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Effect of drought stress on qualitative characteristics of olive oil of c*v* Koroneiki

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RESUMEN

Influencia del estrés hídrico sobre la calidad del aceite de oliva en la variedad Koroneiki

Se han estudiado los valores de calidad reglamentada (acidez, índice de peróxido, los valores de absorbancia en UV (K270, K232), estabilidad oxidativa media en Rancimat 120^{o} C) y características químicas (ácidos grasos, esteroles, triglicéridos) en aceite de oliva virgen variedad Koroneiki sometidos a diferentes regímenes hídricos. El estudio se ha realizado en árboles adultos de 3 años cultivados en contenedor al aire libre. Los árboles se riegan con dos dosis tratando de mantener un potencial de agua en el suelo de unos -0,03 Mpa y -1,5 Mpa.

Los resultados muestran en los olivos de riego un aumento significativo de la riqueza grasa y de la estabilidad del aceite. Los aceites producidos en olivos de riego presentan una mayor proporción de ácidos grasos saturados (palmítico-esteárico). Los esteroles totales se ven significativamente influenciados por el riego. Acidez y absorbancia K_{270}, K_{232} no se han visto afectados por el riego.

PALABRAS-CLAVE: Aceite de oliva virgen – Calidad – Estrés hídrico – Variedad koroneiki.

SUMMARY

Effect of drought stress on qualitative characteristics of olive oil of c*v* Koroneiki.

Quality characteristics (acidity, peroxide values, K_{232}, K_{270}, oxidative stability) and chemical compositional data (fatty acids, sterols and triacylglycerols) were studied in virgin olive oil samples from olive trees, *c v* Koroneiki, subjected to different water regimes. Experimental trials were carried out using three-year old own-rooted olive trees (*Olea europaea* L) variety Koroneiki. Plants were subjected to two irrigation treatments to maintain soil water potential to -0.03 MPa and -1.5 MPa.

Results showed that irrigation significantly increased the fruit oil content and the oxidative stability and peroxide value of the resulting oil. Olive oil from fruits of irrigated trees showed significant higher values in total saturated fatty acids. Total sterols were also significantly influenced by irrigation. Acidity and specific absorption coefficients K_{232}, K_{270}, of olive oils were not significantly affected.

KEY-WORDS: Drought stress – Quality – Variety koroneiki – Virgin olive oil.

1. INTRODUCTION

Any environmental or physicochemical change having the potential to cause a deleterious effect on a living organism is called stress. There are a number of environmental factors that can stress plants. These factors include light, temperature, water, atmospheric or soil constituents (Harwood *et al*., 1998; Levitt 1980).

Olive is one of the major crops in the Mediterranean region, traditionally grown under drought conditions (Rugini and Fedeli., 1990).

As has been proven by many authors, the olive tree (*Olea europaea* L) is remarkably resistant to drought stress. Under water stress conditions -even during prolonged drought period- in Summer, olive tree survives by developing defence mechanisms. (Xyloyianis *et al*, 1999; Chartzoulakis *et al,* 1999). Although olive tree can grow and produce under low annual water supply, irrigation during drought period is essential in order to give high yield (Agabbio., 1983; Scaramuzzi and Roselli., 1986). Irrigation among other increases fruit production by increasing both the size as well as the number of the fruits and oil content (Chartzoulakis *et al,* 1992; Michelakis *et al*, 1995; Inglese *et al*, 1996).

In Greece, and especially in Crete, the dominant cultivated oil variety is 'Koroneiki', which is considered as well adapted to drought conditions. However, its cultivation is continuously extended to irrigated lands.

Although a lot of research work has been done on the defence mechanisms of olive tree to prolonged period of drought stress and the beneficial effect of proper irrigation on growth and yield, little information exists on the effect of drought stress on olive oil qualitative characteristics especially as far as Koroneiki variety is concerned. The results from previous studies on the irrigation effect on qualitative characteristics are contradictory.

Higher acidity, peroxide value, total phenols, and oxidative stability have been reported in irrigated oils compared to water-stressed oils (Dettori and Russo, 1993; Ismail et al, 1999; Motilva *et al*, 1999). On the other hand higher polyhenol content, stability and bitterness have been reported in water- stressed oil by Salas *et al*, 1997; Patumi *et al*, 1999; Andria *et al,* 1999).

