

Full Length Research Paper

Antioxidant capacity of Tunisian virgin olive oils from different olive cultivars

Samia Dabbou^{1*}, Faten Brahmi¹, Sihem Dabbou¹, Manel Issaoui¹, Samira Sifi² and Mohamed Hammami^{1*}

¹ Laboratory of Biochemistry, UR "Human Nutrition and Metabolic Disorders" Faculty of Medicine, Monastir, Tunisia

² National office of oil, Tunis, Tunisia

Accepted 23 April, 2011

This work aimed to study the antioxidant capacity of phenolic extracts of four Tunisian olive oils from Chaïbi, Oueslati and two mixture olive cultivars in relation to their lipid composition and α -tocopherol content. Oleic acid showed high levels (>70%) except for Mix1 (66.2%). α -tocopherol contents were higher than 300 mg kg⁻¹ except for Oueslati oil (171.6 mg kg⁻¹). Total phenol content ranged between 396 and 652 mg kg⁻¹. Furthermore, the highest antioxidant capacity in virgin olive oil measured by the two methods total antioxidant activity by ABTS test (TAA-ABTS) and radical scavenging activity by DPPH assay (RSA-DPPH), was observed in Mix2 (0.9 mmol TE kg⁻¹ and 72.3%, respectively) which showed the correlation between the antioxidant capacity of virgin olive oils studied with polar components and lipid profile, important components to their shelf life. The results obtained from this study imply that Tunisian cultivars are a precious source of bioactive compounds with high antioxidant activities that have a synergistic effect.

Keywords: virgin olive oil, antioxidant capacity, α -tocopherol, fatty acids, pigments, phenolic compounds.

INTRODUCTION

Oil genuineness is a very important aspect of quality edible oils (Bendini *et al.*, 2007). Extra virgin olive oil have been identified as one, if not the major healthful component of the Mediterranean diet, a diet that is synonymous with lowered rates of heart disease, cancers and extended life expectancy. All these properties were attributed to the very high content of mono-unsaturated fatty acids (MUFA) and phenolic compounds (polyphenols and tocopherols) from simple to very complex structures (Robards *et al.*, 1999; Owen *et al.*, 2000^a; McDonald *et al.*, 2001). Nowadays, it is well known that the dietary MUFA healthy effects were essentially attributed to decreased endothelial activation (Massaro and Di Caterina, 2002), and LDL susceptibility to oxidation (Bonanome *et al.*, 1992). Tocopherols are amphipathic molecules consisting of a polar chromanol ring and a lipophilic isoprenyl tail. They behave as potent antioxidants, since they are able to scavenge reactive oxygen species and lipid

peroxyl radicals in cell membranes and other lipid environment, preventing the autoxidation of polyunsaturated fatty acids (Bramley *et al.*, 2000). In addition, several lines of evidence indicate that tocopherols influence cellular responses to various oxidative stresses by modulating signal-transduction pathways (Bramley *et al.*, 2000; Sattler *et al.*, 2003; Maeda *et al.*, 2008).

Polyphenols, although their minor concentrations in olive oil, have shown to be more effective antioxidants in vitro than tocopherols. These antioxidant properties arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol derived radical to stabilise and delocalise the unpaired electron (chain-breaking function), and from their ability to chelate transition metal ions (termination of the Fenton reaction) (Rice-Evans *et al.*, 1997; Covas *et al.*, 2006). Previous studies of possible mechanisms of phenol action indicate that these compounds are also able to interact with the biological systems, act as bioactive molecules and may play a major role in the prevention of certain diseases. Polyphenols are also considered as an important

*Corresponding author E-mail: samia_1509@yahoo.fr, Tel : +216 73 462 200, Fax : +216 73 462 737.

parameter for the evaluation of virgin olive oil (VOO) quality as they contribute greatly to oil flavor and taste (Gutiérrez-Rosales *et al.*, 2003), as long as the oil is protected from autoxidation (Servili & Montedoro, 2002; Mateos *et al.*, 2005).

