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Lipid profile, volatile compounds and oxidative stability during the storage of Moroccan *Opuntia ficus-indica* seed oil

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SUMMARY: The fatty acids, sterol, tocopherol and volatile compositions of Moroccan cold-pressed cactus (*Opuntia ficus-indica*) seed oil were studied. The most abundant fatty acid, tocopherol and sterol were linoleic acid (60.6%), γ-tocopherol (533 mg/kg) and β-sitosterol (6075 mg/kg), respectively. In this study, 23 volatile compounds were identified with perceivable odor attributes for 14 compounds. The oxidative quality of cactus seed oil was monitored over 4 weeks at 50 °C. Increases in PV, K232 and FFA were detected during the first two weeks as well as a decrease in the induction time; whereas no change was reported for the K270 values. The amount of total phenolic content increased until it reached 0.3 mg/kg and then decreased by the end of the storage period; while tocopherols started to decrease after the first week. The fat-free residue extracts showed a very strong effect to reduce the oxidation of linoleic acid. Consequently, the extracts were significantly more effective to bleach β-carotene in the β-carotene-linoleic acid assay in comparison with the control.

KEYWORDS: *β-Sitosterol; γ-Tocopherol; Opuntia ficus-indica; Oxidative stability; Peroxide value.*

RESUMEN: ***Perfil lipídico, compuestos volátiles y estabilidad oxidativa durante el almacenamiento del aceite de semilla de*** **Opuntia ficus-indica** ***marroquí.*** Se estudiaron los ácidos grasos, esteroles, tocoferoles y la composición volátil del aceite de semilla de cactus marroquí (*Opuntia ficus-indica*) prensado en frío. Los ácidos grasos, tocoferoles y esteroles más abundantes fueron el ácido linoleico (60,6%), γ-tocoferol (533 mg/kg) y β-sitosterol (6075 mg/kg), respectivamente. En este estudio, se identificaron 23 compuestos volátiles con atributos perceptibles para 14 de ellos. La oxidación del aceite de semilla de cactus fue monitoreada durante 4 semanas a 50°C. Se observó un aumento en el PV, K232 y FFA durante las dos primeras semanas y una disminución en el tiempo de inducción, mientras que no se apreciaron cambios para los valores de K270. La cantidad de fenoles totales aumentó hasta alcanzar 0,3 mg/kg y luego disminuyó al final del almacenamiento, mientras que los tocoferoles comenzaron a disminuir después de la primera semana. Los extractos de residuos libres de grasa mostraron un efecto muy fuerte para reducir la oxidación del ácido linoleico. En consecuencia, los extractos fueron significativamente más efectivos para blanquear el β-caroteno en el ensayo de β-caroteno-ácido linoleico en comparación con el control.

PALABRAS CLAVE: *β-Sitosterol; Estabilidad oxidativa; γ-Tocoferol; Índice de peróxido; Opuntia ficus-indica.*

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1. INTRODUCTION

Recently, the requirements for natural ingredients, healthy foods and nutraceuticals had increased (Matthäus and Özcan, 2011). Plants are viable sources to satisfy this necessity because they contain a large number of bioactive compounds with several positive effects on human nutrition, cosmetics, medicine or pharmacy.

A fruit with multiple properties such as *Opuntia ficus-indica* (OF) is beneficial to the production of nutraceuticals functional foods due to the presence of essential components, such as amino acids, fatty acids, carbohydrates, minerals, vitamins and soluble fibers (Chahdoura *et al.,* 2017). The cactus plant, which belongs to the Cactaceae family, is native to Mexico. Several parts of the plant have been used in human nutrition for thousands of years. In the 16th century the plant was introduced into the Mediterranean basin as well as South and North Africa (Matthäus and Özcan, 2011).

Interest in *Opuntia ficus-indica* (OF) has increased in many countries as a result of its socio-economic and ecological benefits such as overcoming desertification and erosion, use in animal feed and cosmetic products (Taoufik *et al.,* 2015). The seeds in OF represent about 10% of the fruit and are discarded as waste after the production of cactus juice. (Gharby *et al.,* 2013).

The oil yield from OF seeds in Morocco ranged from 5.4 – 9.9% (Taoufik *et al.,* 2015) and this was similar to the results reported in different studies that used solvent extraction (Salvo *et al.,* 2002; Ramadan and Mörsel, 2003). *Opuntia ficus-indica* seed oil (OFSO) has been characterized to have a very high content of unsaturated fatty acids, in which linoleic acid is recorded as the major fatty acid (Salama *et al.,* 2020). A low amount of saturated fatty acids (palmitic and stearic) was detected in OFSO (Salama *et al.,* 2020). The oil also contains important compounds like sterols and tocopherols (El Mannoubi *et al.,* 2009). Regarding pharmacological activity, OFSO showed relevant antioxidant and antimicrobial activities (Ramírez-Moreno *et al.,* 2017), α-glucosidase inhibitory activity, and cytotoxicity against human tumor cell lines, in addition to anti-inflammatory and analgesic activities (Chahdoura *et al.,* 2017).

