Physicochemical and biochemical characterizations of some Tunisian seed oils

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Abstract – Four Tunisian vegetable oils extracted from seeds (Nigella sativa, Opuntia ficus indica, Pistacia lentiscus and Hibiscus sabdariffa) have been characterized in this study. The following parameters were determined: acidity, peroxide value, saponification value, specific extinction coefficients K232, K270, chlorophylls and carotenoids content. The triglyceride and tocopherol compositions of the oils were determined using reversed phase high performance liquid chromatography with diode array detection (HPLC-DAD) and the fatty acids (FA) and phytosterol compositions were determined using a gas chromatography (GC) with a flame ionization detector (FID). Polyunsaturated fatty acids were predominant in all tested samples except in Pistacia lentiscus oil where monounsaturated fatty acids were predominant. Major FAs were linoleic and oleic acids. β-sitosterol was the most abundant phytosterol. All samples had high content of TAGs with an equivalent carbon number of 44, 46 and 48. Nigella sativa oil had the highest content of tocopherols.

Keywords: Nigella sativa oil / Opuntia ficus indica oil / Pistacia lentiscus oil / Hibiscus sabdariffa oil

Résumé – Caractérisations physico-chimiques et biochimiques de quelques huiles de graines tunisiennes. Quatre huiles végétales tunisiennes extraites de graines (Nigella sativa, Opuntia ficus indica, Pistacia lentiscus et Hibiscus sabdariffa) ont été caractérisées dans cette étude. Les paramètres suivants ont été déterminés : acidoité, indice de peroxyde, indice de saponification, coefficients d’extinction spécifiques K232, K270, teneur en chlorophylles et en caroténoïdes. Les compositions en triglycérides et tocophérols des huiles ont été déterminées par chromatographie liquide haute performance en phase inverse avec détection à barrette de diodes (HPLC-DAD) et les compositions d’acides gras (FA) et de phytostérol ont été déterminées à l’aide d’une chromatographie en phase gazeuse (GC) couplée à un détecteur à ionisation de flamme (FID). Les acides gras polyinsaturés étaient prédominants dans tous les échantillons testés, à l’exception de l’huile de Pistacia lentiscus où les acides gras mono-insaturés prédominaient. Les principaux acides aminés étaient les acides linoléique et oléique. Le β-sitostérol était le phytostérol le plus abondant. Tous les échantillons avaient une teneur élevée en triacylglycérols (TAG) avec un nombre de carbone équivalent de 44, 46 et 48. L’huile de Nigella sativa avait la teneur la plus élevée en tocophérols.

Mots clés : huile de Nigella sativa / huile d’Opuntia ficus indica / huile de lentille de Pistacia / huile d’Hibiscus sabdariffa

1 Introduction

Humans have used vegetable oils for centuries and still using it nowadays in food, medicine, cosmetics and as fuels (Thomas, 2000; Durrett et al., 2008; Montero de Espinosa and Meier, 2011). Plants generally accumulate oil in their seeds and fruits to provide energy for germination and the early stages of seedling development, which make seeds good sources of edible oils.

Oilseed crops are primarily grown for edible oil. Recently, oilseeds attracted more attention due to an increasing demand for their healthy vegetable oils, livestock feeds, pharmaceuticals, biofuels, and other oleochemical industrial uses. The increased interest resulted in an 82% expansion of oilseed crop cultivation areas and about a 240% increase in total world production over the last 30 years (Rahman and Jiménez, 2016).
Fats and oils are the most concentrated kind of energy that all living organisms can use (Odoemelam, 2005; Aliyu et al., 2010; Barua et al., 2011). The suitability of oil for certain application and a particular purpose is determined by its characteristics, fatty acid and triglyceride compositions (Alvarez and Rodriguez, 2000; Akpabio et al., 2012).

