthalein are added. The excess of potassium hydroxide was determined with hydrochloric acid at a concentration of 0.5 mol/l up to the endpoint (discoloration of the solution). Under the same conditions, a blank test was carried out.

### **3.3 Peroxide index: Method ISO 3960: 2001 (AFNOR 2000)**

In a 250 ml Erlenmeyer flask, 1 g of fatty substance is stirred with 25 ml of the solution (chloroform / acetic acid (2: 3 (v: v))) then 1 ml of the potassium iodide solution is added (KI) of concentration (0.01N). The mixture is placed in the dark for 5 min at a temperature between 15 and 25°C. Afterwards, 75 ml of distilled water is added and the mixture is stirred. The iodine released by sodium thiosulphate is titrated in the presence of starch as an indicator.

### **3.4 Relative density: IUPAC Method 2.101 (AFNOR 2000)**

It is the ratio of the mass of one volume of oil to the mass of the same volume of distilled water at a temperature of 20°C.

### **3.5 Refractive index: method ISO 6320: 2000 (AFNOR 2000)**

## **4. Chromatographic analysis CPG-MS**

The chromatographic analyses were carried out after transesterification by gas chromatography coupled with a mass spectrometer (SHIMADZU series GCMS-QP2010), equipped with a split/splitless injector and a column (LxDI :30m x 0.25 mm) apolar (Stationary phase : 95% dimethylpolysiloxane : 5% phenyl; Thickness ; 0.25µm). The carrier gas used was the Helium.

## **5. Tocopherols analysis (HPLC)**

The tocopherol analyses were performed by HPLC-FLD, (Agilent Technologies 1200 series system, Agilent Technologies) using the official method, AOCS 8–89 (AOCS 1989). It was equipped with an automatic injector, on an Uptisphere 120A° NH<sub>2</sub> column (150 mm \* 3 mm, 3 µm) Interchim (Montluçon, France). The temperature was maintained at 30°C. The mobile phase was hexane/2-propanol (99:1, v/v) with a flow rate of 1 ml min<sup>-1</sup>. The different isoforms of tocopherol (α-, β-, γ- and δ-tocopherols) were estimated using

an oil/hexane solution of the oils of the three extraction types of *Opuntia ficus indica*. Tocopherols obtained from Sigma-Aldrich (Steinheim, Germany) were used as external standardization for the identification of tocopherols at 292 nm. The homologues of the tocopherols were quantified by comparing the peak response of each sample with that of the corresponding standard.

## 6. Statistical analysis

Data were statistically tested by a unidirectional analysis of variance (ANOVA) followed by a Tukey test to compare means that showed a significant variation ( $P \leq 0.05$ ) using SPSS 20 software.

## Results And Discussion

### 1. Yields

The oil yields obtained from *Opuntia ficus indica* seeds using the three extraction methods are presented in (Fig. 2). The results varied from 12.46% and 11.49%. The oil yields present a highly significant difference ( $P < 0.05$ ). The mechanical extraction shows a high oil content with  $12.49 \pm 0.09$  followed by the chemical and the maceration extractions ( $11.46\% \pm 0.10$ ,  $10.52 \pm 0.09$ ) respectively. Gharby et al (2015) reveal that the oil yields of cold press extraction are ranged from 6 to 7%. However, the findings of Bertrand Matthäus et al (2015) showed that the oil contents of the seeds of *Opuntia ficus indica* in Turkey varied among the different localities ranging from 5.0% (Ortaören) to 14.4% (Eskioba) which is in accordance with our results, therefore the result of Sawaya and Kahn (1982), which found that the seeds of *Opuntia ficus indica* represented only about 12–15% of the whole fruit and that the oil yield of the seeds was about 13.6% whereas El Hachimi et al (2015) shows that prickly pear oil is a fluid oil with a relatively low extraction yield of ( $7.81 \pm 0.78\%$  to  $10.45 \pm 1.34\%$ ).

