Short Note

Chemical composition and sterol profile of Tunisian *Quercus ilex* oil

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Tunisian *Quercus ilex* kernels were analysed for their main biochemical composition and for their oil properties. On a dry weight basis, results showed that the acorn kernel moisture was 15.95%, whereas proteins, ash, fat and carbohydrates contents were 8%, 1.62%, 10% and 64.43%, respectively. Total phenolic (TPC), total flavonoid (TFC) and total tannin (TCT) contents were 232.56 mg GAE/g DW, 7.08 mg CE/g DW and 2.89 mg GAE/g DW, respectively. The main phenolic acids were protocatechuic acid (1441.21 μg/ml), ferulic acid (1207.91 μg/ml) and gallic acid (1101.23 μg/ml). Besides, acorn kernel extracts showed remarkable DPPH radical scavenging activity with IC_{50} values of 318 μg/ml. Gas chromatography revealed that the major fatty acids in acorn oil were oleic, linoleic, and palmitic acids (67.2%, 16.46% and 11.51%, respectively). Stearic, myristic, arachidic, palmitoleic, gadoleic, linolenic and heptadecanoic acids were also found. The β-sitosterol was the predominant sterols form (82.40%). The antioxidant activities in acorn oil were evaluated using DPPH method and β-carotene/linoleic acid assay with IC_{50} values reaching 31.84 μg/ml and 0.69 μg/ml, respectively. The acid, peroxide, saponification and iodine values of acorn kernel oil were 6.65 mg KOH/g oil, 5.66 meq O_2/kg oil, 182.35 mg KOH/g oil and 84.15 g I_2/100 g oil, respectively. Acorn oil contained high amounts of polyphenols, high essential fatty acids and antioxidant potential for producing specific health promoting antioxidants in food and pharmaceutical industry.

Keywords: Acorn. Antioxidant activities. Fatty acids. Oil properties. Phenolic acids. Sterols.

1. INTRODUCTION

Acorn belongs to the family *Fagaceae* whose botanical name is *Quercus*. Generally, these trees are found in sub-tropical climate areas in various parts of the world, including southern Italy, the Balkans, North America, Eastern Hungary, Eastern Asia and Europe [1]. In Tunisia, the *Quercus* trees are one of the most important woody genera because of their tolerance to different environmental and climatic conditions. Since antiquity, acorn fruits are considered as nutritionally rich products and are known to man for their good properties in medicinal use, human foods and animal feeds. A great range of extracted ingredients from acorns such as flour (generally for bread production), starch (in dairy products) [2], coffee substitute beverage (after a roasting process) [3] and oil were included in the human diet [4].
In recent years, acorn fruit have received growing interest due to its important nutritional and medicinal properties. Moreover, acorns were reported as having high contents in starch (48–50%) and low levels of proteins and fat content [5, 2]. They are also considered as excellent sources of fibres, Vitamins (A and E) [6] and minerals [7]. Many other studies indicated that acorn fruits have a potential

source of various natural antioxidant compounds [8, 9, 10]. These antioxidant components have been reported to protect man's health against many chronic and degenerative diseases due to their anti-tumoral, anti-allergic and anti-inflammatory activities [11, 12].
Nowadays, there is an important need to search natural plant resources to produce crude oils that could be considered as functional ingredients for domestic and industrial uses. The commonly used methods for the industrial production of vegetable oils are solvent and cold-pressing extraction, which both produces crude oils. With a relatively low boiling point (69.8°C), n-Hexane, has been selected as an excellent extraction solvent for many years due to its non-polar properties, easy evaporation, simple recovery and limited energy cost [13].
In addition, in the same extraction conditions, the use of n-hexane in solvent extraction is better than mechanical extraction in terms of oil yield and quality. The residues after solvent extraction have an oil content of less than 3% compared to 8-9% in press cakes [13]. Moreover, several environment regulation authorities consider the n-hexane extraction as the best available technology with safety, environment and health concerns.
During the industrial production of vegetable oils by solvent extraction, several non-glyceride constituents are incorporated into triglycerides. Therefore, before human consumption, the extracted crude vegetable oils must be refined to remove undesirable components such as phospholipids, free fatty acids, metals, colouring pigments, and odorous components [14]. The refined vegetable oils are characterised especially by the loss of its natural antioxidant components, such as tocopherols and sterols, which negatively influence its nutritional quality [15]. In Tunisia no work has been undertaken concerning the acorn oil extraction, characterisation and valorisation. Several studies have reported that acorn oil contents ranged from 12% to 30% in white *Quercus* species [8] and in black and red acorn species [16], respectively. Thus, authors highlighted the valuable potentials use of Tunisian *Quercus ilex* acorn kernels and their extracts [2]. In fact, acorn oil represents pertinent characteristics to be considered for industrial purposes. Acorn oil has a good oxidative stability and contains mainly oleic and linoleic acids with an average value of 53-65% and 24-50% [17], respectively, which showed that it represents similar characteristics to those presented by olive oil and other edible vegetable oils extracted from sunflower, peanut, cotton and avocado [4]. Besides acorn oils are also good sources of bioactive compounds with different industrial applications. Their chemical composition may depend on many factors such as: plant variety, geographic and climatic conditions, extraction techniques, and storage conditions.
In this regard, the objective of this study was to identify the biochemical composition of Tunisian acorn *Quercus ilex* fruit as well as its phenolic compounds and antioxidant activity. Another scope of this work was to determine the physico-chemical properties and antioxidant activity of extracted acorn oil. Results could help improve economic and health utilisation of acorn fruit as new sources of edible oils for human diet and industrial uses.

