

Study of Physicochemicals Characteristics and Antioxidant Capacity of Cork Oak Acorns (*Quercus suber* L.) grown in Three Regions in Tunisia

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ABSTRACT

This study focuses on the valorization of the Tunisian cork oak acorns in minerals, bioactive compounds and antioxidants. It involved the study of physico-chemical characteristics of acorns from *Quercus Suber*. L, of three Tunisian northern regions: Sejnén, Nefza and Ain Drahem. The kernels and hulls of acorns were analyzed for their antioxidant capacity using DPPH free radical scavenging activity. The total phenolics, flavonoid and carotenoid contents were measured using standard methods. The multi-elemental analysis was performed by atomic emission spectrometry with inductively coupled plasma (ICP). Acorns exhibit a degree of organic matter and moisture varies according to the region and part of the studied plant. The ICP analysis shows the abundance of cork oak acorns in calcium, magnesium and especially in potassium (6.942 g.kg⁻¹). Kernels have higher polyphenol contents (66 - 77 mg GAE.g⁻¹). The radical scavenging activity of methanol extracts of cork oak acorns shows that the Sejnén kernels have significant antiradical activity with an IC50 in the order of 6.94 µg.mL⁻¹. Analysis of sugars in the fruits by HPLC revealed the presence of fructose, galactose, sucrose and maltose. This plant can be used as an inexpensive source of natural antioxidants and mineral supplements of food.

INTRODUCTION

The Cork oak is an evergreen tree of the genus *Quercus* of the Fagaceae family formerly Cupulifère, it is best known for its thick bark used for making caps. The wood of the cork oak is used as charcoal or firewood due to its considerable calorific value of 7000 cal at 25% of moisture (Natividade, 1956). In Europe, Asia, North Africa, Middle East, and North America, acorns were once a staple (Brandis, 1972). They are also a valuable food for pets, birds and wildlife. Currently, cork oak acorns (*Quercus Suber*. L.) are traditionally marketed and consumed in North Africa. When dried and ground into flour, it can be transformed into a paste to use instead of bread, or it is used as a stomachic and antidiarrheal when mixed with honey

(Sijelmassi, 1993). Epidemiological studies in Serbia have suggested that consumption of cork oak acorns may promote general human health (Jevtovic, 1980). Oak kernels (*Quercus semen*) were traditionally used in medicine, particularly roasted ones (*Quercus semen tostum*) as astringents, antidiarrhoeals and antidotes (Tucakov, 1971), since it has been established that a diet rich in fruits reduces the risk of cardiovascular diseases, cancer and other diseases caused by oxidative stress. A reevaluation of acorns and their use would be desirable. The acorns of all species must be tested and adjusted for their antioxidant activity. Antioxidants are usually used to prevent chronic and degenerative diseases with intermediate scan of free radicals (Rodriguez et al., 2007; Prior and Gu, 2005). The role of free radical scavenging antioxidants has attracted much attention of not only scientists but also general public, above all, the natural antioxidants contained in fruits, vegetables, spices and dietary supplements (Sies, 2010). Domingues, Ammar et al. and Conde et al. cited several bioactive polyphenols, compounds such as flavonoids, tannins and ellagic

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acid and its diglucoside derivatives that are present in different parts of the cork oak (fruits, leaves and cork) (Domingues, 2009; Ammar *et al.*, 2005; Conde *et al.*, 1998). Flavonoids have not only demonstrated beneficial effects as antioxidants (Garcia-Saura *et al.*, 2005) but also the ability to regulate the expression of adipocytokine gene (Tsuda *et al.*, 2006). Ellagic acid is strongly believed to have antioxidant and antiviral activity. The composition of the cork oak acorns in tocopherol (α -tocopherol and β -tocopherol) are respectively 38 and 74 mg. kg⁻¹ of dry matter (Cantos *et al.*, 2003). Bainbrides (1986) confirmed that cork oak acorns are rich in carbohydrates, lipids, sterols and amino acids and are low in protein. Rodríguez-Estévez *et al.* (2008) have enabled to determine the composition of kernels and hulls in minerals, ash, fat, fiber, crude protein. Given the richness of the fruit in antioxidant and given the traditional use of the flour for bread making in parts of North Africa, this fruit can replace in case of famine, some seeds such as corn wheat or barley. Therefore, we have undertaken this work to explore antioxidant potential minerals and sugar composition of fruits of cork oak. The influence of harvest location and solvent extract was also studied.