### 2. Physicochemical properties

#### 2.1 Prickly pear oil

*Opuntia ficus indica* oil obtained by mechanical extraction has a lower acid index ( $4.37 \pm 0.10$  mg KOH/g oil) compared to the chemical extraction ( $5.85 \pm 0.03$  mg KOH/g oil) and the extraction by maceration ( $5.66 \pm 0.07$  mg KOH/g oil). This significant difference ( $P < 0.05$ ) is probably due to the solubility of the acids in the solvents (Table 1). This high acidity value could indicate a strong enzymatic hydrolysis of the seeds during harvesting, handling or processing of the oil or could also be due to the wrong storage. Compared to our results, the study carried by Kandji (2001) and R'bia et al (2017), showed a low acidity index of OFI oil produced in Tunisia, which was  $1.28 \pm 0.007\%$ .

The results of saponification index obtained from *Opuntia ficus indica* show a significant difference ( $P < 0.05$ ) between the three types of extractions (mechanical, chemical and maceration) which varies from ( $181.12 \pm 0.18$ ,  $183.77 \pm 1.23$  and  $179.08 \pm 3.45$ ) respectively (Table 1). This index was in accordance with the CODEX STAN 210–1999 standard and these results are comparable to that of the castor oil ( $185.83$  mg KOH / g oil and  $181.55$  mg KOH / g oil) (Akpan et al. 2006). Our results are also in agreement

with El Mannoubi et al (2009) which shows a saponification index of 173.3 mg KOH / g oil. The high saponification index could be due to the geographical origin of the seeds and indicates that the oils have a high amount of triglyceride content and are therefore very useful in cosmetology.

The peroxide value (PV) of *Opuntia ficus indica* oil showed a significant difference ( $P < 0.05$ ) for the three extraction types (mechanical, chemical and maceration) with  $5.75 \pm 0.08$ ,  $6 \pm 0.06$  and  $5.97 \pm 0.04$  meq/kg respectively. Compared to our findings, the research performed by Brahmi et al (2020) shown a higher PV value ( $12.0 \pm 0.4$  meq O<sub>2</sub>/Kg,  $P \leq 0.05$ ) of OFI samples.

This difference may be due to the extraction time and the solvent used (Table 1). The PV may also be affected by the oxidation of the oil studied under the extraction and conservation conditions. This can lead to the oxidation of unsaturated fatty acids, and thus their reduction.

The density of *Opuntia ficus indica* oil for the three types of extraction (Mechanical, Chemical and Maceration) are ( $0.926 \pm 0.003$ ,  $0.925 \pm 0.001$ ,  $0.919 \pm 0.005$ ) respectively, which is in accordance with CODEX STAN 210–1999. Therefore, the density of this oil is comparable to both olive oil (0.910) and almond oil (0.917) (Ollé 2002).

The refractive index depends, like density, on oil chemical composition and temperature. The refractive index increases with the level of unsaturation and availability of minor functions in the fatty acid chain. (Boukeloua et al. 2012).

The refractive index shows that there is a significant difference ( $P < 0.05$ ) between the values of the three types of extraction (mechanical, chemical, maceration) with ( $1.475 \pm 0.001$ ,  $1.476 \pm 0.003$  and  $1.476 \pm 0.001$ ) respectively, which is in accordance with CODEX STAN 210–1999.

Table 1  
Physicochemical properties of the oil extracted from *Opuntia ficus indica* by three methods of extraction.

Extractions	<i>Opuntia ficus indica</i>		
	Mechanical	Chemical	Maceration
Acid index (mg KOH / g oil)	4.376 ± 0.10a	5.854 ± 0.03b	5.667 ± 0.07c
Saponification index (mg KOH / g oil)	181.12 ± 0.18b	183.77 ± 1.23c	179.08 ± 3.45a
Peroxide index 20milieq/Kg	5,75 ± 0.08a	6 ± 0,06b	5,97 ± 0,04b
Density (20°C)	0,916 ± 0.003b	0.915 ± 0.001a	0.919 ± 0.005c
Refractive index (n <sub>20d</sub> °C)	1.475 ± 0.001a	1.476 ± 0.003b	1.476 ± 0.001b

### 3. Gas chromatography analysis

The fatty acid composition of vegetable oils depends on several factors: plant origin, genetic factors, fruit ripening and specific climatic conditions (Tlili et al. 2011).