Therefore, the purpose of this study was to analyze the physicochemical parameters, fatty acid composition, tocopherols and phytosterols in OFSO from Morocco. In addition, the volatile compounds and key odorants of Moroccan OFSO were determined by using GC-MS olfactometry. The oxidative stability of OFSO was monitored over 4 weeks at 50 °C to reveal changes in peroxide value, acidity, UV absorptions, total phenolic content, total tocopherols and induction time, all of which could give an overview on the impact of storage on the quality attributes of the oil.

2. MATERIALS AND METHODS

2.1. Materials

Opuntia-ficus indica seeds and its cold-pressed seed oil were purchased from two local coops in Tiznit, Morocco, and Marrakech, Morocco, respectively. The seeds were identified by Professor Fennane (Scientific Institute, Mohammed V University, Rabat) (Nounah *et al.,* 2021). The two coops collected the fruits from the two areas (Tiznit and Marrakech) in 2017. The cactus fruit was peeled and the seeds were manually collected, sundried and cold-pressed using a KOMET D85-type screw press. OF seeds were used for phenolic compound extraction and cold-pressed OFSO was used for oil parameters.

2.2. Methods

2.2.1. Storage of OFSO at 50 °C for one month

Brown bottles were filled with OFSO and tightly closed. To accelerate lipid oxidation, the bottles were put in an air oven at 50 °C for four weeks. After each week, corresponding samples were removed from the oven for analysis. Peroxide value (PV), free fatty acid (FFA), specific extinction coefficient (K232 and K270), total phenolic content (TPC), total tocopherols and induction period (IP) were measured to determine the oxidative stability of OFSO.

2.2.2. Determination of peroxide values (PV)

In brief, 5 g from the oil were dissolved in a mixture of acetic acid-isooctane (60:40 v/v). Zero point five milliliters of potassium iodide (saturated), were put in the previous solution and shaken for 60 sec. After that, 100 mL of boiled Millipore water were added and titration was done to the solution by pH metric titration with alcoholic (KOH) = 0.1 mol/L.

The peroxide value was determined using METHROM Titrando 888 and expressed as mEq of active oxygen/kg oil.

2.2.3. Determination of free fatty acid (FFA)

After the dissolution of 10 g from the oil in 100 mL of diethyl ether/ethanol (1:1 v/v), the oil was titrated with potassium hydroxide (ethanolic KOH) of known titer (0.1N). METHROM Titrando 888 with Tiamo software was used to measure the % FFA.

2.2.4. UV absorption analysis

Measuring the absorbance at around 232 nm and 270 nm allows the detection and evaluation of the primary and secondary oxidation products. OFSO (250 mg) was put into a graduated flask (25 mL) and diluted with spectrophotometric grade cyclohexane (25 mL). The sample was homogenized by using a vortex (30 seconds), and then the resulting solution was placed into a quartz cuvette. A double beam spectrophotometer was used to determine absorbances at 232 and 270 nm using pure cyclohexane as a blank. The specific extinctions were measured according to the following equation:

$$K\lambda = \frac{E\lambda}{C \times S}$$

Where: Kλ: specific extinction at a wavelength λ; Eλ: absorption or extinction at a wavelength λ; C: the solution concentration (g/100 mL). S: quartz cell length (cm).

2.2.5. Oxidative stability (Rancimat analysis)

To study the oxidative stability of the sample, a Metrohm® 743 Rancimat was used. The oxidation process was tested for 3.6 g of OFSO by exposure to 110 °C in a heating block with an air flow rate of 20 L/hour. The volatile components formed as oxidation products were captured in distilled water and the change in conductivity as a result of oil degradation was determined automatically by the instrument. With the steep increase in conductivity the sample had lost the resistance to oxidation and the inflection point of the curve was calculated. The oxidative stability of the samples was given as induction time in hours.

2.2.6. Determination of fatty acid composition

Methods DGF C-VI 10a (00) and C-VI 11d (19) (DGF 2013) were used to measure the fatty acid composition as follows: in a tube, one drop from the oil sample was dissolved in n-heptane (1 mL). Then 50 mg of CH_3ONa were added and vortexed for 1 min. After that, distilled water (0.1 mL) was added and the tube was centrifuged for 5 min at 3000 rpm. Afterwards, the aqueous bottom phase was eliminated carefully. After adding HCl (1 mol with methyl orange (Merck, Darmstadt, Germany)) (50 µL) again the lower phase was removed. Another centrifugation (with the same parameters) was done after adding $NaHSO_4$ (20 mg) (monohydrate, extra pure). The upper phase from the tube (n-heptane) was transferred to a vial and injected into a GC (HP5890, Agilent Tech. Waldbronn, Germany) with CP-Sil 88 capillary column, (100 m, 0.25 mm, 0.2 µm for length, ID and film thickness, respectively). The injector temperature was 250 °C and the oven program started from 155 °C, heated to 220 °C (1.5 °C/min), and held at isotherm for 10 min. Detection was done by a flame ionization detector (FID) monitored at 250 °C. Other parameters were as follow: 36 mL/min for carrier gas (hydrogen); the gas flow was 1.1 mL/min; split ratio 1:50; 30 mL/min for detector gases (hydrogen), air (300 mL/min) and nitrogen 30 mL/min; the injection volume was 1 µL. After automatic integration of the peak areas by the software, the fatty acids methyl esters (FAME) were reported as weight percent by direct internal normalization.