Using the physical and chemical properties, we can judge vegetable oils quality. Therefore, in this perspective, we have decided to compare four Tunisian seed oils Nigella sativa, Opuntia ficus indica, Hibiscus sabdariffa and Pistacia lentiscus and to investigate their physicochemical, fatty acids, sterols and tocopherol compositions in order to conclude their potential uses.

2 Experimental procedures

2.1 Plant material

Four oil samples: extracted from black cumin (Nigella sativa L), prickly pear (Opuntia ficus indica), lentisk (Pistacia lentiscus L) and hibiscus (Hibiscus sabdariffa L) seeds (Fig. 1) were provided by the National Office of Oil in Tunisia during the 2016/2017 season. Oil samples were stored at 4 °C and protected from sunlight prior analysis.

2.2 Determination of the physicochemical characteristics of seed oils

2.2.1 Determination of free acidity

Acidity is one of the chemical characteristics of the oil used to indicate its quality and to determine its grade (IOC, 2015). The expression of free acidity content was as percent of oleic acid. The free acidity of the oil samples was determined according to the ISO 660 method (AOCS Cd 3d-63) amending Regulation (EEC No. 2568/91), which consists in determining the fatty acids released during the hydrolysis of triglycerides with a sodium hydroxide solution.

2.2.2 Determination of the peroxide value

The peroxide value (PV) determined according to ISO 3960 (AOCS Cd 8b-90) measures the number of hydroperoxides present in the oil and formed by auto-oxidation during storage. The expression of PV was as milliequivalent of active oxygen per kilogram of oil (Meq O2/kg oil).

2.2.3 Determination of the saponification index

The saponification number, which is determined according to ISO 3657 (AOCS Cd-25), is the amount of potassium hydroxide necessary to saponify one gram of fat and is expressed as mg KOH/g of fat.

2.2.4 Determination of extinction coefficients specific for $K_{232}$ and $K_{270}$

Extinction coefficients ($K_{232}$ and $K_{270}$) were determined according to (AOCS Ch 5-91).

The use of ultraviolet absorbance coefficients provides information on the presence or absence of primary and secondary oxidation products in the oil (Tanouti et al., 2011). The higher the values of $K_{232}$ and $K_{270}$, the more the oil is rich in oxidation products.

Extinction coefficient ($K_{232}$ and $K_{270}$) is the specific extinction of a 1% (w/v) solution of oil in cyclohexane in 1 cm cell path length, using a CARY 100 Varian UV spectrometer.

2.2.5 Determination of chlorophyll and carotenoids content

The analysis of the pigments (chlorophylls and carotenoids) was determined according to Minguez-Mosquera et al. (1991) method.

The absorbance of a flask filled up with 7.50 g of oil mixed with 25 ml of pure cyclohexane was measured relative to that of the solvent at 670 nm for chlorophylls and at 470 nm for...
carotenoids using a spectrophotometer (T60 UV-Visible spectrophotometer PG INSTRUMENTS). The pigment content was determined by the following formulas:

\[
\text{Chlorophylls (ppm)} = \frac{(A_{470} \times 106)}{(E1 \times 100 \times d)}, \quad (1)
\]

\[
\text{Carotenoids (ppm)} = \frac{(A_{470} \times 106)}{(E1 \times 100 \times d)}, \quad (2)
\]

E1: extinction coefficient; d: diameter of the tank.

### 2.3 Composition of oils

#### 2.3.1 Composition in free fatty acids

Fatty acid composition was determined following regulation EEC/2568/91 (EEC/2568, 2003). Before analysis, fatty acids (FAs) were converted to fatty acid methyl esters (FAMEs) by transesterification of triglycerides with methanolic potassium hydroxide (2N).

FAMEs were analyzed by gas chromatography using an Agilent Technologies 6890 Network GC System chromatograph equipped with a FID. A split injector used and the injected volume was 1 µL. The column used was a RT-2340 type, (60 m × 0.25 mm × 0.25 µm). The carrier gas was helium and the total gas flow rate was 1 mL/min. The initial and final column temperature was 170 and 230 °C, respectively, and steps of 4°C/min increased the temperature. The injector and detector temperature was 230°C. The elution is therefore carried out in order of increasing molecular weights (C16, C18, ...) and the number of insturations (C18, C18: 1, C18: 2, ...). Fatty acids were identified by their retention times.