Nevertheless, the concentration of these compounds in VOO are strongly affected by agronomical and technological factors, such as olive cultivar (Tura *et al.*, 2007; Baccouri *et al.*, 2008), place of cultivar (Cerretani *et al.*, 2006), climate, degree of maturation (Baccouri *et al.*, 2008), crop season (Gómez-Alonso *et al.*, 2002) and production process (Ranalli *et al.*, 2001; Cerretani *et al.*, 2006). However, interactions and recycling are very common mechanisms in the action of antioxidants.

The most extensive cultivation areas of olive in Africa are in the North where Mediterranean climate prevails. Tunisia, which is among the olive growing countries in this region, is considered as a very important country in the olive oil-producing world. It is the largest African exporter and the fourth largest exporter worldwide after Spain, Italy and Greece (IOC, 2009). The olive tree (*Olea europaea* L.) is present in practically every region of the country, up to the border of the southern desert. Many olive varieties are present in Tunisia, but there are two that stand out: Chemlali and Chétoui (Ben Rouina *et al.*, 2002; Dabbou *et al.*, 2009). The other varieties known by the second cultivars are losing their origin location and are forgotten both by farmers and scientists (Issaoui *et al.*, 2007). Although there has been much agreement about the antioxidant effects and composition of olive oil quality and cultivars, there are few reports about the antioxidant profile of field-grown olive cultivars of Tunisian origin. Therefore, this paper was designated to investigate the antioxidant and the free radical-scavenging potential of phenolic extracts of four virgin olive oils obtained from the second Tunisian cultivars in relation to fatty acids, α -tocopherol and pigments using two different tests RSA-DPPH and TAA-ABTS in order to find out the most valuable oil for disease preventing diets.

MATERIALS AND METHODS

Plant material and growing areas selected

Olive fruits (*Olea europaea* L.) of the varieties Chaïbi, Oueslati and two mixture olive cultivars (Mix1 and Mix2) were collected at full maturity in the 2007-2008 season from trees all located in the same orchard (a National Collection, 20 km away from Tunis, in North-East Tunisia) and which benefited from the same cultural practices. The experimental field in Béjaoua is characterised by a rainfall of 500 mm/year and a temperature varying from a minimum of 18 °C to a maximum of 32 °C.

Oil extraction

Olive fruits were hand-picked from the tree, using rakes. After harvesting, the olive samples were transported on the same day

to the laboratory, to be transformed into oil within 24 h. Only healthy fruits, without any kind of infection or physical damage, were processed and olive oil was obtained using a laboratory scale system. The olive fruits were washed, leaves removed, crushed, and the resulting olive paste malaxed at 27 °C for 30 min. Finally, the oil was separated by centrifugation and immediately transferred into dark bottles, which were stored at 4 °C until analysis.

Analytical methods

Quality parameters

Determination of free fatty acids (FFA), peroxide value (PV) and UV absorption characteristics at 232 nm (K232) and 270 nm (K270) were carried out following the analytical methods described in the European Union Regulation EEC 2568/91 and EEC 1429/92 (EUC, 1991).

Fatty Acid Composition

The fatty acid composition of oil samples was determined as fatty acid methyl esters (FAMES) by capillary gas chromatography analysis. The FAMES were prepared as described in the Regulation EEC/2568/91, EEC/1429/92 of the European Union Commission and the International Olive Council (EUC, 1991; IOC, 2009). The chromatographic separation was carried out using a Hewlett-Packard (HP 5890) chromatograph, a split/splitless injector, and a flame ionization detector (FID) linked to an HP Chemstation integrator. A fused silica capillary column HP-Innowax (30 m x 0.25 mm x 0.25 μ m) was used with nitrogen as the carrier gas at a flow rate of 1 ml min⁻¹; flame-ionization detection temperature 280 °C; injector temperature 250 °C and an oven temperature programmed from 180 to 250 °C. Results were expressed as relative percent of total area (Dabbou *et al.*, 2009).

