International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 5, No. 1, p. 124-132, 2014

INNSPUB

RESEARCH PAPER OPEN ACCESS

Oil quality analyses of four autochthon Tunisian olive varieties cultivated in the mountain of Kesra

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Article published on July 26, 2014

Key words: Oil quality, fatty acid composition, phenolic compounds, pigment content, mountain, Kesra.

Abstract

Kesra is a mountainous region characterized by an important olive biodiversity with high oil quality but little is known about this olive germplasm. The aim of this work is to analyze the oil quality of the most predominant varieties 'Chétoui', 'El hor' 'Sradki' and 'Ouesleti' cultivated in this region. The most of the quality indices and fatty acid composition showed significant variations among the studied olive cultivars. Olive oil content is high for the four cultivars, especially for the variety 'Sradki' with approximately 67%. The cultivars 'El Hor' and 'Sradki' had the highest values of oleic acid (72.8% and 74.8%, respectively). While the varieties 'Oueslati' present the highest content of chlorophyll and carotenoid compounds. The cultivar 'Sradki' was also noteworthy for its higher content of phenolic compounds (720 mg kg^{-1}). In conclusion, the oil quality of the different studied cultivars is classified as extra-virgin oils with high oleic acids and low palmitic and linolenic acids. These findings were of interest to protect the specimens studied cultivars, which can be used from the agronomic point of view to substantially improve the production of olive oil in the mountain of Kesra.

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Introduction

Mountain people, who are among the world's poorest and hungriest, are key to maintaining mountain ecosystems and their role in providing environmental services to downstream communities. Mountain communities need to be empowered and their livelihoods improved, to enable them to take responsibility for the preservation of natural resources and to fulfill their role as mountain stewards (Walther 1986, Garcia-Ruiz and Lasanta-Martinez 1990, Blondel and Aronson 1999, MacDonald *et al.*,2001, Romero-Calcerrada and Perry 2004).

In Tunisia, the mountains are characterized by an important olive biodiversity with high oil quality but little is known about this germoplasm (Mnasri *et al.,* 2013). This resource could be used from the agronomic point of view to substantially improve the production of olive in the mountainous orchards, specifically, that olive is one of the few trees that can still produce fruits even on rock and unproductive land (Omrani-Sabbaghali *et al.,* 2007*)*.

On the other hand, virgin olive oil has a delicate and unique flavor that distinguishes it from other edible vegetable oils *(*Boskou., 1996). Quantity and quality of substances existing in the virgin olive oil such as fatty acids, phenolics, chlorophyll and carotenoids are affected by various factors including the type of the olive cultivar (Baccouri *et al.,* 2007a; Cerrtani *et al.,* 2006 and Gomez-Rico.,2008), climatic conditions *(Aguilera et al.,2005)*, ripening stage (Salvadoral *et al.,* 2001), irrigation management *(*Vinha *et al*.,2005) and the extraction methods (Ranalli *et al*.,2000). Among these factors, cultivar is undoubtedly one of the most important. However, it is often ignored, either through lack of varietal information, or because the olive oil is a mixture of various varieties or even because emphasis has been laid only on its place of origin (Lanteri *et al.,* 2002).

The present work was carried out on the extra-virgin olive oils of the four main olive varieties (Chetoui, El Hor, Sradki and Ouesleti) grown in the mountain of Kesra which is localized in the North West of Tunisia. Several analyses were performed to characterize the different olive oils: free acidity, peroxide value, fatty acid composition, pigments content and phenolic compounds by HPLC–MS. This is a preliminary study with the aim of finding any variable able to discriminate among the monovarietal extra-virgin olive oils and evaluate the oil quality of these varieties. Especially that, the olive cultivation could have an important role in the sustainable mountain development.