Results expressed as the relative percentage of each individual FA present in the sample.

#### 2.3.2 Triglyceride composition

Total TAGs were separated according to the equivalent carbon number (ECN), defined as the total CN in the fatty acid acyl chains minus twice the number of DB per molecule (ECN = CN – 2 DB), through the application of a high performance liquid chromatography (HPLC) (Agilent 1100, Santa Clara, Calif., USA), equipped with an auto-injector.

The triacylglycerols were separated using an RP-18 column (250 × 4 mm) with a particle size of 5 µm and eluted from a column with a mixture of acetonitrile/acetone (50/50) at a flow rate of 1 mL/min. Twenty microliters of the mixture (0.5 g of oil diluted in 10 mL of acetone) was injected into the HPLC column.

#### 2.3.3 Sterol composition

Sterol composition was determined using the NFT 60–254 method as previously described (Hilali et al., 2005). The trimethylsilyls were analyzed using gas chromatography (Agilent Technologies 6890 N Network GC System) equipped with a CP-SIL-5CB apolar capillary column (30 m × 0.25 mm × 0.25 µm). The injector’s temperature was of 230°C. At the outlet of the column maintained at 210°C, the compounds were detected by a FID detector (flame ionization detector) brought to a temperature of 250°C. The flow rate of the carrier gas (helium) was 1.5 mL/min and the injected volume was 5 µL.

#### 2.3.4 Quantification of tocopherols

The quantitative analysis of the tocopherols was determined using a Waters e2695 High Performance Liquid Chromatography (HPLC), equipped with an UV detector at 280 nm by injecting a solution of 20 mg of oil mixed in hexane and isopropanol (99:1) and filtered using a 0.45 µm filter. The capillary column (4.6 mm × 25 cm) used is apolar ODS-2 5 mm. The mobile phase was composed of 4% A and 96% B (A = 0.5% H3PO4 in water; B = Acetone/Acetonitrile (50/50). The flow rate was set at 1.5 mL/min. The detection wavelength was at 292 nm. The quantification of tocopherols was performed using an external standard method and expressed in mg/kg. (ISO 9936, 2006).

### 3 Statistical analysis

All data reported are the means ± SE of three repetitions. Significance of differences between samples was calculated by the ANOVA procedure, using a significance level of $P \leq 0.05$.

### 4 Results and discussion

#### 4.1 Physicochemical characterization of oils

According to Table 1, *Opuntia ficus indica* oil represents the lowest free acidity (0.27 ± 0.005) followed by *Hibiscus sabdariffa* oil (0.55 ± 0.003), then *Nigella sativa* (3.11 ± 0.01) and finally *Pistacia lentiscus* oil with a value of 4.14 ± 0.02 of oleic acid. All founded values were within the range normally encountered for crude vegetable oils.

Our results are comparable to that of Boukeloua (2009) who found a free acidity value for *Pistacia lentiscus* oil about 2.955 ± 0.03.

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**Table 1. Oils quality indices.**