2.2.7. Determination of sterol composition

The determination of the sterol composition was made according to method DGF F-III 1 (98) (DGF 2013) and described as follows: cholestan-3-ol as internal standard and 5 mL ethanolic KOH were added to 250 mg OFSO and refluxed for 15 min. Solid-phase extraction was used to isolate the unsaponifiable matter by using a column of Al_2O_3. The sterol fraction was separated from unsaponifiable matter by thin-layer chromatography with n-heptane and distilled diethyl ether (50:50 v/v) as mobile phase. The sterols were silylated using a silylating agent (MSHFBA, with 50 uL methylimidazole dissolved in the MSHFBA-vial). The sterol fraction composition was illustrated by GLC, on a SE 54 CB column (Macherey-Nagel, Duren, Germany; 50 m,

0.32 mm, 0.25 µm for length, ID and film thickness, respectively). The other parameters were set as follows: the temperature of the injection and detection adjusted to 320 °C, the carrier gas was hydrogen, the program temperature, 245–260 °C (5 °C/min). Standard compounds such as β-sitosterol, campesterol, stigmasterol separated from rapeseed oil or Δ7-avenasterol, Δ 7-stigmasterol, and Δ 7-campesterol isolated from sunflower oil were used to identify the peaks. For the first time, the other sterols were reported by GC-MS and then identified in the samples by comparison with the retention time.

2.2.8. Determination of tocopherol composition

Method DGF F-II 4a (00) (DGF 2013) was used to determine the tocopherol composition as follows: 150 mg of oil sample were dissolved in 1 mL of n-heptane. After filtration (1.0 µm and 0.45 µm), the oil sample was directly used for HPLC analysis as follows: low-pressure gradient system (Merck-Hitachi), with a pump (L-6000), F-1000 Merck Hitachi fluorescence spectrophotometer detection system and a ChemStation software for integration. 20 µL of the sample were injected by a Merck 655-A40 autosampler onto the HPLC column (Diol phase 25 cm × 4.6 mm ID). The separation was performed with n-heptane and tert-butyl methyl ether (482:18 mL v/v) as mobile phase at a flow rate of 1.3 mL/min.

2.2.9. GC-MS-olfactometry analysis

Three grams of OFSO were put into a 20 mL headspace vial. The vial was purged with a stream of helium (10 PSI, 20 mL/min) at 80 °C for 20 min using a PTA 3000 dynamic headspace system, and the volatile compounds were trapped with an online Tenax trap cooled to -35 °C using a Peltier element as a cooling device. Afterwards the trap was heated for 10 min at 200 °C to transfer the volatiles via an uncoated fused silica transfer line at 200 °C to the GC system. As GC System a Trace 1300 Series GC equipped with a CPSil 19 fused silica capillary column (14% cyanopropyl-phenyl 86% dimethylpolysiloxane, 60 m, 0.32 mm ID, 1 mm film thickness) was used for the separation of volatile compounds. The temperature program was 40 °C for 5 min, then heated at 3 °C/min to 245 °C, and finally held at isotherm for the least 10 min. Olfactometry volatiles were divided in a ratio of 1:3 between the MS (Thermo Scientific, Darmstadt, Germany) and the olfactometry detection port (ODP).

Through a transfer line set at 200 °C, one part of the volatiles reached the MS ionization source (electronic ionization (EI) set to 230 °C in positive mode). The other volatiles were moved to the ODP set at 260 °C. For each new sequence, a blank consisting only of air was used to ensure good reproducibility of the retention times (DGF 2013).

GC-olfactometry was done by two test persons who noted the retention time of the olfactory perceptions, its intensity and aroma description. The lists of aroma profiles were compared to the chromatograms of the samples and the olfactory impressions were assigned to the corresponding peaks. Peak identification was performed by comparing mass spectra information with the NIST 2005 database as well as known data from the internal library of the instrument. Chemical standards were injected into the GC-MS system to validate some identifications.

2.2.10. Determination of the total phenolic compounds (TPC)

Extraction of phenolic compounds was done according to Owon *et al.* (2021) with some modifications. One gram of the OFSO was extracted by a mixture of methanol-water (80:20 v/v) using an ultrasonic bath for 30 min. After centrifugation for 5 min at 3000 rpm the supernatant was filtered into a 50-mL round-bottom flask. The extraction was repeated twice before removing the solvent by a rotary evaporator at 40 ºC.

The residue was dissolved with 1 mL from the same solvent (ultrasonic bath), transferred into a 5-mL volumetric flask and the 50-mL flask was washed twice. The volumetric flask was filled with 0.3% MeOH-HCl and 2 mL of Na_2CO_2 (2%) were added to a 100-µL aliquot. Fifteen microliters of Folin-Ciocalteu (Merck, Darmstadt, Germany) reagent were added after 2 min.