The prickly pear oil is rich in unsaturated fatty acid (UFA), the predominant one is the linoleic acid in the three types of extraction (chemical (75.80%±0,10), maceration (74.07%±0,14) and mechanical (71.58% ±0,14)). In the second place, we find the saturated fatty acids (SFA), the palmitic acid with (17.32%±0,02 for chemical extraction, 22.41%±0,06 maceration and 26.58%±0,00 for mechanical extraction), followed by the stearic acid (6.87%±0,12 for chemical extraction) and the arachidic acid which represents (3.50% ±0,02 for maceration and 1.83%±0,01 for mechanical extraction).

The chromatographic analysis of prickly pear oil for the three types of extraction clearly shows that the fatty acids are stable whatever the extraction and the temperature used. This stability is remarked for the three major fatty acids. The proportion of linoleic acid always remains around (70%) despite the different temperature extraction (maximum 120°C), it is also the case of the saturated fatty acids where we observe no degradation of those acids (palmitic, stearic and arachidic).

The SFA content varies slightly between 24.19% (chemical extraction), 25.92% (extraction maceration) and 28.41% (mechanical extraction), while there is a clear predominance of UFA with slightly variable proportions and the dominance of linoleic acid (Table 2). This is in agreement with the results of El Hachimi et al (2015) and that of Tlili et al (2011) which show that prickly pear oil is also rich in

polyunsaturated fatty acid with the dominance of linoleic acid. Similar results were obtained by Özcan and Al Juhaimi (2011) which showed that linoleic acid was the most important fatty acid (61.01%), following by oleic acid (25.52%) in OFI oil seeds harvested in the Mersin province of Turkey. These values are in the range of the main fatty acids of OFI oil from another Moroccan regions that are linoleic acid (60.2–64.6%) and oleic acid (18.2–22.3%) (Taoufik et al. 2015), and those of OFI oil of South Africa: linoleic acid ( $56.86 \pm 0.07$  to  $67.32 \pm 0.37\%$ ) and oleic acid ( $15.20 \pm 0.14$ – $22.51 \pm 0.52\%$ ) (De Wit et al. 2017). The results of R'bia et al. 2017 shown that the same fatty acids have been identified in the OFI from the area of Nabeul, Tunisia: linoleic acid (61.42%) and oleic acid (20.55%), and from Palermo, Sicily, Italy: linoleic acid ( $58.5 \pm 1.1\%$ ) and oleic acid ( $15.8 \pm 1.0$ – $18.1 \pm 0.9$ ) (De Wit et al. 2017).

Moreover, according to Ennouri et al (2005; 2006), the rate of linoleic acid overtakes 70% and oleic acid 12% in OFI seed oil of Sfax (Tunisia).

Other parameters related to nutritional aspects are the unsaturated/saturated ratio, in fact, OFI oil has an average value of 3.13 due to its high linoleic acid content. In addition, linoleic acids (omega-6) can be transformed by organisms into a series of long-chain fatty acids (Letawe et al. 1998), a precursor of the biosynthesis of arachidonic acid, a substrate for the synthesis of eicosanoids (Ghazi et al. 2013) which play an important role in vascular level and blood coagulation. Also, this oil has useful properties for the skin that's why it is used in the cosmetics industry (Ramadan and Mörsel 2003).

However, the work of Chaaben et al. 2015 shows that the average content of SFA in prickly pear oil is only 16.64%. This difference is probably due to the genetic, climatic and soil type of the cultivated plant which is in agreement with the work of Ramadan and Mörsel (2003), which shows that the fatty acid composition of the oil of *Opuntia ficus indica*, is very much influenced by climatic factors, soil type and genetic factors in which they are cultivated.