| Oil                  | Acidity      | Peroxide value | Saponification index | Carotenoid | Chlorophyll | $K_{232}$ | $K_{270}$ |
|----------------------|--------------|----------------|----------------------|------------|-------------|-----------|-----------|
| Nigella Sativa       | 3.11 ± 0.01<sup>c</sup> | 4.11 ± 0.025<sup>d</sup> | 190.25 ± 0.05<sup>b</sup> | 1.81 ± 0.002<sup>b</sup> | 2.02 ± 0.01<sup>b</sup> | 2.05 ± 0.001<sup>b</sup> | 0.37 ± 0.005<sup>a</sup> |
| Opuntia ficus indica | 0.27 ± 0.005<sup>a</sup> | 3.71 ± 0.1<sup>ac</sup> | 190.13 ± 0.1<sup>b</sup> | 1.08 ± 0.05<sup>ac</sup> | 2.15 ± 0.01<sup>c</sup> | 1.65 ± 0.01<sup>a</sup> | 0.29 ± 0.005<sup>a</sup> |
| Pistacia lentiscus    | 4.14 ± 0.02<sup>d</sup> | 1.92 ± 0.07<sup>b</sup> | 189.12 ± 0.1<sup>a</sup> | 9.73 ± 0.02<sup>d</sup> | 8.91 ± 0.02<sup>d</sup> | 2.13 ± 0.005<sup>c</sup> | 0.43 ± 0.15<sup>a</sup> |
| Hibiscus sabdariffa   | 0.55 ± 0.003<sup>b</sup> | 0.28 ± 0.08<sup>a</sup> | 193.25 ± 0.25<sup>c</sup> | 1.49 ± 0.002<sup>a</sup> | 1.41 ± 0.008<sup>b</sup> | 2.12 ± 0.005<sup>a</sup> | 0.32 ± 0.004<sup>a</sup> |

Values are given as mean ± SD ($n = 3$). Values followed by the same letter did not share significant differences at $p < 0.05$ (Duncan’s test).
Mahmoud et al. (2016) studied the physicochemical parameters of the oil of Hibiscus seeds grown in Egypt and found a free acidity about 0.78 higher than our findings. This may be due to the presence of fatty acids released by the action of lipase on triglycerides during crushing seeds since free acidity controls the level of hydrolytic, enzymatic or chemical degradation of triglyceride fatty acid chains (Abaza et al., 2002).

Concerning the peroxide values shown in Table 1, Nigella sativa oil has the highest value (4.11 ± 0.025 meq O₂/kg fat), followed by Opuntia ficus indica oil about 3.71 ± 0.1, then the oil of Pistacia Lentiscus about 1.92 ± 0.07 and Hibiscus sabdariffa oil which is characterized by the lowest one about 0.28 ± 0.08.

Detection of peroxide reveals the current level of oxidative rancidity in unsaturated fats and oils and indicates the oxidation state of the fat.

All samples presented PV values inferior to 5 meq O₂/kg fat which lead us to conclude that tested oils are considered to have a low oxidation. Mahmoud et al. (2016) found a higher peroxide value of Hibiscus seeds oil grown in Egypt about 4.82.

The highest saponification index, as shown in Table 1, was found in Hibiscus sabdariffa oil (193.5 ± 0.25 mg KOH/g) and the lowest one was found in Pistacia Lentiscus oil (189.1 ± 0.1 mg KOH/g). On the other hand, the rest of tested oils have almost the same index: Nigella sativa oil was about 190.25 ± 0.05 mg KOH/g and the seed oil of Opuntia ficus indica was about 190.10 ± 0.1 mg KOH/g.

Because there is an inverse relationship between saponification value and weight of fatty acids in the oils, it can be assumed that the oils hold fatty acids with 16–18 carbon atoms with a significant amount of saturated fatty acids in the case of the P. lentiscus oil (Charef et al., 2008).

The chemical examinations of the oils as used in this study were in agreement with the other vegetable oils reported in the literature (Karlenksind 1992).

Our results are comparable to that of Boukeloua (2009) who found a saponification value for Pistacia lentiscus oil about 197.75 to 200.45 mg KOH/g.

Mahmoud et al. (2016) found a higher saponification index of Hibiscus seeds oil grown in Egypt about 196.68 mg KOH/g.

Chlorophylls and carotenoids are involved in auto and photo-oxidation mechanisms (Cheikh-Rouhou et al., 2007). They are responsible for the color of the oil, which is a very important attribute to evaluate its quality.

According to Table 1, the carotenoid and chlorophyll contents for Nigella sativa oil were 1.81 ± 0.002 mg/kg and 2.02 ± 0.01 mg/kg, respectively. Chlorophyll content is much lower than that evaluated by Cheikh-Rouhou et al. (2007) which was about 6.04 ppm.