The absorbance was measured after a further 30 min using a spectrophotometer (U 2000, LTD, Hitachi Tokyo, Japan) at 750 nm wavelength. Total phenolic content was expressed as gallic acid (mg) equivalents GAE/g extract.

2.2.11. Determination of OFS antioxidant activity with the ß-carotene bleaching method

The ß-carotene/linoleic acid assay was used to evaluate the antioxidant activity of OFS. Linoleic acid (40 mg) and Tween-20 (400 mg) were mixed in a flask with 1 mL of a mixture of 12 mg ß-carotene dissolved in 2 mL of chloroform. The chloroform was evaporated at 40 °C (rotary evaporator, IKA, model RV 10 CS93) and an emulsion was formed by adding 100 mL distilled water and mixing with a vortex. 0.2 mL of the OFS extract were added to 5 mL of the emulsion in a tube and the absorbance was immediately measured against a blank (the emulsion without ß-carotene) at 470 nm with a spectrometer (Analytik Jena Specord 250). Afterwards the absorbance of the solution was measured every 15 min for one hour and finally after 120 min. The tubes were incubated at 40 °C in a water bath (HAAKE C 10) between measurements.

2.3. Statistical analyses

Values were determined in three replicates (means ± SD). One-way ANOVA was used to analyze mean values at the 0.05 significance level.

3. RESULTS AND DISCUSSION

3.1. Physicochemical parameters and fatty acids composition

The oil extracted from OFS was a yellow-green oil that had a slightly fruity taste. The results in Table 1 show that very low acidity of about 0.4% (calculated as oleic acid) was reported for OFSO. The PV of OFSO reached about 3.5 meqO_2/kg and extinction coefficients (K232 and K270) were determined to be 2.83 and 0.35, respectively.

The oxidation of lipids is a significant cause of quality degradation in edible oil. Oxidative stability refers to the resistance of the oil to oxidation during processing and storage. The time it takes to reach the critical point of oxidation, whether it is a sensorial transition or a sudden acceleration of the oxidative phase, can be expressed as resistance to oxidation (Silva *et al.,* 2001).

Oxidative stability as a marker of quality has been extensively evaluated by accelerated methods such as the Rancimat method, which uses elevated temperatures and airflow. The induction period (IP) at 110 °C of OFSO was reported to be 6.9 h. The results showed that the oxidative stability of OFSO was higher than that of sunflower (5.3h) (Almoselhy, 2021). However, it was less than moringa (10 h), olive (23 h) and argan (31 h) oils (Gharby *et al.,* 2013; Salama *et al.,* 2020). Generally, this difference is related to the number of antioxidants and the percentage of polyunsaturated fatty acids. The higher the percentage of polyunsaturated fatty acids, the lower the oxidative stability.

OFSO consisted of different fatty acids which are also typical for other seed oils, including saturated fatty acids such palmitic and stearic acids, as well as oleic, vaccenic and linoleic acids, representing the unsaturated fraction (Table 2). The data shows that OFSO contained SFA and PUFA of about 16.0 and 82.7%, respectively. Therefore, the calculated oxidizability (COX) value was determined by the percentage of unsaturated C18 fatty acids as follows:

$$COX = (1\,[18{:}1\%] + 10.3\,[18{:}2\%] + 21.6\,[18{:}3\%])/100$$

In this work, the COX value of OFSO was 6.45, which was greater than that for fresh palm, peanut, camellia and moringa oils (1.61, 4.63, 1.77 and 0.82 respectively) (Xu *et al.,* 2015; Salama *et al.,* 2020). On the other hand, the ratio of PUFA/SFA was 3.80 in OFSO. It is known that the COX value and the ratio of PUFA/SFA are considered as tendency parameters of oils to undergo oxidation (Méndez *et al.,* 1996). Linoleic, oleic and palmitic acids were the most abundant fatty acids in OFSO (60.6, 16.3 and 11.8%, respectively), while stearic and vaccenic acids were found in lower amounts (3.5 and 4.6%, respectively). This result makes OFSO belong to the group of oleic-linoleic acid oils and agrees with the results reported by Özcan and Al Juhaimi (2011); Ciriminna *et al.* (2017); Gharby *et al.* (2021) who showed that oleic and linoleic acids ranged from 17.61 to 25.52% and from 57.98 to 61.80%, respec-

Table 1. Physicochemical parameters of OFSO

Parameters	Value
Free fatty acid (oleic acid %)	0.40±0.04
Peroxide value (meqO_2/Kg oil)	2.1±0.3
K232	2.83±0.01
K270	0.35±0.04
Induction period (h)	6.85±0.02

Values are the mean of three values ± standard deviation.