Total phenols and o-diphenols

The phenolic fraction was determined with Folin–Ciocalteu reagent, according to the method of Montedoro *et al.* (1992). 10 g of olive oil was homogenized together with 10 ml of a methanolic solution [methanol/water (80:20, v/v) and 20 mg of Tween 20 (2%, v/w)] for 1 min at 15,000 g, using an Ultra-Turrax homogenizer (T25, IKA Labortechnik, Janke & Kunkel, Staufen, Germany). The suspension obtained was then centrifuged (10 min, 4 °C and 5,000 g). The extraction was repeated twice. To eliminate the oil droplets, the methanolic extract was kept in a freezer for 24 h. Then, the amount of total phenols and o-diphenols were determined colorimetrically (Montedoro *et al.*, 1992) and the results are expressed as hydroxytyrosol equivalents.

Pigments

Carotenes and chlorophylls were determined colorimetrically at 470 and 670 nm, respectively, using 7.5 g of oil dissolved in cyclohexane (Minguez-Mosquera *et al.*, 1991). The results were expressed as mg of pheophytin “a” and lutein per kg of oil,

respectively.

***α*-tocopherol**

The content of *α*-tocopherol in the VOOs was evaluated following the method reported by Gimeno *et al.* (2000). 200 μ l of a solution of oil was transferred to a screw capped tube, where 600 μ l of methanol and 200 μ l of the internal standard solution (300 mg ml⁻¹ of *α*-tocopherol acetate in ethanol) were added. HPLC analysis was conducted using an Agilent Technologies system model 1100 (Agilent Technologies, DE, Germany), composed of a vacuum degasser, quaternary pump, thermostated column compartment and a photodiode array detection system model 1200 (Agilent Technologies, DE, Germany). A column was Tracer Extrasil ODS-2 (150 mm x 4.6 mm I.D., 5 mm particle size). The injection volume was 50 μ l. The mobile phase was methanol–water (96:4, v/v) and the elution was performed at a flow-rate of 2 ml min⁻¹. The analytical column was kept at room temperature. To determine the compounds in the samples, the working standard solutions were always analyzed together with the samples and peak-area ratios were used for calculations following the internal standard method. Detection was performed at 292 nm and each run lasted 6 min (Dabbou *et al.*, 2010^a).

Determination of the antioxidant activity

The following 2 tests were used

(1) Radical Scavenging Activity (RSA) using DPPH assay

RSA was determined using a 1,1-diphenyl-2-picrylhydrazil radical (DPPH*) scavenging assay as the procedure described previously (Brand Williams *et al.*, 1995; Dabbou *et al.*, 2010^b). One hundred μ l of each methanolic extracts was mixed with 3.9 ml of 6 x 10⁻⁵ M methanolic DPPH. Mixtures were vortexed at the highest setting and left in the dark in a water bath for 60 min at 25°C (sample 60). The decrease in DPPH radical absorption of the resulting solution, after exposure to radical scavengers, was then measured spectrophotometrically at 515 nm. The absorbance of the DPPH* without any antioxidant in methanol (control 0) was measured daily and kept in dark. The RSA toward DPPH* was expressed as the % of scavenging effect = 100 x (1 – absorbance of sample 60/absorbance of control 0). The concentration of sample required to scavenge 50% of the DPPH free radical, was determined from the plot between %inhibition and concentration and labelled as EC50. The experiments were performed in triplicate.

(2) Total antioxidant status (TAA test with ABTS)

A stock solution of 7 mM of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) aqueous solution was prepared. ABTS radical cation (ABTS^{•+}) was generated by the interaction of ABTS (7 mM) and potassium persulfate (2.45 mM final concentration) (Rice-Evans *et al.*, 1996; Re *et al.*, 1999). The stock solution was kept in the dark at room temperature for 16 h, allowing it to form the ABTS radical (ABTS^{•+}). The radical was stable in this form for more than two days when stored in these conditions. Finally, the stock solution

was diluted with ethanol (approx. 1/88) to obtain an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30°C. Reagent blank reading was taken (A0). For the spectrophotometric assay, 3.9 ml of the ABTS^{•+} diluted solution were mixed with 100 μ l of phenolic fraction or trolox. Mixtures were vortexed vigorously for 30 s and allowed to stand for 6 min in the dark at room temperature. Then, the absorbance for each sample (ABTS^{•+} solution plus compound, At) was measured at 734 nm and corrected for the absorbance of a control (ABTS^{•+} solution without test sample, A0). The absorbance reading was taken at 30°C exactly 6 min after initial mixing. The radical-scavenging activity of the samples was expressed as mM Trolox equivalent (TE) per kg.