Experimental Section

Plant material

Samples that are subject of this study (1kg) were collected during the month of December 2011 in three regions of Tunisia Sejnen (202 m altitude), Nefza (500 m altitude) and Ain Drahem (800 m altitude) located in northern Tunisia. Acorns were cleaned to remove all foreign matter (dust, dirt, immature and damaged fruits). Three batches containing thirty fruits for each region were formed; the hull and kernel of fruit of each batch were then separated and cut into small pieces. All samples were preserved in the dark at a dry place until further use.

Chemical reagents

The chemical reagent DPPH (2, 2-Diphenyl picrylhydrazyl) and Folin-Ciocalteu phenol were purchased from Sigma Co. (St. Louis, MO, USA). Catechin, gallic acid (98%) and β -carotene were from Sigma Aldrich (Steinheim, Germany). Concentrated hydrochloric acid (37%), absolute ethanol ($\geq 99.8\%$) and absolute methanol ($\geq 99.8\%$) were purchased from Panreac Química, S.A. (Barcelona, Spain). Aluminum chloride-6-hydrate AlCl₃.6H₂O (>99%), sodium nitrite NaNO₂ and all other chemical reagents used were obtained from Sigma Co.

Physicochemical characterization

The moisture content was determined according to the method of Twidwell *et al.* (2002). The dry matter content was determined in accordance with French standard NF B 51-004 (AFNOR, 1986). A mass of 5 g of plant material was introduced into a porcelain crucible and dried in an oven at 105 °C until a constant mass. The mineral matter content in the fruits of the cork oak was determined according to French standard T 211 OM-93. The formol index was evaluated in the fruit extract after soaking

overnight. It was determined using the AFNOR method (V. 76-102.1985). The titratable acidity was measured by the titrimetric method according to the Tunisian standard NT 52.15 (1982). Titration of free acidity of the aqueous extracts of all cork oak samples was determined by an assay using a sodium hydroxide solution NaOH (0.01 M) in the presence of phenolphthalein as a color indicator.

Ash content and mineral composition

The method NF.EN.1482 (March 2003) was used: 5g sample was incinerated with high pressure in a muffle furnace (MLS 1200) for 24 h at 550 °C. The residue of incineration was extracted with 5 ml of HNO₃ 65% and 50 ml of water. The mixture was heated a few minutes in a boiling water bath to dissolve the ash. After cooling, the solution was filtered and then placed in a 50mL volumetric flask, the volume is adjusted by adding distilled water, where Fe, Cu, Mn and Zn and other mineral composition were directly measured at the suitable wavelength for each element, using standard solutions for calibration purposes. The concentration of each analyte in the different samples was expressed as mg.100 g⁻¹ of fresh material from their calibration curves taking into account the dilution factor.

Antioxidant Content

Preparation of extracts

Different solvent systems were used to evaluate the best extraction solvent on the extraction of phenolic compounds: S₁ methanol/water (80:20, V/V), S₂ ethanol/water (50:50, V/V); S₃ methanol/acetic acid (99:1). 100 mg of fresh samples were extracted by 10 mL of different solvents. The supernatant was centrifuged (2500 rev.min⁻¹) filtered through Whatman N° 40 (Whatman International England).

Total Phenolic Content

The amount of total phenols was determined using the Folin-Ciocalteu reagent according to the method of Singleton and Rossi Jr (1965), and 1 mL of the extract was added to 5 mL of distilled water and 1 mL of Na₂CO₃ (20% ; W/V). After 3 min, 1 ml of the Folin-Ciocalteu reagent was added and mixed. After 30 min of incubation in the dark at 40 °C, the absorbance of all samples was measured at 765 nm using the Shimadzu UV-Vis spectrophotometer T60U. The results were expressed in terms of mg of equivalent of Gallic acid per gram of sample, using a calibration curve of the gallic acid.