**Table 2** Gas chromatography of the fatty acids of *Opuntia ficus indica* and *Argania spinosa* Oils extracted by three methods of extraction.

<i>Opuntia ficus indica</i>					
Fatty acids	Retention Time	% Air			IM
		Extraction			
		Chemical	Maceration	Mechanical	
Palmitic acid, methyl ester C16 :0	23.29	17.32% ±0.02	22.419% ±0.06	26.58% ±0.00	MS
Linoleic acid, methyl ester C18 :2	24.99	75.80% ±0.10	74.07% ±0.14	71.59% ±0.14	MS
Oleic acid, methyl ester C18 :1	25.03	***	***	***	***
Stearic acid, methylester C18:0	24.62	6.88% ±0.12	–	–	MS
Arachadic acid, methyl ester C20:0	25.24	–	3.51%±0.02	1.83%±0.01	MS
Fatty acids	SFA <sup>a</sup>	24.198	25.928	28.413	-
	UFA <sup>b</sup>	75.802	74.072	71.587	-
	UFA / SFA <sup>c</sup>	3.13	2.85	2.51	-

**IM:** Identification Method, **MS:** Mass Spectrometry, **SFA:** Saturated Fatty Acid, **UFA:** Unsaturated fatty acid

## 4. Tocopherols

The major tocopherol founded in *Opuntia ficus indica* oil is  $\beta$ -tocopherol, it represents  $502,04 \pm 2,7$ mg/kg for the mechanical extraction followed by the chemical and the maceration extractions  $499,6 \pm 1,56$  mg/kg and  $315,47 \pm 1,11$ mg/kg respectively (Fig. 3). Our results also shown that  $\gamma$ -tocopherol was detected in the chemical and the maceration extractions ( $257,86 \pm 2,01$  mg/kg and  $260,61 \pm 2,9$  mg/kg) respectively, but missing in the mechanical extraction. The extraction by maceration presents a high value of  $\alpha$ -Tocopherols with  $237,12 \pm 0,33$ , followed by the chemical and the mechanical extractions ( $98,71 \pm 1,06$  and  $93,49 \pm 0,89$ ) respectively. The  $\gamma$ -tocopherol was missing in mechanical extraction and that may be due to temperature of extraction or the absence of the solvent.

Our results are in accordance with those of Matthäus and Özcan (2011) who find that  $\beta$ -tocopherol contents of *Opuntia* seed oil varied between 3.9% (Eskioba) and 50.0% (Adana). But less than Gharby et al (2015) shown that prime level of tocopherols in cactus seed cold press oil 946 mg/kg decided, it's much above that of the Tunisian (447 mg/kg) and therefore the Germany cactus seed oil (403 mg/kg). Total tocopherols content of cactus oil is almost that of sunflower-seed oil (490 mg/kg), however, much above that of vegetable oil (220 mg/kg), and less than that of the soya bean (650 mg/kg)

## Conclusion

This study demonstrates the stability and richness of *Opuntia ficus indica* oil in tocopherol, saturated and unsaturated fatty acids with the dominance of linoleic acid. This richness may interest the production units of food, cosmetics and pharmaceutical products and allow all units exploiting this oil in their artisanal or industrial preparations, and their commercialization on the national and international market, either in full or by the manufacture of new products based on these Moroccan oils.

## **Declarations**

### **Ethics approval**

Not applicable.

### **Consent to participate**

All authors were participated in this work.

### **Consent for publication**

All authors agree to publish.

### **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests.

### **Funding**

Not applicable.

### **Authors' contributions**

S.K. performed the experiments and wrote the paper. A.B responsible for resources figures. H.L and B.H read and correct the paper. S.F. analysed the data and made All the authors read and contributed to the submitted version of the manuscript.

### **Acknowledgements**

We thank all persons who contributed in the elaboration of this paper.