2. MATERIALS AND METHODS

2.1 ACORN PREPARATION

Quercus ilex acorns were manually collected from Ain Drahem, a region in Jendouba, Tunisia, during the month of November 2018. Kernels were hand-peeled, dried at 40°C for 3 days and then milled into flour in a blender. The acorn flour was stored in glass flasks at about 4°C for further oil extraction and analysis.

2.2 CHEMICAL COMPOSITION OF ACORN FLOUR

2.2.1 Moisture, ash, protein, fat, carbohydrates and mineral contents

Acorn flour moisture, ash and protein contents were determined according to AOAC Methods [18]. The fat content was determined by extracting a known weight of acorn flour with n-hexane for 8h, using a Soxhlet apparatus. Total carbohydrates were calculated by difference (100 - sum of (% protein, % fat, % ash and % moisture)). Mineral elements were analysed using an Atomic Absorption Spectrophotometer (Hitachi Z-6100, Japan) according to the method described by the AOAC [18].

2.2.2 Phytochemical analysis and antioxidant activity

Preparation of extracts

Sample extracts were obtained by mixing 2.5 g of dry acorn flour with 25 ml of methanol (80%) for 30 min at room temperature [19]. The mixture was centrifuged at 3000 g for 10 min. The supernatant was kept at -25°C until being used for quantification of total phenolics and antioxidant activity assay.

2.2.3 Total phenolic contents (TPC)

The quantification of TPC was determined by the Folin–Ciocalteu spectrophotometric method (UV–VIS) as described by De Pascual et al. [20]. 1 ml of the methanol extract was mixed with 1 ml of Foline–Ciocalteu's phenol reagent and allowed to react for 5 min. Then, 10 ml of 7% sodium carbonate solution (w/v) were added, and the final volume was made up to 25 ml with distilled water. After 1 h of reaction at ambient temperature, the absorbance at 720 nm was measured by a spectrophotometer. TPC of acorn samples was expressed as mg gallic

acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid. All samples were analysed in three replications.

2.2.4 Total flavonoid contents (TFC)

TFC were measured according to Dewanto et al. [21]. 250 µl of the methanolic extract was mixed with 75 µl of $NaNO_2$ (5%). After 6 min, we added 150 µl of 10% aluminium chloride and 5 min later, 500 µl of NaOH (1M) was added to the mixture. Finally, the mixture was adjusted to 2.5 ml with distilled water. The absorbance was determined at 510 nm. TFC of acorn samples was expressed as mg catechin equivalents (CE) per gram of dry weight (mg CE/g DW). All samples were analysed in three replications.

2.2.5 Total condensed tannins (TCT)

50 µl of the methanolic extract was mixed with 3 ml of 4% methanol vanillin solution and 1.5 ml of H_2SO_4. The absorbance was measured at 500 nm after 15 min. TCT of acorn were expressed as mg catechin equivalents (CE) per gram of dry weight through the calibration curve of catechin. All samples were analysed in three replications.