Total Flavonoid Content.

The total flavonoid content in *Quercus Suber* was determined according to Zhishen *et al.* (2009) using a method based on the formation of complex flavonoid-aluminium. A volume of 125 μ L of methanol extract was added to 75 μ L of a solution of NaNO₂ (5%; W/V). The mixture was left to stand for 6 minutes, then 150 μ L of a solution AlCl₃ 6H₂O (10 %) freshly prepared were added, after 5 min of rest, 500 μ L of NaOH (solution 1M) was added to mixture. The final volume of the

solution was adjusted to 2500 μL with distilled water. The absorbance was measured at 510 nm (Shimadzu UV-Vis spectrophotometer T60U). The total flavonoid content of acorn was expressed in terms of catechin equivalent ($\text{mg}\cdot 100\text{ g}^{-1}$).

Total Anthocyanin Content

The total anthocyanin contents were determined according to the procedure of Swain and Hillis (1959). 5 g of acorn were submitted to extraction with 10 mL of ethanol (80%; V/V) for 2 hours with agitation (magnetic stirrer). Then the samples were filtered. The filtrates were completed to a total volume of 10 mL with 80% aqueous ethanol. Then 0.02 mL of HCl (37%) was immediately added. The absorbance was read at 525 and 595 nm to quantify anthocyanins using the Shimadzu UV-Vis spectrophotometer T60U. The total anthocyanin contents were quantified according the relation:

$TA = (A_1 - A_2) \times 0.879 \times Ve \times 2 \times 10 \times F$, where A_1 is the absorbance measured at 525 nm, A_2 measured at 595 nm, 0.879 is the extinction coefficient of anthocyanins. Ve : is the total volume recovered after extraction 100 g sample, and F is the dilution factor

Determination of total Carotenoid Contents

The total carotenoid contents were determined according to the procedure described by Rousseff *et al.* (1987). 200 mg of fresh samples were extracted by 10 ml of acetone-water (80:20; V/V), then allowed to stand for 30 min at 4 °C. The extract was filtered through Whatman No. 40. The organic phase obtained was extracted repeatedly with hexane ($3 \times 10\text{mL}$). The combined extracts were subjected to a rotary evaporator (Laborota 4000 Germany) under reduced pressure at 30 °C. The absorbances of all samples were measured at 450 nm using the Shimadzu UV-Vis spectrophotometer T60U. The results were expressed in equivalent β -carotene, compared to a range standard prepared starting from a mother ethanolic solution of β -carotene.

Evaluation of Antioxidant Activity

The free radical-scavenging activity was determined using stable 2,2-diphenyl-1-picrylhydrazyl (DPPH \cdot) radical according to Brand Williams *et al.* (1995). 2 mg of each sample were dissolved in 1mL of methanol. An aliquot of this solution was mixed with 1 mL of 0.5 mM DPPH in methanol and the final volume adjusted up to 5 mL, so that final concentrations of the samples were in a range from 1,25 to 20 $\mu\text{g}\cdot\text{mL}^{-1}$. Then, the mixtures were shaken vigorously and left 30 min to stand in the dark. The reduction of DPPH radical is accompanied by a color change from violet (DPPH \cdot) to yellow (DPPH-H). The absorbance of mixture was measured using a spectrophotometer at 517 nm in a Shimadzu UV-2401 PC spectrophotometer using methanol as blank, after establishing the stationary. One milliliter of 0.5 mM DPPH diluted in 4 mL of methanol was used as control. The yellow color of solution indicates the end of the reaction. This reduction capacity is determined by a decrease in absorbance

induced by free radical inhibitors (Majhenic *et al.*, 2007). Antioxidant activity was expressed as percentage of inhibition in relation to control according to the equation proposed by Yen and Duh (1994): $I (\%) = 100 \cdot (A_0 - A_s) / A_0$, where A_0 is the absorbance of the control (containing all reagents except the tested sample), and A_s is the absorbance of the tested sample. The effectiveness of acorn extracts in scavenging free radicals was evaluated as the concentration of acorn extracts in the reaction mixture that caused a decrease in the initial DPPH concentration by 50%, defined as IC50. These values were calculated from the graph plotting inhibition percentage against extract concentration.