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## Figures

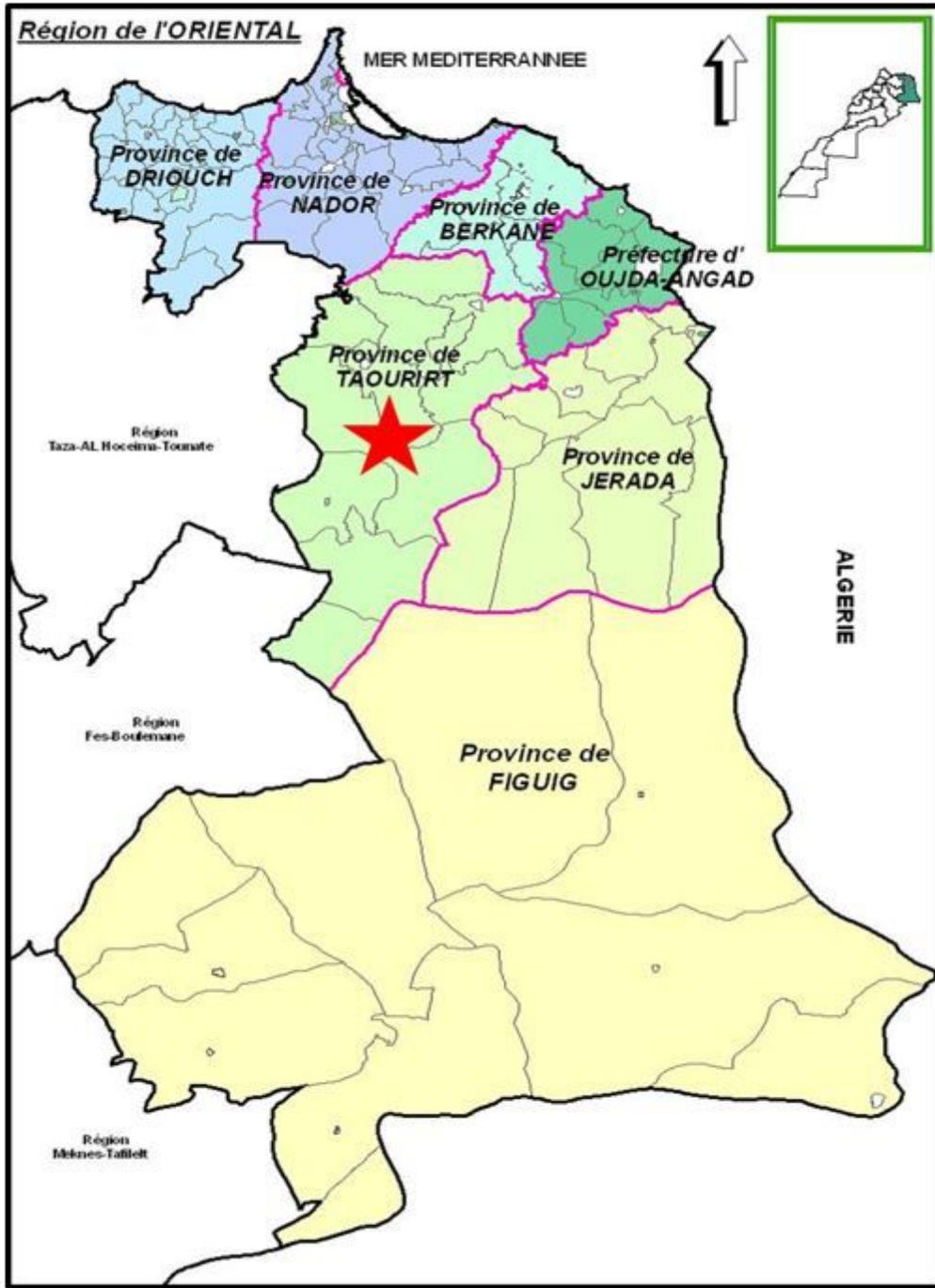
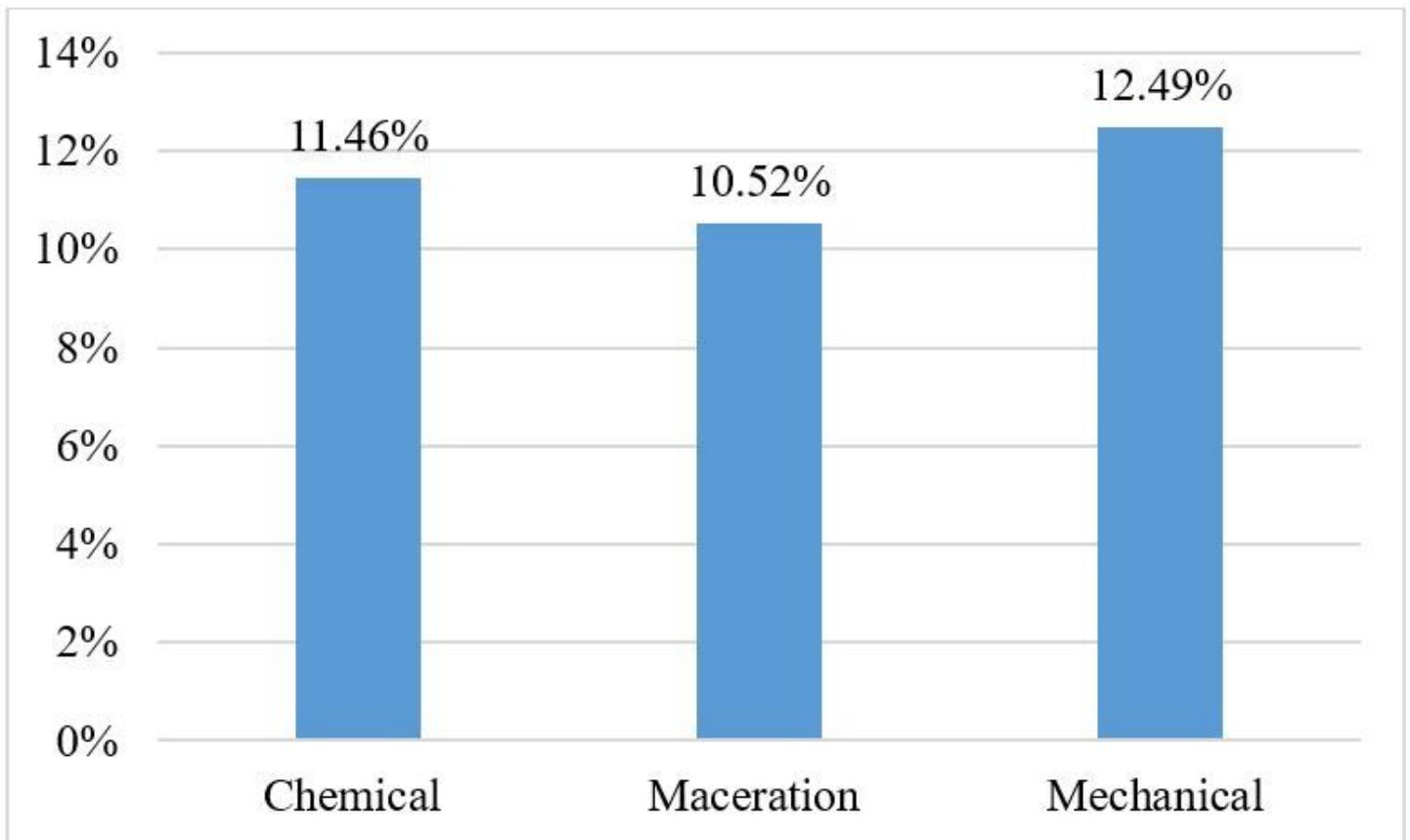