Opuntia ficus oil contains the lowest carotenoid content (1.08 ± 0.05 mg/kg) and chlorophyll content about (2.15 ± 0.01 mg/kg).

Pistacia lentiscus oil contains the highest amounts of carotenoids (9.73 ± 0.02 mg/kg) and chlorophyll (8.91 ± 0.02 mg/kg).

The carotenoids and chlorophyll contents in Hibiscus sabdariffa oil were 1.49 ± 0.002 ppm and 1.41 ± 0.008 ppm, respectively.

In a study done by Ramadan and Mörsel (2003), the assessment of carotenoids levels was limited to beta-carotene, which represented 0.42 mg/kg in pulp oil, but higher than that in the seed oil. They also explained that pigment content depends on the stage of fruit maturity, extraction process and storage conditions.

The highest extinction coefficient K₂₃₂ was found in Pistacia lentiscus oil (2.13 ± 0.005), followed by that of Hibiscus sabdariffa oil (2.12 ± 0.005), then Nigella sativa which is equal to 2.05 ± 0.001 and finally Opuntia ficus indica oil (1.65 ± 0.01).

Concerning K₂₇₀, results for all tested oils were close to each other, respectively; for Opuntia ficus indica oil (0.29 ± 0.005); for Pistacia lentiscus (0.43 ± 0.15), for Hibiscus sabdariffa oil (0.32 ± 0.004) and finally for Nigella sativa oil which is equal to 0.37 ± 0.005.

The K values measured at 232 nm and 270 nm are associated with changes in the content of conjugated dienes and trienes formed due to polyunsaturated fatty acids oxidation. It is a measure of oxidation/rancidity and oil quality (Abdulkarim et al., 2007).

Generally, K₂₃₂ increase due to inappropriate storage of fruits or old-fashioned extraction or standardization procedure. On the other hand, K₂₇₀ increase when the oil is not fresh and results from a previous harvesting.

### 4.2 Composition of oils

Fatty acid composition is an important characteristic for vegetable oils analyzed using a gas chromatography (GC). Values listed in Table 2 showed a significant variation between the tested samples due probably to the quality of seeds (maturity, storage conditions...), and genetic causes (plant cultivation...).

Polyunsaturated fatty acids constitute the major fraction of total fatty acids in Nigella sativa oil, representing a total of 58.79% and containing basically linoleic acid (C18:2), oleic acid (C18:1) and palmitic acid as major saturated fatty acid in the case of the P. lentiscus oil (Charef et al., 2008).

The fatty acid profile and the high levels of polyunsaturated fatty acids make Nigella sativa oil a special component for nutritional applications.

Results in the Figure 2 showed that PUFA represents the major fraction of total fatty acids (59.56%) in the Opuntia ficus indica seed oil, followed by monounsaturated fatty acids representing a content of 24.07%. Almost the same result was observed with Nigella sativa, in which the PUFA represents 58.79% and monounsaturated fatty acids represents a content of 24.81%. These criteria make the classification of both samples to the category of polyunsaturated oils like most vegetable oils. It is composed mainly by linoleic and oleic acid giving it a great similarity with corn oil. This result is in agreement with the result of Bhira (2012).
The works of Ramadan and Mörsel (2003) have shown that the fatty acid composition of prickly pear oil is highly influenced by climatic factors, soil type and genetic factors in which the seeds are grown.

Tlili et al. (2011) showed that the unsaturated fatty acids of *Opuntia ficus indica* seeds harvested for three different years represent a high content of unsaturated fatty acids (83.2%), linoleic acid, with 56.60%, was the main fatty acid, followed by oleic acid (20.10%).

**Pistacia lentiscus** oil has a major fraction of monounsaturated fatty acids (55.3%), of which oleic acid is the major compound (52.88%). This result is similar to that found by Charef et al. (2008) who demonstrated that oleic acid represents a proportion of 55.3 ± 0.8% in the oil of *Pistacia lentiscus*.