2.2.6 Identification of phenolic compounds using RP-HPLC

The analysis phenolic compounds are done by RP–HPLC of the type Agilent Technologies 1100, equipped with a visible UV detector to length variable wave and provided with a C18 Hypersil ODS column (250 × 4.6 mm, 4 µm), at ambient temperature. The mobile phase is given as follows: solvent A: acetonitrile and solvent B: H_2O at 0.2% sulfuric acid. The elution gradient chosen was as follows: 15% A/85% B 0-12 min, 40% A/60% B 12-14 min, 60% A/40% B 14-18 min, 80% A/20% B 18-20 min, 90% A/10% B 20-24 min, 100% A 24-28 min [22]. The flow rate is done at 0.5 ml/min.

2.2.7 DPPH assay

The antioxidant activity was determined using the diphenylpicrylhydrazyl radical (DPPH) test according to Hantano et al. [23]. 2 ml of methanolic extracts with different concentrations ranging from 1 to 200 µg ml^{-1} were added to 0.5 ml of a 0.2 $mmol^{-1}$ DPPH methanolic solution. The mixture was then left standing at room temperature for 30 min in the dark, and then the absorbance was measured at 517 nm. For each dilution of the extract, the DPPH scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A0 - A1) / A0 \times 100$$

Where:
A_0 is the absorbance of the control at 30 min,
A_1 is the absorbance of the sample at 30 min.

The antiradical activity was finally expressed as IC_{50} (µg ml^{-1}). All samples were analysed in three replications.

2.3 OIL EXTRACTION

An ultrasound cleaning bath (Kunshan Meimei instrument Co., Ltd., Jiangsu, China) with a 3L used capacity was used to extract acorn oil. The acorn flour (5 g) was mixed with 150 ml n-hexane into a 250 ml beaker. The beaker was immersed into an ultrasonic clearer bath (70 kHz, 140 W), with the liquid level in the beaker kept lower than that of the cleaner tank, for 30 min at 40°C. The extract was filtered, and the acorn oil was separated from the organic solvent by using a rotary vacuum evaporator at 40°C. The obtained acorn oil was drained under a nitrogen stream (N_2) and was then stored at -8°C until further analysis.

2.4 OIL ANALYSIS

2.4.1 Chemical analysis

The determination of the acid value, peroxide value, iodine value, saponification value, specific gravity, the refractive index at 20°C and the specific absorptivity values K_{232} and K_{270} of the extracted acorn oil were determined according the Official methods [24].

2.4.2 Thermal oxidation

The oxidative stability of the acorn oil was determined using a Rancimat 743 Metrohm apparatus. The stabilization level was measured by the oxidative-induction time using 2.5±0.01 g of oil at 121.6°C. Sample of oil was placed in the apparatus and analysed simultaneously. The induction times were recorded automatically by the apparatus' software and taken as the breaking point of the plotted curves [25].

2.4.3 β-carotene / linoleic acid assay

The evaluation of oxidation inhibition by β-carotene/linoleic acid oxidation system was performed by means of a spectrophotometric assay based on the discoloration of β-carotene due to oxidation induced by oxidative degradation products of linoleic acid [26]. The content of total carotenoids was determined by measuring the absorbance of 0.25 g of oil dissolved in 10 ml of hexane at 449 nm. Quantitative analysis was performed by a calibration curve of β-carotene and the total carotenoid content expressed as mg of β-carotene equivalent per kg of oil.

2.4.4 Identification of fatty acids by GC/FID analysis

Fatty acid composition was determined as methyl esters following the procedures described in the enclosures of the European Parliament and the European Council in EEC regulation 2568/91 [27]. In general, fatty acids were converted to fatty acid methyl esters (FAMEs) before being injected into a gas chromato-

graph (GC agilent system 7890A) equipped with a capillary column HP-5MS (30 m × 250 μm × 0.25 μm). The separation was carried out with a programmed temperature (110°C held for 5 min, increase of 3°C min^{-1} to 150°C and held for 16.33 min, increase of 4°C min^{-1} to 230°C and held for 27 min) and an FID detector at 150°C. The results are expressed in percentages for chromatographic areas.