Table 2. Fatty acid composition of OFSO

Fatty acid	Value (%)
Myristic	0.1±0.0
Palmitic	11.8±0.0
Stearic	3.5±0.0
Arachidic	0.4±0.0
Behenic	0.2±0.0
Lignoceric	0.1±0.0
Palmitoleic	0.6±0.0
Elaidic	0.1±0.0
Oleic	16.3±0.0
Vaccenic	4.6±0.0
Linoleic	60.6±0.1
Linolenic	0.2±0.0
Gondoic	0.2±0.0
SFA	16.00
MUFA	21.84
PUFA	60.87
Total unsaturated fatty acids (USFA)	82.71
PUFA/SFA	3.80
Total fatty acids	98.71
Cox value	6.45

SFA: Total saturated fatty acids, MUFA: Mono unsaturated fatty acids. PUFA: Polyunsaturated fatty acids, USFA: Total unsaturated fatty acids. COX: Calculated oxidizability value.

Values are the mean of three values ± standard deviation.

tively. The fatty acid composition of OFSO was similar to those of grape seed and sunflower oils, which also belong to the oleic-linoleic acid oils (Özcan and Al Juhaimi, 2011).

Linolenic acid was detected at a low level in OFSO (0.2%). This small amount of linolenic acid helps in detecting adulteration of this oil with other oils if other cheaper oils with higher amounts of linolenic acid were added. Palmitoleic, arachidic, gadoleic and behenic acids were also reported in OFSO in very small amounts. Ciriminna *et al.* (2017) reported a higher amount of stearic acid (3.92%) in comparison to the present results. The observed difference may be due to the difference in fruit maturity and geographical origin. The dietetic quality of OFSO is high as the total unsaturated fatty acids/total saturated fatty acids ratio is approximately 5.2, which is similar to that of argan oil (Gharby *et al.,* 2013).

3.2. Sterols and tocopherols composition

Sterols constitute the major part of the unsaponifiable matter in many oils. The phytosterol content can play a role in detecting adulterations in vegetable oils (Taoufik *et al.,* 2015). It has been shown that phytosterols lower blood LDL cholesterol by around 10–15% as part of a healthy diet (Ramadan and Mörsel, 2003). Table 3 shows the sterol composition measured in this study. β-sitosterol represented the highest amount of all phytosterols in OFSO (6075 mg/kg). This high amount of β-sitosterol in OFSO makes it very useful in inhibiting dietary cholesterol absorption (Taoufik *et al.,* 2015). Other sterols were determined in OFSO such as campesterol (877 mg/kg), Δ-5-avenasterol (411 mg/kg), sitostanol (266 mg/kg) and cholesterol (189 mg/kg). The total sterol amount of OFSO (855 mg/100 g) was lower compared to the Tunisian (1606 mg/100 g) and German (933 mg/100 g) OFSO. β-sitosterol was the predominant component in all samples, varying from about 71.6% in the Tunisian samples to 72.3% in German samples (Ramadan and Mörsel, 2003; El Mannoubi *et al.,* 2009).

Table 3. Sterol and tocopherol compositions of OFSO

Compound	Value (mg/kg)
β-Sitosterol	6075±38
Campesterol	877±38
Δ-5-Avenasterol	411±11
Sitostanol	266±26
Cholesterol	189±3
Δ-7-Avenasterol	180±5
Δ-7-campesterol	133±21
Stigmasterol	124.9±0.01
Δ-7-Stigmasterol	111±26
Δ-5-2,4-stigmastadienol	103.0±0.9
Δ-5-2,3-stigmastadienol	101±2
Total sterol	8554±80
α-tocopherol	11±0
γ-tocopherol	533±3
δ-tocopherol	4±0
β-tocotrienol	7±0
γ-tocotrienol	4±0
Total tocopherol	559±3

Values are the mean of three values ± standard deviation.

Tocopherols are natural antioxidants that have some antioxidant properties. OFSO contains large amounts of tocopherols and tocotrienols (Taoufik *et al.,* 2015). In our study, the vitamin E active compounds identified were α, γ, δ-tocopherols and β, γ-tocotrienols while β-tocopherol was absent in the seed oil. Table 3 shows the tocopherol content in OFSO. γ-tocopherol was the major tocopherol in OFSO, accounting for 533 mg/kg (95.28%), whereas α-tocopherol, ß-tocotrienol, δ-tocopherol and γ–tocotrienol accounted for 11, 7, 4, and 4 mg/kg, respectively. These findings were close to the results reported by Ramadan and Mörsel (2003), who found that γ-tocopherol, α-tocopherol and δ-tocopherol represented 330, 56 and 5 mg/kg. The higher biological activity of α-tocopherol in comparison to the other tocopherols makes it interesting for human consumption, but γ-tocopherol shows the highest antioxidant capacity (Gharby *et al.,* 2013). The high antioxidant activity of the tocopherols against the oxidation of polyunsaturated fatty acids helps to enhance oil quality by preserving it from rancidity during storage and prolonging the shelf-life (Matthäus and Özcan, 2011). The amount of tocopherols in Moroccan OFSO (559 mg/kg) is much higher than that of the Tunisian (447 mg/kg) and the German OFSO (403 mg/kg), which were solvent extracted oils (Ramadan and Mörsel, 2003; El Mannoubi *et al.,* 2009). In comparison with olive (220 mg/kg) and sunflower oils (490 mg/kg), OFSO had higher amounts of tocopherols, but it was lower than the amounts in soybean (650 mg/kg) and argan oils (850 mg/kg) (Gharby *et al.,* 2013).