2.6. Sugar content. Soluble sugars were determined by High Performance Liquid Chromatography HPLC method AOAC 980-13 982-1 (1990), using a refractive index detector (RID) (Perkin Elmer). The separation was carried out on a SGE SS Exsil amino column NH_2 (150 x 4.6mm I.D.). The elution solvents used were acetonitrile and water (82:18 v/v). The column was operated at 25 °C with 1 mL/min flow rate. Sample injection volume was 5 μL . The resultant peak areas in the chromatograms were plotted against calibration curves obtained from multiple standard solutions (external standard method), in a concentration range of 1 $\text{mg}\cdot\text{mL}^{-1}$ for each compound. All the experiments were run in triplicate. The mean values and standard deviations were calculated using the Microsoft Excel software (Microsoft Corporation, Redmond, WA).

RESULTS AND DISCUSSION

Physicochemical characteristics

Some chemical properties of cork used in this study are presented in table 1. As can be seen from these data, the moisture content in the samples ranges from 38% to 51%. The highest values are those of Ain Drahem where rainfall exceeds 1559 mm per year. There is the weak acidic character in acorns, the measurable acidity varies between 5.2×10^{-3} and 7.1×10^{-3} $\text{mmol}\cdot\text{L}^{-1}$. The kernels have higher acidity than the hulls. Formaldehyde indexes were similar, however, the lowest value of the acidity and formol index was observed in Sejnán variety. The mineral content of cork fruits is presented in table 2. Based on these results, the fruits are rich in calcium, magnesium, sodium and especially in potassium ($6.942\text{ g}\cdot\text{kg}^{-1}$). Besides potassium, the other minerals are present in the hulls. These results were similar to those reported by Özcan and Bayçu (2005) for potassium content ($7.849\text{ g}\cdot\text{kg}^{-1}$) of fruit of the Turkish variety *Quercus petraea* subsp. Several epidemiological studies have established a link between a diet rich in potassium and a reduced risk of hypertension (Houston and Lanham-New, 2008); stroke and renal calculus (Bazzano *et al.*, 2001; Curhan *et al.*, 1993). In studies that have established these relationships, this contribution was closely associated with the consumption of fruits and vegetables which contain other beneficial compounds for health (He and Mac Gregor, 2008). WHO (2005) recommends increasing dietary potassium intake to lower blood pressure and reduce the risk of cardiovascular disease, stroke and coronary heart disease in adults.

Table 1: Some chemical properties of cork oak varieties.

Samples	Moisture %	Titratable acidity (10^{-3} mmol.L ⁻¹)	Formol index
HuA	50.99 ± 0.07	5.6	4.66 ± 0.41
HuS	49.12 ± 0.08	5.2	4.11 ± 0.14
HuN	49.91 ± 0.19	5.7	4.61 ± 0.29
KeA	40.83 ± 0.12	7.1	6.51 ± 0.29
KeS	38.31 ± 0.14	6.8	6.17 ± 0.05
KeN	39.13 ± 0.21	7.1	6.48 ± 0.53

HuN – fruit hull of Nefza, HuA – fruit hull of Ain drahem, HuS– fruit hull of Sejnan
KeN – fruit kernel of Nefza, KeA – kernel of Ain drahem, KeS – fruit kernel of Sejnan

Table 2: Mineral content of acorn cork oak expressed as mg/kg of fresh mater.