The studies of Dhifi et al. (2013) showed that the first class of fatty acids contained in Tunisian *Pistacia lentiscus* oil were dominated by monounsaturated fatty acids representing...
52.40% of all fatty acids. Saturated fatty acids and polyunsaturated fatty acids accounted for 26.42% and 21.18% of total fatty acids, respectively. The major fatty acid was oleic acid with a quantity of 51.06%. Linoleic acid (C18:2), which is an essential fatty acid, accounted for 20.71% of total fatty acids.

Polyunsaturated and monounsaturated fatty acids are the major fatty acids in *Hibiscus sabdariffa* oil with proportions of 40.98 and 33.69%, respectively, of which the major fatty acid is linoleic acid with a content of 40%. Then, there is oleic acid with a rate of 33.23%. This result is contradictory with that found by Eltayeib and AbdElaziz (2014) who showed that the main fatty acid is oleic acid with a percentage of 47.88% followed by linoleic acid (30.79%).

Cissouma *et al.* (2013) found in their study that oleic and linoleic acids had the highest values of 33.07 and 35.16%, respectively. Of the unsaturated fatty acids, palmitoleic acid was the lowest (0.32%), while palmitic acid (18.76%) was the most abundant saturated fatty acid.

The fatty acid composition showed a high content of unsaturated fatty acids, particularly linoleic acid (35.16%).

TAGs with an equivalent carbon number of 44 represent major proportions (38.41% and 39.15%) respectively in *Nigella sativa* and *Opuntia ficus indica* seeds oils as shown in Table 3. HPLC analyses revealed three individual TAGs: OLL, PLL and OOL. Gharby *et al.* (2015) identified nine triacylglycerols in *Opuntia* oil. The most important are LLL (24.94%), LLO (21.31%), LLP (15.90%) and OOL (13.76%).

TAGs with an equivalent carbon number equal to 46 represent the major fraction in *Hibiscus sabdariffa* oil with 37.16%. HPLC analyses revealed two individual and one combined TAGs: OOL, SLL+PLO and PLP.

However, for *Pistacia lentiscus* oil, TAGs with an equal carbon number equal to 48 represent the major fraction with 53.44% as shown in Figure 3. HPLC analyses revealed three individual TAGs OOO, SOL and POO. This result is in agreement with that reported by Dhifi *et al.* (2013) who found that the majority of the triacylglycerols are in mono and polyunsaturated form and the main constituents were SO + OOP representing 27.58% of total TAG.

Using sterols in vegetable oils, we can identify their composition and determine their quality. It has been reported to be unaffected by environmental factors and/or culture (Ramadan and Mörsel, 2007). Sterols make up about 2% of the oil and are free and esterified sterols.

β-sitosterol is the major sterol in all tested samples (88.22–67.04%) as listed in Table 4, which is the main phytosterol of olive oil and found in many seeds (Ramadan and Mörsel, 2003). This sterol is known for its ability to reduce prostate tumors and strengthening immunity (Rakel, 2018).

β-sitosterol is the major sterol found in *Nigella sativa* oil with a content of 67.04%, followed by stigmasterol (14.14%) and campesterol (13.43%) and then 5-24 stigmastadienol, 5-7-stigmastenol and 5-7-avenasterol. Cheikh-Rouhou *et al.* (2007) found lower proportion of β-sitosterol in Tunisian and Iranian *Nigella sativa* oil (44.5 and 53.9%, respectively).

The major sterols found in *Opuntia* oil were β-sitosterol (82.31%) and campesterol (10.66%). Ramadan and Mörsel (2003) found lower percentage of β-sitosterol in their study that was around 72%.

The major sterols found in *Pistacia lentiscus* oil was β-sitosterol with a proportion of 82.31% followed by campesterol (13.43%) and stigmasterol (2.25%) and cholesterol (0.50%). Dhifi *et al.* (2013) had found in their study a much lower proportion of β-sitosterol (55.55%) and a higher percentage of cholesterol about 44.45%.