3.3. Volatile compound composition

The volatile compounds present in trace amounts in the oil are of great interest because they mainly reflect the quality of the oil and influence consumer acceptance. The combination of these substances in a specific concentration ratio gives a characteristic flavor to the oil which in many cases is experienced as a unique sensation (Morales and Przybylski, 2013). The analysis of Moroccan OFSO volatile compounds and key odorants was done using GC-MS-olfactometry.

The results are reported in Table 4. In this work 23 volatile compounds were identified, 14 of them with perceivable odor attributes. The volatile composition of OFSO was mainly characterized by alkanes, aldehydes and alcohols. Most of the volatile compounds, aroma-active or not, were crucial to the final smell of OFSO. A previous study was done to determine the volatile composition of the whole fruit of three varieties of *Opuntia ficus-indica* (Oumatou *et al.,* 2016). In this study, the authors used Solid-Phase Microextraction (SPME) connected with GC-MS. The study recorded 46 volatile compounds. The most considerable compounds in the three varieties were n-hexanol and 2-hexanal. In addition, nonanal, 1-octanol and n-hexanol were found in the fruit (Oumatou *et al.,* 2016) and the seed oil of the present investigation.

From our results, 3-methylbutanal and 2-methylpropanal were determined in the volatile composition of OFSO. Hu *et al.* (2014) found these two aldehydes as typical volatile compounds in rapeseed, sunflower, and soybean oils. Indeed, these two aldehydes are also known as products of microorganism activities such as bacteria or fungi (Bonte *et al.,* 2017).

Table 4. Volatile compounds of OFSO

VOLATILE COMPOUNDS	ODOR IMPRESSION
Acetaldehyde	Sweet - fruity - roasted
Ethyl ether	
2-methyl pentane	
3-methyl pentane	
n-hexane	Burnt – Gas
2-methyl propanal	Moldy – sweet
Cyclohexane	
2,2,4 trimethyl pentane	Cheese
3-methyl Butanal	Detergent - chocolate
2-methyl Butanal	
Pentan-1-ol	
Formic acid	
Acetic acid	Vinegar
Acetoin	Fatty- pleasant buttery
1 butanol,3-methyl-acetate	Burnt
1-Hexanol	grille - green – cut grass
[R- (R; R)]-2,3-Butanediol	
Benzaldehyde	mint - sweet - orange - nutty
Hexanoic acid	Wood-like - cheese - fermented – medicine
1-Octanol	Stinky
Benzeneacetaldehyde	
Nonanal	Detergent, soap, lemon, Sweet, Fatty
phenylethyl Alcohol	Nutty, rye, gum

The pleasant buttery odor indicates the existence of acetoin (3-hydroxy-2-butanon) in OFSO. Peroxidation reaction products can be found as by-products from the degradation of linoleic acid (Bonte *et al.,* 2017). In the case of OFSO, alkanes (propane and butane), aldehydes (propanal, butanal, nonanal), and alcohols (propanol, butanol, pentanol) were found as secondary products of degradation.

3.4. Oxidative stability of OFSO for one month at 50 ºC

Many factors such as temperature, light and oxygen availability reduce the organoleptic and nutritional assets through oxidation. In this study, the influence of temperature on OFSO was evaluated. OFSO was placed in brown closed flasks to eliminate the effect of the unrestrained access of oxygen and light and stored in the oven at 50 °C. The evolution of the oxidative state was reported by PV, FFA, the UV absorptions (K232 and K270) and induction period (IP). Also, total phenolic content and total tocopherols were estimated (Figure 1a, 1b, 1c, 1d). Fatty substances can be oxidized in the presence of oxygen and certain factors (e.g.: temperature). This oxidation, called autooxidation, leads initially to the production of hydroperoxides by fixing oxygen to the unsaturated fatty acids (Mohdaly *et al.,* 2010). To estimate the first stages of this oxidation, PV was measured. PV is a simple and commonly used method for determining the oxidation and rancidity of oil caused by unsaturated fatty oxide saturation.

Temperature had a remarkable effect on the PV of OFSO. Three phases were observed, and the first one showed a very strong increase during the first week to reach the highest value of about 8.3 meqO_2/kg oil. Due to the oxygen impermeability of the container, the available oxygen was consumed and the production of further peroxides was avoided. In the second phase, a decrease in PV to reach 6.9 meqO_2/kg oil by the end of the third week was noticed (Figure 1a). Although the level of PV had decreased, this did not reflect the quality of the oil, because in general oil oxidation goes through two stages, a first with the production of hydroperoxides and a second stage with a proliferation of hydroperoxides which are transformed into secondary