Minerals	[mineral] mg.kg ⁻¹			
	HuA	HuS	KeA	KeS
Copper	4.49 ± 0.06	62.30 ± 7.20	3.60 ± 0.12	3.35 ± 0.03
Iron	27.94 ± 3.20	19.41 ± 2.41	10.62 ± 1.20	13.98 ± 2.16
Manganese	27.14 ± 2.13	89.10 ± 7.36	11.64 ± 1.02	9.04 ± 0.86
Zinc	9.22 ± 1.06	11.86 ± 2.41	5.37 ± 0.52	7.26 ± 0.72
Calcium	1396 ± 676	1636 ± 846	23.85 ± 3.12	25.01 ± 2.12
Magnesium	781.6 ± 37.1	784.65 ± 54.10	453.23 ± 43.12	476 ± 43
Sodium	504.95 ± 27.10	691.68 ± 42.20	214.16 ± 23.12	228.48 ± 31.12
Potassium	5942 ± 483	3112 ± 347	36825 ± 870	6942 ± 880

Table 3: Bioactives extract content in hulls and kernels of cork oak.

Samples	Carotenoids (mg EβC.100g ⁻¹ DM)	Anthocyanins (mg .100g ⁻¹)	Flavonoids (mgECat.g ⁻¹ DM)
HuA	30.16±0.45	0.22±0.10	0.20±0.02
HuS	13.19±0.15	0.49±0.13	0.27±0.03
HuN	20.61±0.06	0.43±0.11	0.19±0.06
KeA	6.39±1.05	0.19±0.05	0.35±0.05
KeS	5.90±0.25	0.59±0.07	0.49±0.07
KeN	7.04±0.95	0.32±0.06	0.38±0.02

Table 4: Antioxidant activity and total phenolic content of acorn cork oak

Samples	Total phenolic (mg of GAE.g DM ⁻¹)			
	Solvent S ₁	Solvent S ₂	Solvent S ₃	IC50 in S ₂ (μg /mL)
KeS	68.10 ± 0.09	77.11 ± 1.20	42.94 ± 0.10	6.94
KeA	52.24 ± 0.19	66.18 ± 1.06	36.35 ± 0.71	8.80
KeN	51.24 ± 0.61	67.37 ± 0.13	30.73 ± 0.75	8.11
HuS	47.75 ± 0.31	757.24 ± 0.19	29.64 ± 0.44	9.94
HuA	35.52 ± 1.05	4 49.52 ± 0.15	21.23 ± 0.19	10.34
HuN	37.62 ± 0.75	551.38 ± 1.02	19.04 ± 0.08	9.90

S₁: methanol / water (80/20 : v/v ; S₂ : methanol / acetic acid (99/1 : v/v) ;
S₃: ethanol / water (50/50 : v/v)

Table 5: Contents of sugars in hulls and kernels of the cork oak (% DM; mean±SD, n=3)

Samples	Sugars (% DM)				
	Fructose	Glucose	Sucrose	Maltose	Total sugars
HuA	0.54± 0.04	0.12± 0.003	---	0.07± 0.002	0.73± 0.05
HuS	0.76± 0.06	0.42± 0.01	0.02± 0.001	0.07± 0.003	1.28± 0.08
KeA	3.00± 0.23	1.06± 0.03	0.06± 0.001	0.43± 0.03	4.55± 0.44
KeS	3.94± 0.34	1.50± 0.05	1.30± 0.040	0.34± 0.02	7.08± 0.62

Antioxidant Content in *Quercus Suber*

Carotenoids

The content of carotenoids has been quantified (Tab.3). The results concerning the hulls of acorn of the three regions studied show high levels of carotenoids (13.19 to 30.16 mg EβC.100 g⁻¹ DM), kernels are less rich in carotenoids.

Anthocyanins

The anthocyanin contents of the studied acorns are low (0.05 to 0.13 mg.100g⁻¹), with hulls having maximum values. The low values observed could be explained by the immature acorns studied which are collected in December. In spring, acorns stain red or orange which is accompanied by an influx of anthocyanins.

Indeed, light, temperature, wind and humidity are factors involved in the rate of anthocyanins.