Accelerated Oxidation Tests

Measurement of the induction period (IP) was performed using the well-established Rancimat method. A Rancimat apparatus, model 734 (Metrohm, Herisau, Switzerland) was operated at 120°C (Tura *et al.*, 2007). A dry air flow of 20 l h⁻¹ was passed through the oil sample (3 ± 0.001 g). The volatile oxidation products, arising from the oxidation of the oil, were dissolved in cold milliQ water (60 ml) causing an increase of the electrical conductivity parameter value. This test is based on the change of conductivity of the distillate collected from oil subjected to an accelerated oxidation at a prefixed temperature. The change of conductivity is due to the production of formic and other carboxylic acids because of the oxidation of secondary products during the forced oxidation. All tests were performed in triplicate. The time taken to reach an inflection point at the induction curve was considered as the IP.

Statistical analysis.

The results are reported as mean values and standard deviation. Significant differences among varieties studied were determined by an analysis of variance which applied a Duncan test with a 95% significant level (P<0.05), using the SPSS programme, release 11.0 for Windows (SPSS, Chicago, IL, USA). All analyses were carried out in triplicate and the results were presented as means of three repetitions.

RESULTS AND DISCUSSION

Analytical parameters of olive oils. Physico-chemical characteristics of all the oils produced and analysed showed values (Table 1) completely within the limits established by IOC (2009) as required for the 'extra virgin' category.

Fatty acid composition

The distribution of fatty acids, from all tested samples, covered the normal expected range for olive oil (Table 1). Results showed that fatty acid composition of the oils, one of the characteristics predominantly genetically determined (Hrnčirik & Fritsche, 2005) differ according to the variety. In fact, olive oils were low in palmitic acid and

Table 1. Quality indices and fatty acids composition (%) evaluated in the virgin olive oils studied.

<i>Cultivars</i> <i>Parameter</i>	<i>Mix1</i>	<i>Chaïbi</i>	<i>Mix2</i>	<i>Oueslati</i>
FFA (% of Oleic acid)	0.28±0.03c	0.51±0.02b	0.17±0.01d	0.75±0.03a
PV (meq O ₂ kg ⁻¹)	10.07±0.24c	12.66±0.09b	8.67±0.02d	15.67±0.07a
K232	2.16±0.13a	2.14±0.00c	2.29±0.07b	2.19±0.04b,c
K270	0.20±0.02a	0.21±0.02a	0.19±0.01a	0.20±0.02a
Fatty Acids %				
Palmitic	11.25±0.14b	10.40±0.01a	9.38±0.03c	9.45±0.04b
Palmitoleic	0.48±0.01a	0.57±0.01b	0.39±0.01c	0.49±0.01c
Margaric	0.09±0.01a	0.08±0.01b	0.09±0.01a	0.05±0.01c
Margaroleic	0.12±0.00b	0.11±0.01c	0.12±0.00a,b	0.09±0.01d
Stearic	2.95±0.05b	2.82±0.02c	3.13±0.02a	2.60±0.02d
Oleic	66.21±1.12c	69.94±0.40b	69.03±0.01b	72.81±0.66a
Linoleic	14.92±0.19b	13.41±0.11c	15.47±0.03a	10.92±0.11d
Linolenic	0.89±0.01a	0.82±0.02b	0.90±0.01a	0.73±0.01c
Arachidic	0.37±0.01a	0.38±0.01a	0.36±0.01a	0.32±0.01b
Lignoceric	0.10±0.05a	0.10±0.01a	0.15±0.05a	0.09±0.09a
Oleic/Linoleic ratio	4.44±0.02d	5.22±0.01b	4.46±0.00c	6.67±0.01a