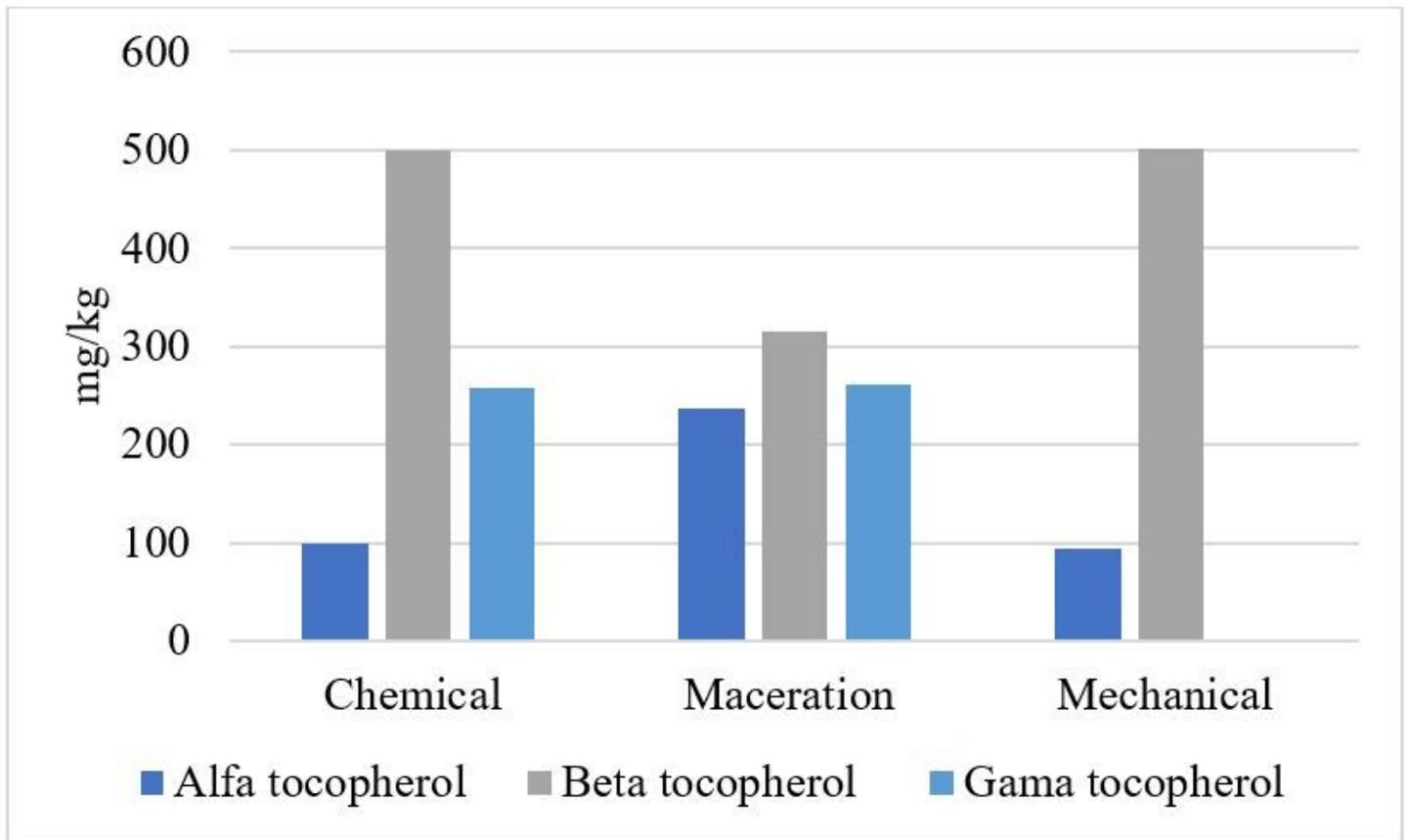
Figure 1

Localization of collection area, eastern of Morocco. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



**Figure 2**

Oil yields extracted from *Opuntia ficus indica* seeds using different extraction methods.



**Figure 3**

Tocopherols extracted from *Opuntia ficus indica* oil using different methods of extraction.