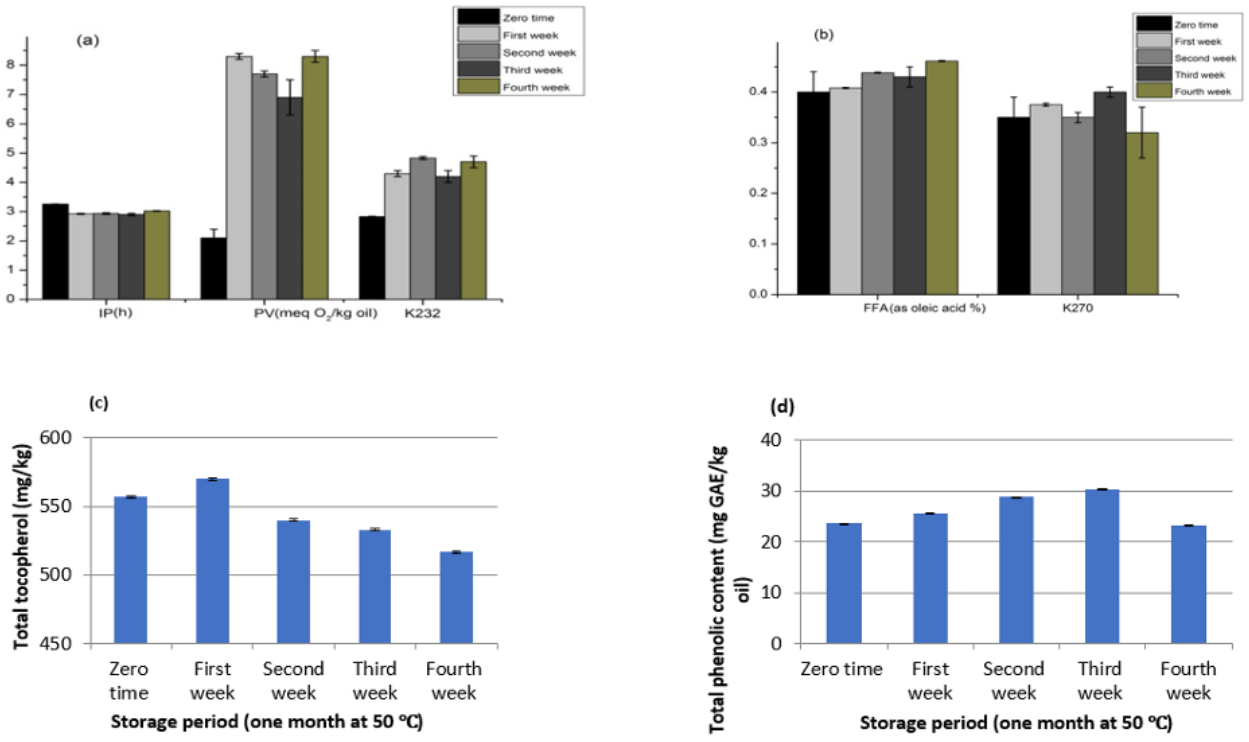

Figure 1. Effect of storage period of OFSO for one month at 50 °C on, (a): Induction period (IP) (h), Peroxide value (PV) (meqO_2/Kg oil), and K232, (b): Free fatty acid (FFA) (as oleicacid %) and K270, (c): Total tocopherol (mg/kg), (d): Total phenolic content (mg GAE/kg oil). Values are the mean of three values ± standard deviation.

products. In the third phase, PV started to increase again and reached about 8.3 meqO_2/kg oil. This rapid increase in PV may be due to the high content of linoleic acid.

The IP of OFSO was significantly affected by temperature during the storage period. It could be observed from the results that the oil lost around 30 min of resistance after one week of storage at 50 °C and IP became almost stable during the next three weeks with no significant differences between them (Figure 1a).

Spectrophotometric examination in the ultraviolet range can indicate oil quality. The absorption at the mentioned wavelengths was due to the existence of conjugated dienes and trienes resulting from the oxidation process. These results were expressed as extinctions E, which is typically denoted by K232 and K270 values referring to conjugated dienes and compounds of secondary oxidation (ketones, aldehydes, etc.). Figure 1a shows the evolution of K232 values during storage for 4 weeks at 50 °C. The K232 value of OFSO at the initial analysis was 2.83 and after one month of storage at 50 °C, the K232 reached 4.7. During the first two weeks of storage, an increase in the absorbance values as a result of peroxide formation and the existence of fatty acid oxidation products could be noticed. After the second week, as the oxygen available was depleted, and the amount of compounds measured at 232 nm was seen to remain relatively constant. This measure confirmed what was found previously for PV.

The evolution of the formation of secondary oxidation products was followed by measuring the absorbance at 270 nm under storage for one month at 50 °C. The storage led to little or no effect on the K270 values (Figure 1b). This result could be explained by the fact that the production of secondary oxidation products had not started yet and oxidation stopped at the production of hydroperoxides. Salama *et al.* (2020) showed that the value for K270 during the storage period of cactus oils (one month) increased and then started to decrease and after that, although it increased again by the end of the storage period. This finding was also observed by Torres *et al.* (2006), who found that the K232 and K270 values for soybean and jojoba oils increased, then decreased and by the end of storage period (240 h) increased again.

The percentage of FFA gives information on the alteration of oil by hydrolysis. FFA occurs in fats because of enzymatic hydrolysis by lipases, metal ions promoting degradation of free radicals or at an elevation of temperature (Goswami *et al.,* 2013). The changes in FFA values during storage at 50 °C are depicted in Figure 1b. The initial FFA value was seen to be 0.40% (calculated as oleic acid), which increased slowly and steadily to 0.46% after a storage period of one month. OFSO showed good resistance to the hydrolyzation of triglycerides during one month of storage at 50 °C.