Values are the means of the three different VOO samples (n=3) ± standard deviations. Different letters indicate significant differences ($p < 0.05$) between cultivars.

high in oleic acid (>70%) except for Mix1. Furthermore, studied olive oils seem to be rich in linoleic acid (>13.4%) except for Oueslati. The values obtained for Oueslati and Chaïbi olive oils were similar to those obtained previously by these cultivars planted in the north of Tunisia (Hannachi et al., 2007; Taamalli et al., 2010). However, when grown in the south and the centre, Oueslati oils behave differently showing higher values for palmitic and linoleic acid but lower contents for oleic acid (Dhifi et al., 2004; Krichène et al., 2007; Dabbou et al., 2010b; Ouni et al., 2011). Furthermore, the Oleic/Linoleic acid ratios of Chaïbi and Oueslati (5.22 and 6.67, respectively) were relatively higher than those of Mix1 and Mix2 oils (<4.5).

Pigments

Results showed significant differences between cultivars ($p < 0.05$) where the levels of these pigments ranged from 8.28 to 12.51 mg kg⁻¹ and from 13.05 to 24.17 mg kg⁻¹, for carotenes and chlorophylls respectively (Table 2). These levels of green–yellow colors of VOO are influenced by olive cultivar (Minguez-Mosquera et al., 1991), and could be considered as a product freshness indicator. Furthermore, pigments showed quite similar contents in Mix1 and Chaïbi oils whereas Mix2 oil had the lowest levels.

Total Phenols and O-diphenols

The total phenols and o-diphenols evaluated by the colorimetric method (Table 2) showed significant

differences ($p < 0.05$) between the different studied VOO where the ranges were wide from 395.67 to 651.55 mg kg⁻¹ for total phenol concentration and from 191.81 to 376.31 mg kg⁻¹ for o-diphenols. However, the highest amount of the total phenols and o-diphenols were present in Mix2 olive oil followed by Mix1 oil. Oueslati and Chaïbi oils presented approximately the same content in total phenols whereas a wide difference was observed for o-diphenols. Previous researches on Oueslati cultivar grown in the centre and the south of Tunisia showed lower values for total phenols (Dhifi et al., 2004; Krichène et al., 2007; Dabbou et al., 2010b).

α -tocopherol is the major constituent in olive oils with respect to tocopherols (Hrncirik & Fritsche, 2005). As shown in Table 2, the range of α -tocopherol contents in the studied olive oils is wide. There were significant differences in the α -tocopherol contents which are highly variety-dependent ($p < 0.05$) (Owen et al., 2000b). In fact, α -tocopherol amounts ranged from 171.64 mg kg⁻¹ (Oueslati) to 457.85 mg kg⁻¹ (Mix1). The α -tocopherol contents of Oueslati oils, when compared with various studies, are the same although planted in different locations of Tunisian area (Krichène et al., 2007; Dabbou et al., 2010b).

Accelerated Oxidation Tests

The accelerated oxidation test (Table 2) is usually useful to evaluate the effects of natural or chemical antioxidants on olive oil resistance against oxidation, and to compare the storage stability of different oils. Results showed that these values varied according to the cultivar. Oueslati

Table 2. Stability parameters evaluated in the virgin olive oils studied.

<i>Cultivars</i> <i>Parameter</i>	<i>Mix1</i>	<i>Chaïbi</i>	<i>Mix2</i>	<i>Oueslati</i>
Carotenes (mg kg ⁻¹)	12.51±1.15a	12.50±1.48a	8.28±1.29b	10.53±0.55a
Chlorophylls (mg kg ⁻¹)	24.17±2.80a	23.55±3.01a	13.05±2.24b	22.25±1.47a
α-Tocopherol (mg kg ⁻¹)	457.85±2.08a	299.28±34.25c	386.75±15.95b	171.64±5.14d
O-diphenols (mg kg ⁻¹)	247.63±7.90b	240.01±28.74b	376.31±14.05a	191.81±5.28c
Total phenols (mg kg ⁻¹)	492.35±2.81b	403.07±3.82c	651.55±29.94a	395.67±18.01c
IP (hours)	7.00±0.09c	6.97±0.15c	7.31±0.08b	9.11±0.06a
DPPH (%RSA at 60 min)	48.50±1.36b	33.16±0.08d	72.33±0.46a	41.77±0.53c
TAA (mM TE kg ⁻¹)	0.38±0.01c	0.47±0.02b	0.90±0.04a	0.33±0.01d