Materials and methods

Fruit samples

Healthy olive fruit samples of the varieties 'Chétoui', 'El Hor' 'Esradki' and 'Oueslati' were picked at industrial optimum ripening stage. The maturity index of all the olives was of 3 and was based on the degree of skin and pulp pigmentation according to the method developed by the Agronomic Station of Jaén (Uceda and Hermoso; 1998). This experiment was conducted during the crop season of 2012-2013 in the mountainous olive orchard of Kesra localized in North West of Tunisia. The average annual precipitation was 539 mm with the majority in October, December, and January. Average annual temperature of the experimental orchard site is 13.9°C; the altitude is 1078 m, 35°48' N of latitude and 9°21′ E of longitude.

Oil Content

For oil content determination, 40 g of olive fruits was dried in an oven at 80°C to constant weight. The dried olives were crushed and extracted with hexane using a Soxhlet apparatus (Bettach *et al.,* 1996). The results were expressed as percentage of dry matter (DM).

Analytical indices

Determination of free acidity, peroxide value and specific ultraviolet absorbance were carried out following the analytical methods described in the EC Regulation (1995).

Fatty acids, peroxide value, and UV

Spectrophotometeric indices (K232, K270)

The quality indices of fatty acids, peroxide value, and specific extinction coefficient o(f K232 and K270 and ΔK were calculated from absorption at 232 and 270 nm, respectively, by a UV spectrophotometer (JENWAY - 6405 UV Visible spectrophotometer, England) according to the European Commission Regulation EEC/2565/91.

Determination of chlorophyll and carotenoid compounds

Pigments of chlorophyll and carotenoid were determined by a spectrophotometer according to (Minguez-Mosquera *et al.,* 1991): 1 g of olive oil was dissolved in 10 ml of iso-octane. The absorbance of the solution was measured at 670 and 470 nm for chlorophyll and carotenoid, respectively.

Fatty Acid analyses

The fatty acid composition of oil samples was determined as methyl esters by capillary gas chromatography analysis after alkaline treatment. The gas chromatograph (VARIAN CP-3800 Gas Chromatograph) was equipped with an autosampler (CP-8400), a capillary column HP Innowax (Agilent Technologies, USA) (30 m × 0.53 mm, 1 μm), a split–splitless injector and a flame ionization detector (FID). Alkaline treatment was carried out by mixing 0.1 g of oil dissolved in 3 ml of n-hexane with 0.5 ml of 0.2 N methanolic potassium hydroxide solution according to the method of Reg EC 2568/91.

Determination of total phenols

Phenolic compounds were isolated by a 3-time extraction of a solution of oil in hexane with a water/methanol mixture (60:40, v/v). The Folin-Ciocalteau reagent (Merck Schuchardt OHG, Hohenbrunn, Germany) was added to a suitable aliquot of the combined extracts, and the absorption of the solution at 725 nm was measured. Values were given as milligrams of caffeic acid per kilogram of oil (Vàsquez 1978; Gutfinger 1981).

Determination of phenolilc compounds by GC and GC-MS analyses

Fruits, destoned and immediately frozen in liquid nitrogen, were triturated in a blender. Approximately 5 g of the powder obtained were homogenized four times in 30 ml of methanol/water solution (80:20, v:v), containing 0.5% sodium metabisulfite, and centrifuged at 5000 rpm at 3 °C for 20 min. An ethanolic solution of resorcinol (0.5 g/l) was added as internal standard. The combined supernatants were concentrated under reduced pressure and washed with hexane. The remaining aqueous solution, partitioned four times with ethyl acetate in a water to phase ratio of 1:1, was filtered on sodium sulphate (anhydrous) and evaporated to dryness at 30 °C under vacuum. The dry residue was converted into tri-methylsilyl derivatives with a silylation mixture made up of pyridine, hexame-thyldisilazane and trimethyl-chlorosilane (2:1:1) for 1 h at room temperature. The silanized extracts were dried, dissolved in isoctane and further analyzed by GC and GC-MS. An HP model 5890A, equipped with an on-column injection system, and coupled with a mass selective detector model HP 5970B, was employed. Phenolic compounds extracted by ethyl acetate were identified by comparing both their retention times and mass spectra with those of authentic compounds or reference standards.