2.4.5 Sterols analysis

The content and composition of sterols were determined according to the official method of the International Olive Oil Council [28]. 5 g of acorn oil were added to 500 µL of the 0.2% α-cholestanol solution, used as internal standard and then evaporated to dryness. After that, sample was saponified with 50 mL of potassium hydroxide in ethanolic solution (2 N). The unsaponifiable fraction was removed with diethyl ether. The unsaponifiable sterol fraction was separated using thin layer chromatography. Separation and quantification of the silylated sterol fraction were performed by capillary gas chromatography (GC2010, Shimadzu, Japan) using a Supelco (SPBTM-5 24034, Bellefonte, USA) capillary column (30 m, 0.25 mm i.d. and 0.25 mm film thickness) and a flame ionization detector (FID). The column temperature was 260°C. Detector and injector temperatures were 290 and 280°C, respectively. Helium was used as a carrier gas with 1 ml/ min flow rate and the split ratio was 50:1.

2.5 STATISTICAL ANALYSIS

All experiments were performed in triplicate. The values of different parameters were expressed as the mean ± standard deviation ($\bar{X}$ ±SD).

3. RESULTS AND DISCUSSION

3.1 CHEMICAL COMPOSITION, PHENOLIC COMPONENTS AND ANTIOXIDANT ACTIVITY OF ACORN FLOUR

The chemical composition of acorn flour is shown in Table I. Carbohydrates, protein, ash and moisture contents were 64.43%, 8%, 1.62% and 15.95%, respectively. These results were in accordance with those reported by Zarroug et al. [2] and Masmoudi et al. [29]. The acorn flour was found also to contain significant amounts of minerals whereas calcium (Ca) was the most abundant element (5617 ppm), followed by potassium (K) (3262 ppm) and sodium (Na) (2371ppm). The fat content of the studied acorn (10%) was within that reported by Charef et al. [30] on Algerian acorn, but higher than those found in *Quercus lobata* (4.25%) [31], *Quercus shrubs* (4.05%) [32] and *Quercus robur* (5.3%) [33]. In fact, the variation in fat content of acorns species could be attributed to many factors such as plant variety, cultivation climate, ripening stage and the extraction method used [34]. Note that these results

Table I - Chemical composition, phenolic components and antioxidant activity of Tunisian *Quercus ilex* flour

Components	Contents
Moisture (%)	15.95±0.02
Fat (%)	10±0.03
Proteins (%)	8±0.05
Ash (%)	1.62±0.05
Carbohydrates (%)	64.43±0.01
K (ppm)	3262±0.25
Ca (ppm)	5617±0.55
Na (ppm)	2371.5±0.75
TPC (mg GAE/g DW)	23.56±0.05
TFC (mg CE/g DW)	7.08±0.04
TCT (mg CE/g DW)	2.89±0.05
IC_{50} (µg/ml)	318±0.23
Reducing power EC_{50} (µg/ml)	200±0.16

TPC: Total phenolic content; TFC: Total flavonoid content; TCT: Total condensed tannins; IC_{50}: The half-maximal inhibitory concentration; Values are means ±SD of three determinations.

indicated that the acorn kernel is fairly poor in fat and cannot be considered an oleaginous seed, which possesses 30-45% oil content [35]. However, acorn oils might be used for industry application because of their healthy features, like other plant oil sources, such as wheat germ (8-14%) or amaranth (4.9-8.1%) [36].

As illustrated in Table I, TPC, TFC and TCT were 23.56 mg GAE/g DW, 7.08 mg CE/g DW and 2.89 mg CE/g DW, respectively. In contrast to our finding, the study of Custódio et al. [37], on acorns collected from Portugal, revealed lower contents of TPC and TCT, but higher levels of TFC. However, the content of TPC was in accordance with that (33.11%) registered by Masmoudi et al. [29] on *Quercus suber*. L.

The TCT determined in our study was close to that of cork acorns (3.1 mg CE/g DW) [38] and lower than that of *Pinus parviflora* (21 mg CE/g DW) [39, 40]. With their higher tannin concentrations, acorn *Quercus* species are known to be a very astringent fruit. In addition, it the importance of the nature of solvent used in extraction has been mentioned, as demonstrated by many recent works such as that of Custódio et al. [37]. They showed a variation in phenolic contents of the same plant extract using different solvents. For example, in leaves, the methanol extracts had the higher TPC followed by water and hexane extracts. Variability in polyphenols, flavonoids and tannins contents seems to be due to the nature of species and the geographical growth sites [40]. The obtained data on the antioxidant activity shows that the methanolic extract of acorn can be used to reduce the stable DPPH radical with IC_{50} values of 318 µg/ml. The obtained results found were in line with others suggestions that acorns are a source of biologically active compounds with an antioxidant status [8].