Values are the means of the three different VOO samples (n=3) ± standard deviations. Different letters indicate significant differences ($p < 0.05$) between cultivars.

olive oil had very high value of this parameter (>9h) followed by Mix2 olive oil (7.31h) whereas the others olive oils (Mix1 and Chaïbi) showed similar values (7h). The different oxidative stabilities of the four samples are resulting from the fatty acid composition and the effect of various pro/antioxidants present in the oils (Hrncirik and Fritsche, 2005). The explanation of the considerably higher stability of Mix2 and Mix1 oils lies probably in a relatively low content of polyunsaturated fatty acids, traditionally expressed by the O/L ratio and the higher content of antioxidants (phenols and tocopherols). Although the considerably higher levels of α-tocopherol, total phenols and fatty acids in Mix1 oil, no significant differences had been observed in its stability compared to Chaïbi oil. These results can be attributed to the similarity in the contents of o-diphenols and pigments composition.

Antioxidant capacities

The results of the free radical scavenging properties of all olive oils studied evaluated by the two different methods are shown in Table 2. Differences had been observed between the two tests. In fact, Oueslati and Mix1 olive oils showed the lowest antioxidant capacities (<0.4 mM) when evaluated by TAA, while oils from the cultivars Chaïbi and Oueslati had the lowest values by RSA. However, Mix2 oil showed the same behavior for the two tests with the highest values (72.33% and 0.9mM). Contrary to the accelerated oxidation tests showing the highest stability for Oueslati oil, the higher stability evaluated by TAA and RSA was observed for Mix1 and Mix2 oils. This result can be explained by the higher contents of total phenols or o-diphenols when these tests were realized on the methanolic extract containing polar phenols.

CONCLUSION

The results of this study demonstrated that the four Tunisian olive cultivars studied produced virgin olive oils

with different composition where Chaïbi and Oueslati oils showed the best fatty acid composition (lowest palmitic and linoleic acid and the highest oleic acid) and phenolic composition. Moreover, we also found that VOOs varied greatly in oxidative stability, which depends on many intrinsic factors as fatty acid composition and natural level of pro/antioxidants. These natural antioxidants, despite their lower concentrations prevent them more from oxidation where Mix1 and Mix2 oils showed better RSA activity. Therefore, besides the single role of each antioxidant compound to monitor the oils stability, the resulting stability may be the effect of synergisms of all antioxidants matrix such as phenols, lipidic profile, α-tocopherol, and pigments. Based on the assays presented here, it can be concluded that Tunisian olive oils obtained from second cultivars are an accessible source of natural antioxidants that provides the expected health benefits.

ACKNOWLEDGEMENTS

This research was supported by a grant from the 'Ministère de l'Enseignement Supérieur et de la Recherche Scientifique' UR03ES08 "Nutrition Humaine et Désordres Métaboliques" and 'DGRST-USCR-Spectrométrie de masse. The authors are grateful to the staff of experimental farms of the National Office of Oil (Tunis) for their help and advice.

REFERENCES

- Baccouri O, Guerfel M, Baccouri B, Cerretani L, Bendini A, Lercker G, Zarrouk M, Daoud Ben Miled D (2008). Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. *Food Chem.* 109: 743-754.
- Bendini A, Cerretani L, Carrasco-Pancorbo A, Gómez-Caravaca A.M, Segura-Carretero, A, Fernández-Gutiérrez, A, Lercker, G (2007). Phenolic Molecules in Virgin Olive Oils: a Survey of Their Sensory Properties, Health Effects, Antioxidant Activity and Analytical Methods. An Overview of the Last Decade. *Molecules* 12: 1679-1719.
- Ben Rouina B, Trigui A, Boukhris M (2002). Effect of climate and the