Statistical analysis

The results reported in this study are the averages of at least three repetitions (n = 3), unless otherwise stated. Chemical data were analysed by the XLSTAT (version 2010.4.01). The significance of differences at a 5% level between averages was determined by one-way ANOVA using Tukey's and Duncan's multiple range tests.

Results and discussion

Oil yield of olives

As reported in fig 1, all the studied cultivars are characterized by a high oil yield according to the classification of (Tous and Romeo, 1993). Expressed as percentage of dry matter, the oil yield presented significant differences between the four olive varieties; the content of oil in the samples ranged from 50% for the cultivar 'El Hor' to 67% for the

cultivar 'Esradki'. Therefore following classification of Tous and Romero, we can qualify the four tested cultivars by varieties with high oil yield (> 46%).

Free acidity, peroxide value and UV spectrophotometric indices

All the analyzed oils (Table 1) showed very low values for the regulated physicochemical parameters (acidity ≤0.8%; peroxide value ≤20 m equiv. O2 kg^{-1}; K270 ≤0.22; K232 ≤2.5). Free acidity of the studied oils was in a range from 0.25 to 0.42%. The peroxide values, samples ranged from 2.03 meqO2/kg for 'Oueslati' cultivar to 3.66 meqO2/kg for 'El Hor' cultivar. These low values are a measure of the high freshness of the oils analyzed (Cerretani *et al.,* 2006 and Rotondi *et al.,* 2004). The specific ultraviolet absorbance K232 varied from 1.89 to 2.17 having the highest values in Oueslati variety, while K270 ranged from 0.20 for Oueslati cultivar to 0.17 for Chetoui. With all of them falling within the ranges established for 'extra virgin olive oil' category, as required by Regulation EC/1989/2003(EEC, 2003) and Codex Alimentations, (2003).

Table 1. Fatty acids composition, Free acidity and peroxide value of virgin olive oils from cultivars 'Chetoui', 'El Ho'r, 'Sradki' and 'Oueslati' cultivated in the mountain of Kesra according to the norm of the IOC, (1997).

Quality indicates	*Cultivar*				*Norm (IOC, 1997)*
	Chetoui	El Hor	Esradki	Oueslati	
C16 :0	29.21±0.11d	14.5±0.5c	8.89±1.59a	11.06±0.4b	7,5–20
C16 :1	2.68±0.1c	0.94±0.02b	0.83±0.03a	0.7±0.1a	0,3–3,5
C18 :0	3,21±0.11c	2,97±0.04b	2,02±0.02a	2,1±0.1a	0,5–5
C18 :1	66,58±0.6a	72,8±0.4b	74,82±1.8c	74,5±0.5c	55–83
C18 :2	10.7±0.3a	20.79±0.42b	11.12±0.12a	10.7±0.5a	3,5–21
C18 :3	0.51±0.02a	0.9±0.06b	0.59±0.09a	0.6±0.3a	<0,9
C20 :0	0.07±0.01a	0.45±0.04d	0.32±0.01c	0.25±0.06b	≥0,6
Free acidity (oleic acid, %)	0,31±0,01a	0,42±0,02b	0,28±0,02a	0,25±0,05a	
Proxide value (meq O2.kg oil)	3.16 ± 0.16c	3.66± 0.06d	2,29±0,02b	2.03±0.03a	≤20
K232	1.89 ± 0.09a	2.00± 0.2a	2,1 ± 0.1a	2.17±0.07b	≤2.5
K270	0.17 ± 0.02a	0.15± 0.01a	0,17±0,01a	0.2±0.01b	≤0.22
MI*	3	3	3	3	

Mean ± SD (n=3): Means in each column with the same letters are not significantly different at 5% of probability by Duncan's multiple range test.

MI* :Maturity index fruits olive.