Flavonoids

Were present in very small quantities in all investigated samples, with higher content in kernel of acorn of Sejnan (0.49 mg ECat.g⁻¹). This is in agreement with the results of some studies conducted in Spain in 2009 by Domingues *et al.* on cork oak fruits that showed a content of 0.6 mg ECat.g⁻¹.

Total Phenolic Content

The total polyphenol contents of the extracts obtained by the three solvents S₁, S₂ and S₃ are listed in Table 4. Significant

differences were observed, the amounts of total phenolics in cork oak fruits extracts for different parts of cultivars show that the methanol 1% acetic acid provide the highest levels varied widely, ranging from 77 to 49 mg of GAE.g⁻¹ DM. The lowest levels of total polyphenols were recorded for the extracts obtained from ethanol/water (42.9 to 19 mg of GAE.g⁻¹ DM). This study reported the potential effects of extracting solvent on antioxidant activity estimations for acorns. The methanol 1% acetic acid is the recommended solvent to prepare antioxidant extracts from acorns samples. We observe that for all the extraction solvents used, the kernel has higher polyphenol contents than the hulls. The highest content of polyphenols was obtained from Sejnán plant extract. Additional research is required to investigate the influence of extraction solvent on the chemical composition of acorns antioxidant extracts.

Evaluation of Antioxidant Activity

The evaluation of the antioxidant capacity of the samples was carried out by determining the concentrations required to inhibit stable radical by 50% (IC₅₀), which are shown in Table 3. It can be noted that all the extracts showed appreciable free radical scavenging activities in the DPPH assay. However, kernel extracts (IC₅₀ = 6.94- 8.11) inhibit DPPH radical more rapidly than hulls extracts (IC₅₀ = 9.9 - 10.3). This is deduced by the results obtained by comparing the IC₅₀ (Figures 1(a) and 1(b)).

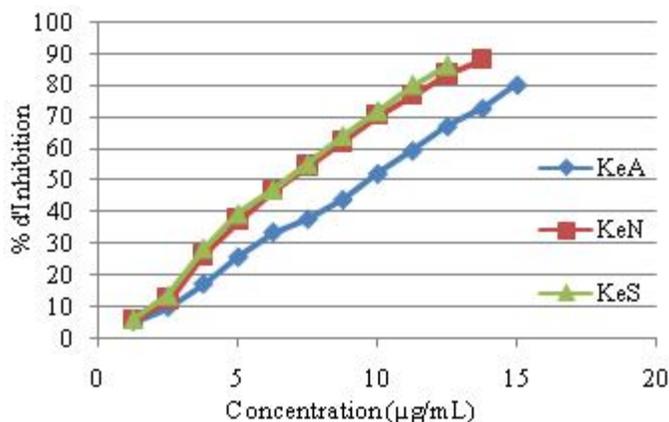


Fig.1.a : DPPH scavenging activity of methanol extracts of kernels acorns.

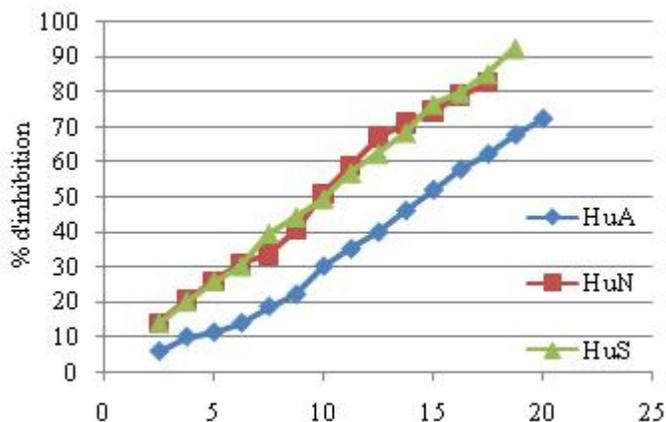


Fig.1b : DPPH scavenging activity of methanol extracts of hulls acorns.