- soil conditions on crops performance of the Chemlali de Sfax "olive tree". *Acta Horticulturae* 586: 285–289.
- Bonanome A, Pagnan A, Biffanti S, Opportuno A, Sorgato F, Dorella M, Maiorino M, Ursini F (1992). Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification. *Arterioscler. Thromb.* 12: 529–533.
- Bramley PM, Elmadfa I, Kafatos A, Kelly FJ, Manios Y, Roxborough HE, et al. (2000). Vitamin E (review). *J. Sci. Food Agric.* 80: 913–938.
- Brand-Williams W, Cuvelier M.E, Berset C (1995). Use of a free radical method to evaluate antioxidant. *Lebensm. Wiss. U. Technol.* 28: 25–30.
- Cerretani L, Bendini A, Del Caro A, Piga A, Vacca V, Fiorenza Caboni M, Gallina Toschi T (2006). Preliminary characterisation of virgin olive oils obtained from different cultivars in Sardinia. *Eur Food Res. Technol.* 222 (3-4): 354-361.
- Covas MI, Ruiz-Gutiérrez V, De la Torre R, Kafatos A, Lamuela-Raventós RM, Osada J, Owen RW, Visioli F (2006). Minor components of olive Oil: evidence to date of health benefits in humans. *Nutr. Rev.* 64(9):20-30.
- Dabbou S, Rjiba I, Nakbi A, Gazzah N, Issaoui M, Hammami M (2010a). Compositional quality of virgin olive oils from cultivars introduced in Tunisian arid zones in comparison to Chemlali cultivars. *Sci Hort.* 124: 122–127.
- Dabbou S, Brahm F, Taamali A, Issaoui M, Ouni Y, Brahm M, Zarrouk M, Hammami M (2010b). Extra Virgin Olive Oil Components and Oxidative Stability from Olives Grown in Tunisia. *J. Am. Oil Chem. Soc.* 87:1199–1209.
- Dabbou S, Issaoui M, Servili M, Taticchi A, Sifi S, Montedoro GF, Hammami M (2009). Characterisation of virgin olive oils from European olive cultivars introduced in Tunisia. *Eur. J. Lipid Sci. Technol.* 111: 392-401.
- Dhifi W, Hamrouni I, Ayachi S, Chahed T, Saidani M, Marzouk B (2004). Biochemical characterization of some Tunisian olive oils. *J. Food Lipids* 11: 287–296.
- E.U.C. (1991). European Union Commission Regulation 2568/91, Characteristics of olive and olive pomace oils and their analytical methods. *Off. J. Eur. Commun.* L248: 1-82.
- Gimeno E, Castellote AI, Lamuela-Raventós RM, de la Torre MC, López-Sabater MC (2000). Rapid determination of vitamin E in vegetable oils by reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* 881: 251-254.
- Gómez-Alonso S, Salvador MD, Fregapane G (2002). Phenolic compounds profile of Cornicabra virgin olive oil. *J. Agric. Food Chem.* 50: 6812–6817.
- Gutiérrez-Rosales F, Ríos JJ, Gómez-Rey L (2003). Main polyphenols in the bitter taste of virgin olive oil. Structural confirmation by on-line high-performance liquid chromatography electrospray ionization mass spectrometry. *J. Agric. Food Chem.* 51: 6021–6025.
- Hannachi H, Msallem M, Ben Elhadj S, El Gazzah M. (2007). Influence du site géographique sur les potentialités agronomiques et technologiques de l'olivier (*Olea europaea* L.) en Tunisie. *C. R. Biologies* 330: 135–142.
- Hrnčirik K, Fritsche S (2005). Relation between the endogenous antioxidant system and the quality of extra virgin olive oil under accelerated storage conditions. *J. Agric. Food Chem.* 53: 2103-2110.
- I.O.C. (2009). International Olive Council. Trade standard applying to olive oil and olive pomace oils. COI/T.15/NC n° 3/Rév. 4. November.
- Issaoui M, Dabbou S, Echbili A, Rjiba I, Gazzah N, Trigui A, Hammami M (2007). Biochemical characterisation of some Tunisia virgin olive oils obtained from different cultivars growing in Sfax National Collection. *J. Food Agric. Environ.* 5:17–21.