Fatty acids compound

Methyl ester fatty acid composition and their levels in the analyzed oils are shown in Table 2. As it can be observed, palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids are the major fatty acids present in the studied samples. The fatty acid composition of olive oils varies widely depending on the cultivar. These findings are in good agreement with those of other authors working on Tunisian olive oil varieties (Baccouri *et al.,* 2007a; Haddada *et al.,* 2007 and Mnasri *et al.,* 2013). All the studied varieties except the cultivar 'El Hor' showed a percentage of oleic acid (C18:1) > 55%, a palmitic acid rate which did not exceed 20 % and a low amounts of a linoleic acid (C18:2) varies from 10.17% to 21%.

The Sradki olive oil showed the highest percentage of oleic acid (C18:1) and the lowest percentage of palmitic acid (74.82 and 8.89% respectively).Concerning palmitoleic (C16:1), stearic

(C18:0), linolenic (C18:3) and arachidic (C20:0) acids, the studied olive oil varieties presented low amounts of all of them. The highest percentage of palmitoleic and stearic acid was found in 'El Hor' cultivar, while 'Chetoui' olive oil was the richest in linolenic acid.

Table 2. Pigments and phenolic compounds of virgin oils from cultivars Chetoui, El Hor, Esradki and Oueslati (results are expressed as mg/kg).

Pigments and phenolic compound	*Cultivar*			
	Chetoui	El Hor	Esradki	Oueslati
Total phenol	323±7b	384±4c	720±8d	245±4.04a
Total chlorophylls (mg/kg)	3.46±0.25b	2.17±0.02a	4,1±0.1c	4.77±0.07d
Total carotenoid (mg/kg)	1.49±0.04b	0.86±0.06a	1,54±0,04b	1.57±0.05b
Hydroxytyrosol (mg/kg)	7,22±0,06b	7,68±0,13c	1,81±0,02a	7,18±0,08b
Trysol (mg/kg)	3,53±0,03d	3,25±0,05c	2,27±0,07a	2,39±0,06b
Cinamic acid (mg/kg)	0,7±0,04a	0.82±0.04a	1,5±0,2b	0,63±0,03a
Vanilic acid (mg/kg)	0.93±0.03b	1,06±0,03c	1,18±0,08d	0,81±0,01a

Mean ± SD (n=3): Means in each column with the same letters are not significantly different at 5% of probability by Duncan's multiple range test.

Other interesting points for the chemical characterization of studied oils are the proportions of some classes of free fatty acids. The monounsaturated fatty acids have great importance because of their nutritional implication and effect on oxidative stability of oils (Martinez de Victoria and Manas, 2001). Fig 2 show that the proportion of monounsaturated fatty acids changed according to the cultivar. It varies from 69.05% for the cultivar 'El Hor' to 76.34% for the cultivar Esradki. The C18:1/C18:2 ratio has the most marked relationship with stability, and it is said that oil presents a good stability index if this value is over 7. Nevertheless, Tunisian olive oils are described in bibliography to present lower C18:1/C18:2 ratios compared to most of the European ones (Baccouri *et al.,* 2007b; Zarrouk *et al.,* 2009). We found that the studied olive varieties, except the cultivar 'Chetoui' present oil with C18:1/C18:2 ratios higher than 6.51.

Pigment contents

The levels of chlorophyll and carotenoids of virgin olive oils from the four cultivars presented significant differences (P<0.05). The 'Oueslati' olive oil showed the highest level of chlorophyll and carotenoids with the mean values of 4.77 mg/kg and 1.57mg/Kg respectively. While the 'El Hor' olive cultivar being the poorest in terms of both of them with respectively mean values of 2.17mg/Kg and 0.86 mg/Kg (Table 2).The olive oil color is directly related to the chlorophyll and carotenoid contents, and it has been proposed as a characterizing factor and as a quality index related to the oil extraction method and to the olive variety (Minguez-Mosquera *et al.,* 1991). Besides, the color is the first attribute of virgin olive oil evaluated by consumers.