The objective of this work was to investigate the fruit characteristics and quality of the corresponding olive oil in relation to irrigation and water-stress conditions.

2. MATERIALS AND METHODS

2.1. Plant material

The experiment was carried out at the Subtropical Plants and Olive Tree Institute, Chania, in the island of Crete. Three-year old own-rooted olive trees (*Olea europea*, L. *cv* Koroneiki) were used. The plants were grown outdoors in 50 l pots, containing freely drained light soil sandy-clay with electric conductivity (1:1) of 0.48 ds x m^{-1}, pH 7.5, field capacity 22% and permanent wilting point 9.2% on dry weight basis. Different soil water regimes were imposed during the dry season (April to November) of the harvesting year 1996. Tensiometers and soil moisture sensors (MC-314 Cell, Soil Moisture, Inc.) placed at 20-cm depth were used for monitoring soil water tension. Two soil water regimes were applied during the dry season (ten plants for each treatment):

a) Irrigation treatment (control), where the plants were irrigated daily or every two days to maintain soil water content close to field capacity (-0.03 Mpa, soil water content 19-21%) throughout the experimental period.

b) Drought treatment (stress), where plants were stressed by withholding water until the soil water potential reached - 1.5 Mpa (soil water content 9.5-11%). In the stress treatment the plants were irrigated on average every eight days.

Trunk diameter was measured every 30 days during the reporting period. In mid June-July-August and September the predawn leaf water potential (ψ) was estimated using a pressure chamber (PMS Instruments, USA). At the same time, photosynthetic rate (Pn) was measured by means of a portable photosynthesis device (Li-6200, Li-Cor, USA). The fourth or fifth fully expanded leaf from the top was selected for the measurements.

Plants were harvested in the first week of December, and the parameters recorded include weight of 100 fruits, moisture content (%) and percentage oil content on dry weight basis.

2.2. Olive oil extraction

Olive oil was extracted from the fruits using a laboratory-scale olive mill under the following operational conditions:

Olives were washed, deleafed and crushed using a hammer mill operating at 3000 rpm. A sieve with 5 mm holes was used at the exit of the crusher. The resulting paste was mixed at 50 rpm, at 28 ± 2 ^{o}C for 30 min and pressed in a laboratory press at 205 Kg cm^{-2}. After decanting, the oil was centrifuged and filtered.

2.3. Moisture content determination

50 g of olive paste was dried in the oven at 102-105 ^{o}C to constant weight and the determined weight loss was taken as moisture.

2.4. Oil content determination

Oil content was determined following the official methods of AOAC. Dry paste was extracted with hexane using the soxhlet apparatus for 12 h. Oil content was expressed as percentage of dry weight basis (w/w).

2.5. Analytical methods

Determination of acidity, peroxide value, UV light absorption (K_{232}, K_{270}), fatty acid composition, sterol composition and triacylglycerol content were carried out following the analytical methods described in EC Regulation 2568/91.

Fatty acids were measured and quantified as their methyl esters produced by a cold saponification method described in previous paper (Stefanoudaki *et al* 1999). A Hewlett Packard, Gas Chromatography HP 6890 series (Hewlett Packard, 76337 Walbronn Germany) with auto injector and flame ionization detector was used for the determination of FAMEs. The analytical separation was achieved on a capillary Column BPX 70 (SGE scientific Pty Ltd-Australia) length 50 m, internal diameter 0.22 mm, film thickness 0.25μm.

Sterols were separated from the unsaponifiable fraction by Thin Layer Chromatography on silica-gel plates. After recovery from the plates the sterols were converted to TMS derivatives and separated by a Hewlett Packard 6890 gas chromatograph with a split injector and FID detector on a HP-5 5% Phenyl methyl siloxane capillary column (30 m x 320 μm x 0.25 μm) at 285 ^{o}C. Sterols were quantified using a-cholestanol as internal standard. Results were expressed as percent of total sterols and their sum as total sterols in ppm.