The major sterols found in *Hibiscus sabdariffa* oil were β-sitosterol (79.08%), campesterol (12.28%) and stigmasterol.

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Table 3. Triglyceride composition of the vegetable oils (%).

|               | Nigella sativa oil | Opuntia ficus indica oil | Pistacia lentiscus oil | Hibiscus sabdariffa oil |
|---------------|-------------------|-------------------------|------------------------|------------------------|
| ECN42 LLL     | 21.84             | 23.41                   | 0.98                   | 11.7                   |
| ECN 44 PLL    | 38.41             | 39.15                   | 10.6                   | 27.01                  |
| OOL           | 30.11             | 27.05                   | 33.76                  | 37.16                  |
| ECN 46 OOL    | 9.13              | 9.98                    | 53.44                  | 21.09                  |
| ECN50 POP     | 0.51              | 0.41                    | 1.22                   | 3.04                   |

LLL: trilinoleoyl-glycerol; OLL: oleyl-dilinoleoyl-glycerol; PLL: palmitoyl-dilinoleoyl-glycerol; OOL: dioleyllinoleoylglycerol; SLL: stearoyldilinoleoylglycerol; POL: palmitoyl-oleyl-linoleoylglycerol; PPL: dipalmitoyllinoleoylglycerol; OOO: trioleylglycerol; SOL: stearoyloleyllinoleoylglycerol; POO: palmitoyldioleylglycerol; PPO: dipalmitoyl-oleylglycerol.
In addition, cholesterol is present in a rate of 1.41%. These results are comparable to those of Ramadan and Mörsel (2007) who found that the percentage of \( \beta \)-sitosterol was 71.9%, followed by campesterol (13.6%) and cholesterol (1.35%).

According to Mohamed et al. (2007), the sterols of \textit{Hibiscus sabdariffa} L seed oil include \( \beta \)-sitosterol (71.9%), campesterol (13.6%), \( \Delta^5 \)-avenasterol (5, 9%), cholesterol (1.35%) and clerosterol (0.6%).

\textit{Opuntia ficus indica} and \textit{Hibiscus sabdariffa} seed oils were characterized by the highest amount of total sterols followed by that in \textit{Pistacia lentiscus} and \textit{Nigella sativa} oils, as shown in Table 5.

The interest in phytosterols these recent years lies in their potential to decrease coronary mortality and reduce plasma low-density lipoprotein cholesterol level (Gul and Amar, 2006).

Cholesterol found in trace amounts about 1% in all tested samples.

Tocopherols are naturally occurring antioxidants with biological activity (Matthäus and Özcan, 2011). They are responsible for the oxidative stability of the oil and protect fatty acids by eliminating free radicals and reactive oxygen species. The composition of tocopherols varies according to the species and, within the same species according to the genotypes (Demir and Cetin, 1999).

![Fig. 3. HPLC Chromatogram of triglycerides of \textit{Pistacia lentiscus} oil.](image)

| Sterol Composition (%) | \textit{Nigella sativa} oil | \textit{Opuntia ficus indica} oil | \textit{Pistacia lentiscus} oil | \textit{Hibiscus sabdariffa} oil |
|------------------------|----------------------------|---------------------------------|-------------------------------|--------------------------------|
| Cholesterol            | 0.91                       | 0.91                            | 0.9                           | 1.41                           |
| Brassicasterol         | 0.06                       | 0                               | 0                             | 0.17                           |
| Campesterol            | 13.43                      | 10.66                           | 6.41                          | 12.85                          |
| Stigmasterol           | 14.14                      | 1.4                             | 2.25                          | 3.3                            |
| Chlerosterol           | 0.82                       | 0.99                            | 0.72                          | 0.49                           |
| \( \beta \)-sitosterol | 67.04                      | 82.31                           | 88.22                         | 79.08                          |
| \( \Delta^7 \)-24stigmastadienol | 1.22                      | 0.96                            | 0.41                          | 0.75                           |
| \( \Delta^7 \)-stigmastenol | 0.7                        | 1.14                            | 0.58                          | 1.01                           |
| \( \Delta^7 \)-avenasterol | 1.65                       | 1.62                            | 0.34                          | 0.89                           |