Table II - Phenolic acid contents (µg/ml) of Tunisian *Quercus ilex* flour

Phenolic compounds	Contents (µg/ml)
Gallic acid	1101.23±0.26
Catechin hydrate	15.27±0.03
Protocatechuic acid	1441.21±0.01
3,4-dihydroxybenzoic acid	86.50±0.32
Gentisic acid	627.03±0.02
Caffeic acid	82.50±0.01
Chlorogenic acid	665.04±0.34
Syringic acid	115.05±0.61
Valoneic dilactone acid	208.87±0.31
Vanillic acid	680.37±0.25
P-coumaric acid	154.25±0.02
Ferulic acid	1207.91±0.01
Naringin	19.12±0.05
Salycilic acid	14.03±0.12
Ellagic acid	123.51±0.21
Quercetin	7.99±0.31
Naringenin	15.07±0.13
Kaempferol	3.30±0.14

Values are means ±SD of three determinations.

3.2 RP-HPLC ANALYSIS

The phenolic acid contents determined by RP-HPLC method of acorn flour are presented in Table II. Results were indicating that protocatechuic acid was the dominant phenolic compound (1441.21 µg/ml), followed by ferulic acid (1207.91 µg/ml), gallic acid (1101.23 µg/ml), vanillic acid (680.37 µg/ml), chlorogenic acid (665.04 µg/ml), gentisic acid (627.03 µg/ml), valoneic dilactone acid (208.87 µg/ml), p-coumaric acid (154.25 µg/ml), ellagic acid (123.51 µg/ml) and syringic acid (115.05 µg/ml). The contents of protocatechuic, ferulic and gallic acids were much higher than those reported for other species such as *Quercus salicina*, and *Quercus serrata* [41]. However, the concentration of gallic and ellagic acids were lower than those noticed in the *Quercus ilex* collected from Spain [8]. In addition, Santos et al. [42] have already declared that the most abundant phenol in *Quercus super* species were gallic, protocatechuic and ellagic acids. According to studies conducted by Phung et al. [41] the amounts of phenolic compounds varied among *Quercus* species, plant organs and extracting solvents. In this study, the observed phenolic acids may be used for anti-mutagenic, antimicrobial and antioxidant activities in plants.

3.3 PHYSICOCHEMICAL COMPOSITION OF ACORN OILS

In order to justify the extraction of acorn oil, it was necessary to study its functional properties. Thus, the physicochemical properties of acorn oil are presented in Table III. The specific gravitational value (0.9) and refractive index (1.45) were close to those reported by Karabas [43] for Turkish acorn oil. However, the iodine value (84.15 g I_2/100 g oil) was lower than those reported by Karabas [43] (99.6 g I_2/100 g oil) and Makhlouf et al. [44] (103.68 g I_2/100 g oil) for *Quercus ilex* oil from Algeria. A high iodine value indicated a high degree of unsaturation (abundance in unsaturated fatty acids) and a good quality of oils [45]. The acid and peroxide values of acorn oil were on the average of 6.65 mg KOH/g oil and 5.66 meq O_2/kg oil, respectively, indicates a higher oxidative stability which is confirmed by the low values of K_{232} and K_{270} (2.47 and 1.46, respectively). The saponification value of acorn oil was recorded as 182.35 mg of KOH/g of oil, which was within range 175-250 normally found in other seed oils such as raspberry seed, sunflower and corn [46]. The obtained saponification value was like those reported for other vegetable oils such as soybean or olive oils [47]. However, the saponification value of Tunisian acorn oil was lower than those reported by Al-Rousan et al. [36] for different acorn species oils from Jordan (*Quercus aegilops* (204.2 mg of KOH/g of oil), *Quercus infectoria* (219.4 mg of KOH/g of oil) and *Quercus calliprinos* (192.6 mg of KOH/g of oil).