Triacylglycerols were determined by HPLC. The apparatus consisted of liquid chromatography Jasco (Model PU 980) coupled with a RI detector (Jasco 830-RI). Injection was by means of a Rheodyne injection valve with 20μl fixed loop (Rheodyne, California, USA). The chromatographic separation was achieved on a Kromasil 100 C18, 5ìm column (250 x 4 mm i d), obtained from MZ Analysentechnik (Wohlerstr, Mainz) at 40 ^{o}C. Isocratic elution was carried out at a flow rate of 0.7 ml min^{-1} with a mixture of acetone: acetonitrile (60:40 v/v) as mobile phase. Peak identification was done on the basis of retention times by comparison with mixtures of triacylglycerols analysed under the same conditions. Results were expressed as percentage composition of total triacylglycerols.

Oxidative stability was measured by a 679 Rancimat apparatus (Methrohm Co, Basel, Switzerland) at 120 ^{o}C with an air flow 20 L h^{-1}. This method provides the induction time for the decomposition of hydroperoxides, produced by the oil oxidation. (Frank J. *et al.,* 1982, Laubli and Brutel, 1986).

2.6. Statistical analysis

The experiment included two treatments with 5 trees per treatment. Each analysis was carried out in duplicate (n=5x2=10 per treatment). The Statistical package used was SPSS, version 8.0 (SPSS 1986). Students' t-test was performed for equality of means at 0.05 level of significance. Further multivariate analysis based on Principal component analysis was applied. The first two Principal Components were saved as variables and the scatter plot of oil samples was constructed on the two axis.

3. RESULTS AND DISCUSSION

Irrigation at different soil water potentials had a significant positive effect on stem diameter, leaf water potential and photosynthesis of olive tree (Table I). Although withholding irrigation during the summer period had negative effects on plant growth and photosynthesis, since both are turgor-dependent processes, olive tree is able to survive and produce by developing defence mechanisms (Chartzoulakis *et al*, 1999; Xiloyiannis et al, 1999). Drought tolerance is associated with turgor maintenance through osmotic adjustment. By lowering the water content and water potentials of its tissues, olive tree establishes a high potential gradient between leaves and roots and therefore utilizes soil water up to –2.5 Mpa (Xiloyiannis *et al,* 1999).

Table I

Mean values[1] ± SE of trunk growth, predawn leaf water potential and photosynthetic rate of olive trees c*v*. Koroneiki, in relation to soil water regime

Soil water regime	Trunk increase (mm)	Predawn leaf water potential (MPa)	Photosynthetic rate (μmol cm^{-2} sec^{-1})
Irrigated	4,51 a	-0,83a ± 0,16	14,5a ± 0,7
Stressed	2,82 b	-2,61b ± 0,34	6,4b ± 1,2

1 Values are means of forty determinations (n=40).
2 Means followed by different letter within column indicate significant differences (p<0.01).

Table II

Mean values[1] ± standard deviation of olive fruit characteristics of c*v* Koroneiki from water-stressed and irrigated olive trees

	Weight of 100 fruits	Moisture (%)	Oil content on dry wt. basis (%)
Water-stressed	66,16 ± 8,42a[2]	56,59 ± 0,71a	35,03 ± 1,94b
Irrigated	72,16 ± 5,65a	51,47 ± 1,15b	40,60 ± 1,14a

1 Values are means of forty determinations (n=10).
2 Means followed by different letters within column indicate significant differences (p≤0,05).

Table III

Mean values[1] ± standard deviation of several qualitative characteristics of virgin olive oils obtained from drought-stressed and irrigated olive trees of cv Koroneiki

	Water-stressed	Irrigated
Acidity (% oleic acid)	0,41 ± 0,02a[2]	0,38 ± 0,07a
Peroxides value (meq O_2Kg^{-1} oil)	3,85 ± 0,24b	4,21 ± 0,26a
OD 232 nm	1,51 ± 0,09a	1,59 ± 0,03a
OD 270 nm	0,17 ± 0,04a	0,17 ± 0,01a
Stability (hours at 120°C)	11,20 ± 0,59b	12,76 ± 0,80a

1 Values are means of forty determinations (n=10).
2 Means followed by different letters within each row are significantly different at (p≤0,05).