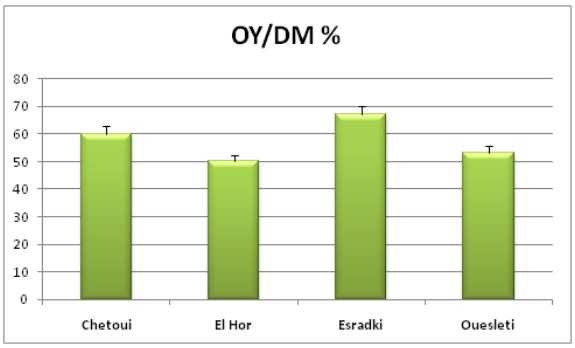

Fig. 1. Comparison of the oil yield rate expressed as percentage of dry matter of the four analysed olive cultivars with the method of Tous & Romeo (1993).

Phenolic compounds

In this research, four major phenolic compounds of virgin olive oil (tyrosol, hydroxytyrosol, cinamic acid, vanilic acid) have been studied by HPLC. Table 2 shows the concentrations of the phenolic compounds that were identified and the data were expressed as mg/kg for samples of virgin olive oils obtained from 'Chetoui', 'El Hor, 'Sradki' and 'Oueslati', cultivars. Significant differences between cultivars (p<0.05) were observed in the amounts of phenolic compounds. In comparison with 'Oueslati' and 'Sradki' cultivars which had 2.39 and 2.27 mg/kg of the phenolic compound of tyrosol, in their oils respectively, 'Chetoui' and 'El Hor' cultivar had oil with higher levels of tyrosol- 3.53 mg/kg and 3.25mg/Kg. The oil of 'Sradki' cultivar had the highest levels of the phenolic compounds of cinamic acid and vanilic acid (1.5 and 1.18 mg/kg, respectively). Moreover, the total level of phenol in the oils of the cultivars showed significant differences and the oil of 'Sradki' cultivar had higher mean amounts of total phenol-720 mg/kg in comparison with the oils of 'Oueslati' cultivar which had the lowest content of total phenol 246mg/kg (Table 2).The results here, confirmed that there were significant differences in the features and characteristics of virgin olive oils of 'Chetoui', El hor', 'Sradki' and 'Ouesleti' varieties cultivated in the mountain of Kesra. High content of total phenol compound is positively correlated with the stability of olive oil (Hanachi *et al.,* 2008; Taamalli *et al.,* 2010).

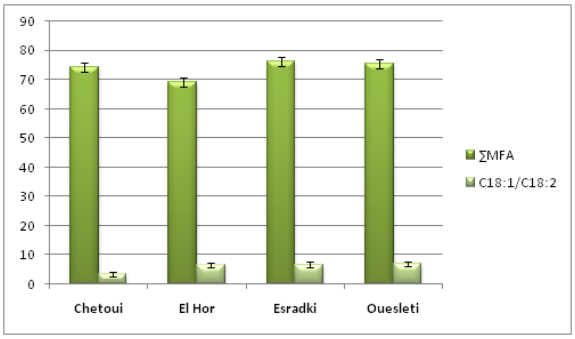

Fig. 2. Rate variation of the monounsaturated fatty acids and the C18:1/C18:2 ratio of the tested oils according to the norm of IOC, (1997).

Conclusion

Although many efforts have been made in the last years to study the oil composition of the Tunisian olive varieties, little is known about olive oil quality of the mountainous cultivars. The present study revealed the height olive oil quality of the varieties cultivated in the mountain of Kesra and significant differences in the features of olive oils in studied cultivars. Variations in fatty acid composition and phenolic compounds were observed in the olive oil samples probably due to the genetic factor. These preliminary findings would be hopefully confirmed by the analysis of a larger number of samples that take into consideration the variation of phenolic profile and chemical composition of virgin olive oil, which may also arise from agronomical practices, maturation index, harvesting years and processing technologies. Specifically, that this resource could be used from the agronomic point of view to substantially improve the production of olive oil in the mountainous orchards, where the olive is one of the few trees that can still produce fruits even on rock and unproductive land.

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