(3.3%). In addition, cholesterol is present in a rate of 1.41%. These results are comparable to those of Ramadan and Mörsel (2007) who found that the percentage of \( \beta \)-sitosterol was 71.9%, followed by campesterol (13.6%) and cholesterol (1.35%).

![Table 3. Sterol composition of the vegetable oils (%).](image)
**Table 5. Total sterols and tocopherols content of different oils.**

| Vegetable oil                  | Total sterols (mg/Kg) | Tocopherols (mg/Kg) |
|--------------------------------|-----------------------|---------------------|
| Nigella sativa oil             | 1034                  | 480                 |
| Opuntia ficus indica oil       | 1623                  | 479                 |
| Pistacia lentiscus oil         | 1380                  | 273                 |
| Hibiscus sabdariffa oil        | 1613                  | 371.67              |

Nigella sativa oil have a tocopherol content about 480 mg/kg followed by the one in Opuntia ficus indica (479 mg/kg), then Pistacia lentiscus oil (273 mg/kg) and finally in Hibiscus sabdariffa oil (371.67 mg/kg), as shown in Table 5.

Ramadan and Mörsel (2002) reported that seed oil of Nigella contains a significant amount of tocopherols in which α-tocopherol is the major compound (48%).

According to Gharby et al. (2015), the total tocopherol content of Opuntia oil from Morocco, Tunisia and Germany was 946, 447.2 and 403 mg/kg, respectively. Y-tocopherol (90%) was the major constituent.

According to Dhi et al. (2013) Pistacia lentiscus oil contained 8111.137 mg of tocopherols/kg of oil higher than our findings. α-tocopherol, which has the highest antioxidant activity accounted for 97% of the total tocopherols in Pistacia lentiscus oil.

Tocopherols detected by Mohamed et al. (2007) and Ramadan and Mörsel (2007) were at an average concentration of 2000 mg/kg, including α-tocopherol (25%) γ-tocopherol (74.50%) and δ-tocopherol (0.50%).

Consumption of food rich in natural antioxidants is protective against some types of cancer and may reduce the risk of cardiovascular and cerebrovascular events Aruoma (1998). These actions of antioxidants attributed to their ability to scavenge free radicals, thereby reducing oxidative damage of cellular biomolecules such as lipids, proteins, and nucleic acids (Ferguson, 1995). This richness in tocopherols, including the predominance of α-tocopherol, which is a very good antioxidant fatty phases, contributes to the natural protection and conservation of the oil against oxidation.

5 Conclusion

Recently, there has been an increased interest in antioxidant activity and the ability to improve health. It is therefore important to promote aromatic and medicinal plants (AMP) that have the most popular source of these active substances and incorporate them into our eating habits.

Highlighting physicochemical and biochemical analyzes of four vegetable oils extracted from the seeds in this study permitted a conclusion of their oxidative activity and their potential incorporation in some foods in order to ameliorate their storage.

According to this study, the seeds of Nigella sativa, Opuntia ficus indica, Pistacia lentiscus and Hibiscus sabdariffa seemed to be a good source of antioxidant agents. Different parameters were studied to check the sample’s quality and freshness and a deep investigation of their composition revealed interesting fatty acids, sterols and TAGs content. Linoleic, oleic and palmitic acids were predominant which are important in cognition and motor activity and have beneficial physiological effects in the prevention of cardiovascular diseases and cancer. β-sitosterol is a phytosterol with high benefits in prevention of cancer and strengthening immunity.

An interesting nutritional composition characterized all tested oils. Such study could lead to many industrial applications.

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