The mean values of several olive fruit characteristics including oil content, moisture content and average weight of 100 fruits are presented in Table II. Percent oil content on dry weight basis was significantly higher (p<0.05) in irrigated fruits in accordance with previous studies (Michelakis *et al*, 1995; Inglese et al 1996; Ismail *et al*, 1999). Regarding the weight of 100 olive fruits the differences among stressed and not stressed fruits were possibly minimized due to rainfall one day before harvest. According to Balatsuras *et al* 1988, olive fruit withholds its moisture tenaciously during the period of prolonged drought and reabsorbs the lost moisture immediately after rainfall.

The oxidative stability of the oils as measured by Rancimat apparatus showed significant higher values in oils extracted from fruits of irrigated trees (Table III). These results are in accordance with those reported by Ismail *et al*, 1999 while they are opposed to those reported by Salas *et al*, 1997; Motilva *et al*, 1999.

Qualitative characteristics of the oil such as acidity, peroxide value and spectrophotometric absorption in the UV region do not vary significantly. It is interesting to note that these values are considerable lower than the limits established by the European Economic Community legislation (1992) for extra virgin olive oil.

The triacylglycerol composition of olive samples under investigation is shown in Table IV. OOO, the major TAG of olive oil, showed significant higher values in stressed in contrast to POO+SOL which showed higher values in irrigated trees. The value of [LLL+ OLLn+ PLLn] was significant affected by water stress and in all cases this sum did not exceed the maximum limit for LLL of 0.5 % determined by EC regulation.

Fatty acid composition of oils from fruits of water-stressed and irrigated trees is tabulated in Table V. Total saturated fatty acids were significantly

Table IV

Mean values[1] ± standard deviation of triacylglycerol content of virgin olive oils obtained from drought-stressed and irrigated trees of c*v* Koroneiki

	Stressed	Irrigated
LLL+OLLn +PLLn	0,34 ± 0,05a[2]	0,28 ± 0,03b
OLL	1,46 ± 0,19a	1,35 ± 0,18a
OOLn	1,56 ± 0,08a	1,38 ± 0,20a
PLL+PoOL	0,66 ± 0,09 a	0,67 ± 0,14a
OOL	10,44 ± 0,61 a	10,23 ± 0,25 a
POL	6,44 ±0,32 a	5,93 ± 0,40 a
PPL	0,55 ± 0,10 a	0,64 ± 0,25 a
OOO	43,29 ± 0,89 a	41,33 ± 1,09b
POO+SOL	25,73 ± 0,71 a	28,05 ± 0,57b
POP	2,81 ± 0,26 a	3,04 ± 0,30 a
SOO	4,77 ± 0,23 a	4,78 ± 0,29 a
POS	1,02 ± 0,11 a	1,00 ± 0,15 a
AOO	0,69 ± 0,17 a	0,83 ± 0,08 a
SOS	0,28 ± 0,04 a	0,30 ± 0,03 a

1 Results are means of ten determinations (n=10).
2 Means followed by the same letter within each row are not significantly different (p≤ 0,05)
Abbreviations: P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid.

higher in the oils from fruits of irrigated trees. Palmitoleic acid showed significantly lower values in the oil from the fruits of irrigated trees. The unsaturated/saturated and oleic/palmitic ratios were higher in the samples of water-stressed trees. These results confirm previous reports (Salas *et al.*, 1997).

The sterol fraction composition as well as total sterol concentration are presented in table 6. Total sterols were significant higher in the oils from water stressed trees. In accordance with previous reports (Inglese *et al,* 1996), b-sitosterol and campesterol were also found significantly higher in oils extracted from water stressed fruits.