Our results are in agreement with some previous investigations concerning types of *Quercus acorns* polyphenols and their antioxidant activity. Rakić *et al.* in 2007 have also suggested *Quercus robur* and *Quercus cerris* to be convenient nutritional components, with significant antioxidant effects (resp., IC₅₀ = 8.04 and IC₅₀ = 8.88).

Gallic acid, digallic acid and gallotannin were identified in the ethyl-acetate fraction of *Q. acutissima* acorns and caused its high antioxidant efficacy (Lee *et al.*, 1992). The obtained results have provided further grounds for establishing *Q. suber* acorn kernels as a source for functional food preparation

Correlation between Total Polyphenol Content and Anti-radical Activity

The phenolic contents and flavonoid contents of kernel was higher than hulls, and the kernel exhibit good antioxidant activity. A positive correlation ($r = 0,925$) was found between the free radical scavenging DPPH (IC₅₀) and the total phenolic compounds content. The polyphenols in extracts of cork oak acorns are probably responsible for the anti-radical activity of these extracts. The capacity of free radical scavenging of antioxidant does not always correlate with the capacity of antioxidation such as lipid peroxidation inhibition. The antioxidation capacity can be accessed from the extent of suppression of lipid peroxidation by antioxidant compared with that in its absence. It is determined not only by the capacity for scavenging free radicals but also by the localization of antioxidant, interaction with other antioxidants, and mobility of antioxidant at the microenvironment. These factors should be considered to understand the action and capacity of antioxidants (Niki, 2010).

Sugars

The cork oak acorns are the sweetest and most popular oak acorns, we therefore measured and identified sugars in the Tunisian variety by high performance liquid chromatography HPLC.

Soluble sugars identified and quantified in the acorns are listed in Table 5. Fructose and glucose are the major kinds of sugar while sucrose and maltose are present in smaller quantities. The lower amounts of sucrose found in these fruits may be due to the enzymatic hydrolysis of this sugar into glucose and fructose during ripening process. The kernel of acorns contains more sugars (4.55 % to 7.08% by dry weight) than the hull (0.735 to 1.28%). Acorns grown in Sejnán have the highest sugar levels. The sugars are transformed under the action of invertase, sucrose is converted by enzymatic hydrolysis in fructose (levulose) and glucose (dextrose) at the stage of maturation. Fruits with a high invertase activity, such as prickly pear fruit, kaki or pomegranates, reach a glucose/fructose ratio near 1 (Ayaz *et al.*, 2000). A ratio higher than 1 has been found in some wild fruits, such as wild red-bilberries (*Vaccinium vitis-idaea L.*) or blackthorn fruits (*Crataegus monogyna Jacq.*), which usually have more than 3-fold more glucose than fructose (Barros *et al.*, 2010; Souci *et al.*, 2008). On the contrary, all oak acorns samples analyzed in this study

showed an inverse behavior, with a ratio glucose/fructose between 0.30 and 0.55, which means that fructose content represented twice glucose content. Moreover, this profile can be related to the intense and pleasant sweet taste of oak acorns when they are completely ripe, since fructose is the sweetest of all the naturally occurring carbohydrates (Hanover and White, 1993).

CONCLUSION

This study conducted on the fruit of the Tunisian cork oak has allowed to determine its physical and chemical characteristics and mineral composition. We observe the richness of this plant in minerals (Ca, K); it can therefore compete with wheat, rice and corn.

This plant showed the presence of phenolic compounds and showed a certain level of antioxidant activity in the kernels as well as in the hulls of acorns. It revealed the presence of four sugars, fructose and glucose being the main sugars.

The results showed that the samples with the highest levels of polyphenols have the lowest IC50. Thus, the antioxidant power of the extracts studied is mainly due to the polyphenols. Great variability in antioxidant and sugar contents of these acorns was observed depending on geographic location, stage of maturation, climate and nature of the extraction solvent.

This natural product can be used as an inexpensive source of natural antioxidants and mineral supplements, especially for food industry which is expanding fast nowadays. Further investigations such as isolation and characterization of the active compounds polyphenols and carotenoids are currently in progress.

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