3.4 FATTY ACIDS COMPOSITION

The fatty acid composition of acorn oil was presented in Table IV. As, shown, most of the fatty acids in acorn oil were unsaturated fatty acids (UFA), in which monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were 67.94% and 17.35%, respectively. The most abundant fatty acids were oleic acid (67.2%), linoleic acid (16.46%) and palmitic acid (11.51%). The literature offers similar data for the percentages of the mentioned fatty acids for acorn oil [43, 48]. In addition, myristic, arachidonic, palmitoleic and gadoleic acids were minor fatty acids constituting 0.09%, 0.37%, 0.08% and 0.61%. Many researchers show that the fatty acid composition of acorn oil was

Table III - Physicochemical characteristics of *Quercus ilex* oil

Parameters	Acorn oil
Refractive index (20°C)	1.45±0.12
Specific gravity (25°C)	0.90±0.11
Acid value (mg KOH/g oil)	6.65±0.01
Peroxide value (meq O_2/kg oil)	5.66±0.04
Saponification value (mg KOH/g oil)	182.35±0.01
Iodine value (g I_2/100 g oil)	84.15±0.01
K_{232}	2.47±0.01
K_{270}	1.46±0.04
Oil stability index (h)	10.72±0.01

K_{232}, K_{270}: Specific extinctions coefficients at 232 and 270 nm; Values are means ±SD of three determinations.

Table IV Fatty Acids contents (% total fatty acids) of *Quercus ilex* oil

Fatty acids	Acorn oil
Myristic acid (C14:0)	0.09±0.22
Palmitic acid (C16:0)	11.51±0.52
Heptadecanoic acid (C17:0)	0.08±0.11
Stearic acid (C18:0)	2.66±0.65
Arachidic acid (C20:0)	0.37±0.41
Palmitoleic acid (C16:1)	0.08±0.52
Cis-10-heptadecanoic acid (C17:1)	0.05±0.22
Oleic acid (C18:1)	67.2±0.12
Gadoleic acid (C20:1)	0.61±0.42
Linoleic acid (C18:2)	16.46±0.92
Linolenic acid (C18:3)	0.89±0.40
∑ SAFA	14.71±1.20
∑ MUFA	67.94±0.57
∑ PUFA	17.35±0.84

Values are means ±SD of three determinations. SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

similar to that of olive oil in terms of the most abundant fatty acids (oleic, linoleic and palmitic acid). Since the acorn oil was rich in both oleic and linoleic acids, it might be considered healthier for the human diet [36]. It has long been acknowledged that plant oils containing relatively low concentrations of omega-6, and higher levels of monounsaturated fatty acids (MUFA) (mainly oleic acid) may contribute to the lower rate of CHD and a nutritional perspective [49].

3.5 STEROL COMPOSITION

Tunisian *Quercus ilex* oil was characterized for its sterol composition and results were presented in Table V. 10 sterols were identified at different significant contents. These results confirmed the high nutritional value of the genus *Quercus ilex*, since sterols are known to have a wide range of biological activity. In this study, the β-sitosterol was the predominant sterol (82.40%), followed by the Campesterol (9.39%). The Stigmasterol and Δ^{5}-Avenasterol contents accounted for 2.95% and 2.04%. In acorn oil, cholerosterol, $\Delta^{5,24}$-stigmastadienol, $\Delta^{5,23}$-Stigmastadienol, Δ^{7}-Stigmastenol and Δ^{7}-Avenasterol were present with lower amounts (<1%). The obtained results for β-sitosterol were lower than those reported for *Quercus ilex* (91.29%) and *Quercus suber* (90.38%) acorns from Tunisia [50], but higher to those reported in *Quercus robur* (64.3%) and *Quercus rubra* (68.1%) acorns collected in Latvia [33]. However, the Campesterol and Stigmasterol values were higher than those found in *Quercus ilex* (2.44% and 1.80%) and *Quercus suber* (2.22% and 1.61%) oils [50]. The differences in the sterol contents are not only affected by the species, but also by the genotype. As it was known in plant sterols, β-sitosterol and campesterol are membrane constituents of plants that effectively reduce serum LDL cholesterol risk [51] and protect from heart disease. Now, these sterols are added to many dairy products, bakery goods, sausages and fruits juices [36].