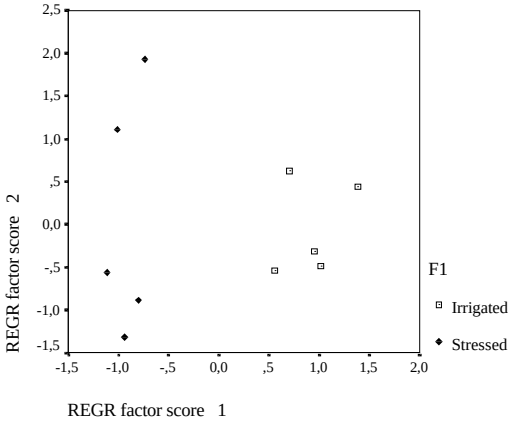

Figure 1
Scatter plot of olive oil samples based on regression variables extracted from principal component analysis.

Table V

Mean values[1] ± standard deviation of fatty acid concentration in virgin olive oils from water-stressed and irrigated olive trees of c*v* Koroneiki

	Stressed	Irrigated
Palmitic (C16:0)	12,49 ± 0,65 a[2]	13,35 ± 0,42 a
Palmitoleic (C16:1)	1,35 ± 0,06 b	1,14 ± 0,10 a
Eptadecanoic (C17:0)	0,08 ± 0,02 a	0,07 ± 0,02 a
Stearic (C18:0)	2,59 ± 0,10 a	2,70 ± 0,20 a
Oleic (C18:1)	75,38 ± 0,78 a	74,71 ± 0,89 a
Linoleic (C18:2)	6,22 ± 0,29 a	6,20 ± 0,49 a
Linolenic (C18:3)	0,85 ± 0,04 a	0,83 ± 0,05 a
Arachidonic (C20:0)	0,45 ± 0,01 a	0,46 ± 0,03 a
Eicosenoic (C20:1)	0,27 ± 0,01 a	0,29 ± 0,01 a
Behenic (C22:0)	0,14 ± 0,00 a	0,14 ± 0,02 a
Lignoceric (C24:0)	0,05 ± 0,01 a	0,06 ± 0,02 a
C18:1/C16:0	6,05 ± 0,37 a	5,60 ± 0,22 b
saturated	15,72 ± 0,72 b	16,70 ± 0,28 a
unsaturated	77,85 ± 0,81 a	76,96 ± 0,79 a
unsaturated/saturated	4,96 ± 0,27 a	4,61 ± 0,13 b

1 Results are means of ten determinations (n=10).
2 Means followed by the same letter within each row are not significantly different (p≤ 0,05)

Table VI

Mean values[1] ± standard deviation of sterol composition of virgin olive oils obtained from olive fruits of cv Koroneiki from drougth -stressed and irrigated olive trees

	Stressed	Irrigated
Campesterol (%)	4,46 ± 0,18 a[2]	3,98 ± 0,14 b
campestanol (%)	0,43 ± 0,02 a	0,47 ± 0,03 b
Stigmasterol (%)	0,49 ± 0,06 a	0,59 ± 0,04 b
b-sitosterol (%)	83,52 ± 1,83 a	80,25 ± 1,13 b
Sitostanol (%)	0,42 ± 0,02 a	0,28 ± 0,03 b
Δ-5_Avenasterol (%)	9,88 ± 2,11 b	11,39 ± 1,29 a
D-7_stigmasterol (%)	0,23 ± 0,07 b	0,26 ± 0,02 a
D-7_Avenasterol (%)	0,28 ± 0,03 b	0,36 ± 0,03 a
Total sterols (ppm)	1173,2 ± 1,38 a	1033,8 ± 5,95 b

1 Results are means of ten determinations (n=10).
2 Means followed by the same letter within each row are not significantly different (p≤ 0,05)

The scatter plot of olive oil samples of irrigated and water stressed fruits in two axes is presented in figure 1. It is evident from the plot that the qualitative parameters of the oils –used as variables in the statistical analysis– allow classification of the oil samples in two separate groups, irrigated and stressed.

Considering the results obtained in this investigation we can conclude that the oil content as well as several chemical characteristics of olive oil are affected by water stress. Irrigation exerts a positive effect on fruit development and oil

accumulation. The increased concentration of total sterols and unsaturated fatty acids in oils from water stressed fruits impose a more advanced stage of ripeness in water stressed fruits compared to the irrigated ones. Therefore, further studies are to be carried out to study the effect of irrigation on the ripening evolution of olive fruit as well as composition and total quality including sensorial evaluation of the resulting oils.

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