- Krichène D, Taamalli W, Daoud D, Salvador MD, Fregapane G, Zarrouk M (2007). Phenolic compounds, tocopherols and other minor components in virgin olive oils of some Tunisian varieties. *J. Food Biochem.* 31: 179–194.
- Maeda H, Sage TL, Isaac G, Welti R, DellaPenna D. (2008). Tocopherols modulate extraplastidic polyunsaturated fatty acid metabolism in *Arabidopsis* at low temperature. *Plant Cell.* 20: 452-70.
- Massaro M, De Caterina R (2002). Vasculoprotective effects of oleic acid: epidemiological background and direct vascular antiatherogenic properties. *Nutr. Metab. Cardiovasc. Dis.* 12(1): 42–51.
- Mateos R, Trujillo M, Pérez-Camino C, Moreda W, Cert JA (2005). Relationships between oxidative stability, triacylglycerol composition, and antioxidant content in olive oil matrices. *J. Agric. Food Chem.* 53: 5766–5771.
- McDonald S, Prenzler PD, Antolovich M, Robards K (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chem.* 73: 73–84.
- Minguez-Mosquera I, Rejano JL, Gandul B, Higinio A, Garrido J (1991). Colour pigment correlation in virgin olive oil. *J. Am. Oil Chem. Soc.* 68: 669-671.
- Montedoro GF, Servili M, Baldioli M, Miniati E (1992). Simple and hydrolysable compounds in virgin olive oil. 1. Their extraction, separation and quantitative and semi quantitative evaluation by HPLC. *J. Agric. Food Chem.* 40: 1571-1576.
- Ouni Y, Flamini G, Issaoui M, Ben Youssef N, Pier Luigi C, Hammami M, Daoud D, Zarrouk M (2011). Volatile compounds and compositional quality of virgin olive oil from Oueslati variety: Influence of geographical origin. *Food Chem.* 124: 1770–1776.
- Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalter B, Bartsch H (2000a). The antioxidant /anticancer potential of phenolic compounds isolated from olive oil. *J. Eur. Cancer* 36 (10): 1235-1247.
- Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalter B, Bartsch H (2000b). Phenolic and lipid components of olive oil: identification of lignans as major components of olive oil. *Clinical Chem.* 46: 976-988.
- Ranalli A, Contento S, Schiavone C, Simone N (2001). Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil. *Eur. J. Lipid Sci. Technol.* 103: 228-238.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an important ABTS radical cation decolorization assay. *Free Rad. Biol. Med.* 26: 1231-1237.
- Rice-Evans CA, Miller NJ, Paganga G (1997). Antioxidant properties of phenolic compounds. *Reviews. Trends Plant Sci.* 2: 152-159.
- Rice-Evans CA, Miller NJ, Paganga G (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Rad. Biol. Med.* 20: 933-956.
- Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.* 66: 401-436.
- Sattler SE, Cahoon EB, Coughlan SJ, DellaPenna D (2003). Characterization of tocopherol cyclases from higher plants and cyanobacteria. Evolutionary implications for tocopherol synthesis and function. *Plant Physiol.* 132: 2184–2195.
- Servili M, Montedoro G F (2002). Contribution of phenolic compounds to virgin olive oil quality. *Eur. J. Lipid Sci. Technol.* 104: 602–613.
- Taamalli A, Gomez-Caravaca AM, Zarrouk M, Segura-Carretero A, Fernandez-Gutierrez A (2010). Determination of apolar and minor polar compounds and other chemical parameters for the discrimination of six different varieties of Tunisian extra-virgin olive oil cultivated in their traditional growing area. *Eur. Food Res. Technol.* 231:965–975.
- Tura D, Gigliotti C, Pedo S, Failla O, Bassi D, Serraiocco A (2007). Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (*Olea europaea* L.) and correlations with oxidative stability. *Sci. Hort.* 112: 108-119.