Table V - Sterols composition (%) of *Quercus ilex oil*

Sterols	Composition (%)
Cholesterol	1.51
Campesterol	9.39
Stigmasterol	2.95
Chlerosterol	0.78
$\Delta^{5,23-}$ Stigmastadienol	0.51
β-sitosterol	82.40
Δ^{5-}Avenasterol	2.03
$\Delta^{5,24-}$ Stigmastadienol	0.18
Δ^{7-} Stigmastenol	0.10
Δ^{7-} Avenasterol	0.15

3.6 TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF ACORN KERNEL OIL

Table VI reports the TPC and the IC_{50} values for the tests of antioxidant activity according to the free radical DPPH scavenging assay and β-carotene/linoleic-acid oxidation of the acorn oils. Results showed that acorn oil witnessed a higher amount of TPC (378.47 mg GAE/g oil) when compared to those found by Al-Rousan et al. [36] for the oils of *Quercus aegilops* (84 mg/kg), *Quercus infectoria* (95 mg/kg) and *Quercus calliprinos* (109 mg/kg). Certainly, this higher number of phenolic compounds will increase the oxidative stability of acorn oil. This amount of TPC is compared to most edible oils except olive oil, which is considered as the richest source of phenolic compounds in the Mediterranean diet. Results showed that the IC_{50} value of acorn oil was about 31.84 μg /ml and were comparable with the IC_{50} value of the β-carotene/linoleic acid

Table VI - Total phenolic content and antioxidant activity of *Quercus ilex* oil

	TPC (mg GAE/g oil)	IC_{50} (μg/ml)	
		β-carotene/linoleic acid	DPPH
Quercus ilex oil	378.47±0.02	28.87 ±0.14	31.84±0.52

TPC: Total phenolic content; DPPH: 1.1-diphenyl 2-picrylhydrazyl; IC_{50} : The inhibitory concentration (μg/ml), amount of antioxidant needed to decrease the initial DPPH concentration by 50%; Values are means ±SD of three determinations.

test (28.87 µg /ml). These lower IC_{50} values correspond to a higher antioxidant activity of the studied oil. This result indicates that the acorn oil was stable against oxidation and capable of capturing and neutralising radicals. Therefore, the high content of TPC (378.47 mg GAE/g DW) in acorn oil might be a factor that influences the antioxidant activity observed. These results on antioxidants were similar to those obtained by Rakić et al. [52] that indicated that acorns from *Quercus robur* and *Quercus petraea* are materials rich in polyphenols and tannins. In the same context, Kamalak et al. [53] evaluated the nutritive values of five oak species, namely, *Quercus branti*, *Quercus coccifera*, *Quercus cerris*, *Quercus libari*, and *Quercus infectoria* based on their phenolic composition and antioxidant activity. On the other hand, it can be mentioned that among Quercus species, *Quercus ilex* presented the lowest β-carotene contents (473±497 µg/g DW), while the other species presented similar values (1301-1421 µg/g DW) [4].

3.7 OXIDATIVE STABILITY

The oxidative stability of acorn oil has never been investigated in previous studies. The result of the Rancimat test was illustrated in Table I. The stability of acorn flour oil expressed as the oxidation induction time was about 10.72 h. The acorn oil was more resistive to oxidation than cactus (7 h) [54], melon (3.8 h), pumpkin (3.74 h) and watermelon (1.72 h) [55] seed oils. The oxidative stability of acorn oil was lower than that of date seed oil (45 h), argan (31 h), sesame (28.5 h) and olive oil (27 h) [54, 56]. This high oil stability might be justified by the high contents of MUFA, PUFA and natural antioxidants, such as phenolic compounds [57]. The same tendency was observed by Aparicio et al. [58], who exposed a strong correlation between the oleic/linoleic ratio, the tocopherols contents and the olive oil oxidative stability measured by Rancimat.

CONCLUSION

This study revealed that Tunisian *Quercus ilex* kernels are a source of many essential nutrients and natural antioxidant having a beneficial effect on human health. Results showed also that *Quercus ilex* oil was a source of several bioactive substances such as phenolic compounds and sterols. Besides, acorn oil revealed a high content of unsaturated fatty acids showing a similar composition with many other edible vegetable oils. All these good qualities make acorn oil a suitable ingredient for many industrial applications in food, pharmaceutical, nutraceutical and cosmetic domains. Highlighting the good chemical composition, oxidative stability and the richness in natural antioxidants of acorn oil, in this study, permitted to establish its potential applications in food industries as crude or refined oil.

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