The total amount of tocopherols decreased by the end of the storage period (517 mg/kg) in comparison with time zero (Figure 1c). After the first week, the value of total tocopherols started to decrease until it reached the lowest amount by the end of the storage period at about 517 mg/kg. The same results were found by Lerma-Garcia *et al.* (2009) who found that the amount of tocopherols (181 mg/kg at zero time) decreased greatly after the third week of storage until the end of the storage period (25 mg/kg) (7 weeks at 60 ºC).

Total phenolic content from OFSO at time zero was 23.6 mg GAE/kg oil (Figure 1d). During the storage period, the total phenolic content increased until it reached 30.3 mg GAE/kg oil in the 3rd week. By the end of the storage period, the total amount of phenolic compounds decreased to 23.3 mg GAE/kg oil. This agreed with Nounah *et al.* (2021), who reported that the exposure of OFS to high temperature (roasting at 110 ºC) for a certain time caused an increase in total polyphenol content. Also, Prabakaran *et al.* (2018) reported that significant increases among different storage times were observed after 12, 24, and 48 weeks of storage of raw soybean flour. Among the three-storage times, the raw soybean flour stored at the 45 ºC had the highest total phenolic content. The raw soybean flour for 48 weeks had the highest total phenolic acid content in all three storage times. Therefore, the increase in total phenolic content in OFSO under higher temperatures and longer storage periods could be explained by the degradation of complex polymerized phenolic structures into simple phenolic structures.

3.5. Antioxidant activity of OFS by β-carotene bleaching method

The results of the antioxidant activity of the extract from OFS by β-carotene linoleic acid are

shown in Fig. 2. In the absence of an antioxidant, carotene decolorizes rapidly because the free linoleic acid radicals attack the double bonds of the β-carotene, causing it to lose its double bonds and, as a result, its orange color. By scavenging the linoleate free radical and any other free radicals produced inside the system, the existence of a phenolic antioxidant will limit the degree of β-carotene destruction.

A reduction in absorbance of β-carotene in the presence of methanolic extract obtained from cactus seeds in the coupled system of β-carotene and linoleic acid was observed (Figure 2). The antioxidant activity of the methanolic extract from OFS was compared to a synthetic antioxidant (TBHQ) which was added to foods to prevent or delay oxidation and to a control sample without any added antioxidant. By using a concentration of 10 mg/mL it was noticed that TBHQ possessed a more powerful antioxidant effect than the extract from OFS. In addition, it was reported that the control sample, without the addition of extract solution, oxidized most rapidly. OFS methanolic extract was markedly effective in inhibiting linoleic acid oxidation and subsequently reduced the bleaching of ß-carotene in comparison with the control.

Many studies showed a good correlation between the antioxidant activity and OFS content of bioactive compounds. The results of the present work are in accordance with those of Zeghad *et al.* (2019), who demonstrated that OFS extracts have potent antioxidant capacities.

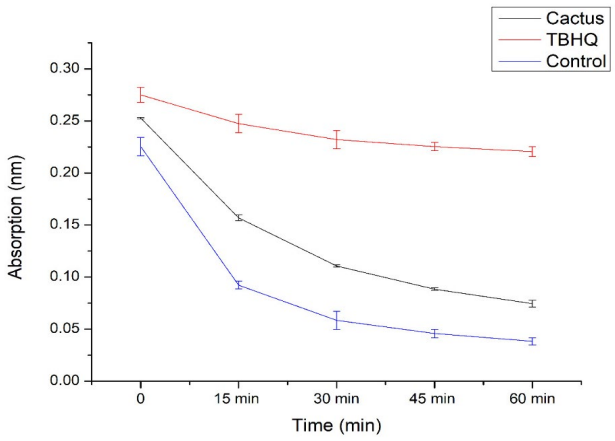

FIGURE 2. Antioxidant activity of OFS extract (10 mg/mL) using the ß-Carotene/linoleic acid bleaching method against TBHQ and control. Values are the mean of three values ± standard deviation.

4. CONCLUSIONS

Nowadays, cold-pressed cactus seed oil is becoming more and more popular in the cosmetic industry, but it can also be interesting for human nutrition. It has a good fatty acid profile with low acidity and peroxide value. Cactus seed oil is a very rich source of tocopherols and phytosterols. The storage of the oil at 50 °C for 4 weeks served to make a determination regarding the oxidative stability of the oil. The high content of linoleic acid is likely to be responsible for the oxidative sensitivity of cactus seed oil. Therefore, special care, such as storage under an inert atmosphere in dark packaging should be taken into consideration for prolonged storage. Indeed, an additional storage experiment considering different oxidation factors and storage facilities is needed in order to determine the shelf-life of cactus seed oil more in detail. The methanolic extract from OFS has a potent antioxidant activity which may be used in order to improve the stability of cactus seed oil and extend its storage time.

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