Increase in the selenium content of extra virgin olive oil: quantitative and qualitative implications

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SUMMARY: The biofortification of food crops for human consumption is a direct strategy increasing dietary intake of selenium (Se). The aim of this study was to evaluate the possibility of increasing the Se content of extra virgin olive oil (EVOO) by spraying the olive tree canopy with sodium selenate and the effect of the increase in Se on the chemical properties and sensory characteristics of the EVOO. Se treatments were up to 50 times more effective in enhancing Se content in the EVOO compared with the untreated controls. Se concentration in all the EVOO samples can be considered adequate and useful for providing the human diet with the correct dose of Se. Se-enriched EVOO showed a significant increase in pigment and phenol content. Also, Se treatment does not produce negative effects on fruit characteristics or the sensory quality of EVOO.

KEYWORDS: *Biofortification; Chemical parameters; Extra virgin olive oil (EVOO); Fruit characteristics; Nutraceutical value; Selenium (Se); Sensory quality*

RESUMEN: ***Aumento del contenido de selenio en aceites de oliva virgen extra: implicaciones cuantitativas y cualitativas***. La biofortificación de cultivos alimenticios para el consumo humano es una estrategia directa para aumentar la ingesta de selenio (Se) en la dieta. El objetivo de este estudio fue evaluar la posibilidad de aumentar el contenido de Se en aceites de oliva virgen extra (AOVE) pulverizando la copa de los olivos con selenato de sodio y el efecto del aumento en el contenido de Se en las propiedades químicas y características sensoriales del AOVE. Los tratamientos con Se fueron muy eficaces consiguiendo aumentar el contenido de Se en el AOVE hasta 50 veces más en comparación con los controles no tratados. La concentración de Se en todas las muestras EVOO puede considerarse adecuada y útil para proporcionar a la dieta humana con la dosis correcta de Se. EVOO-Se enriquecido mostró un aumento significativo en pigmentos y contenido de fenoles. Además, el tratamiento de Se no implica efectos negativos sobre características frutales ni sobre la calidad sensorial de AOVE.

PALABRAS CLAVE: *Aceite de oliva virgen extra (AOVE); Biofortificación; Calidad sensorial; Características de los frutos; Parámetros químicos; Selenio (Se); Valor nutracéutico*

1. INTRODUCTION

Selenium (Se) is a very important element for human health, and is involved in defensive mechanisms and the biosynthesis of hormones in adults and babies. Se is mainly involved in the production of the active thyroid hormone, in muscle function, in the reproduction process and in the immune response to some infections (Dhur *et al.*, 1990). Se is also involved in membrane protection and has an anti-cancer action. In addition, Se plays an important role in the antioxidant protection of cells, and as a component of different enzymes such as glutathione peroxidase and thioredoxin reductase (Combs and Grey, 1998, Birringer *et al.,* 2002; Ferrarese *et al.*, 2012).

The essentiality of Se in higher plants is still under debate. Although it is harmful to plants in high concentrations, it exerts beneficial effects at low concentrations. Se increases the tolerance of plants to UV-induced oxidative stress, it delays senescence and promotes the growth of ageing seedlings. Recently, it has been shown that Se is able to regulate the water status of plants under drought conditions (Kuznetsov *et al.*, 2003, Tadina *et al.*, 2007, Germ *et al.*, 2007, Proietti *et al.*, 2013).

The bio-fortification of food crops for human consumption is a direct strategy to increase dietary Se intake; moreover, Se-enriched crops can take advantage of its beneficial effect (Germ *et al.*, 2007, Gissel-Nielsen, 1998).

The content of Se in plants can be increased in different ways: by adding Se to the soil, soaking seeds in an Se solution before sowing, hydroponic and aeroponic cultivation in a nutrient solution containing Se, and the foliar application of Se solutions to plants (Bittman *et al.*, 1997, Pifferi and Poggi, 1998, Salt *et al.*, 2000, Zhao and Shewry, 2011).

Gupta *et al.* (1988) showed that foliar spraying was preferable in comparison to soil application, and it was due to the more efficient uptake of Se and the absence of residual effects. Furthermore, foliar spraying involves a minimum consumption of Se salts and is the safest and most economically acceptable way of improving Se content in crops (Djujic *et al.*, 2000). The foliar spraying of Se is effective in increasing the Se content in many crops, such as barley and wheat (Gupta *et al.*, 1988). In green tea, Se foliar spraying increased not only the Se content, but also the plant yield, total amino acids, and the amounts of vitamin C (Hu *et al.*, 2003).

Whereas many papers on the effects of fortifying herbaceous crops with Se are present in the literature, very few studies were aimed at investigating its effects on fruit trees. Nonetheless, it was ascertained that spraying peach and pear with sodium selenate affected the shelf-life of the fruit, delaying the reduction in flesh firmness and fruit ripening (Pezzarossa *et al.*, 2012).

Dugo *et al.* (2004) studied the correlation between the Se content of Sicilian olive oil and the olive cultivars and areas of provenance; the results obtained provided evidence that both genetic factors (cultivars) and geographic factors (olive-growing zone) may influence the Se content.

Normally, the Se content in olive oil is highly dependent on the amount of Se in the soil and on the ability of plants to take up and accumulate it (Terry *et al.*, 2000).

Mediterranean cuisine is widely considered a healthy and disease-preventive diet. Olives and olive oil, especially extra virgin olive oil (EVOO), are important components of this diet, since they contain a large variety of bioactive compounds (phytochemicals), which are considered to be beneficial for human health (Cicerale *et al.*, 2009). The healthy properties of olive oil are mainly due to the presence of some antioxidant micro-nutrients: phenols and tocopherols.

EVOO contains a considerable amount of phenols that have a great effect on both the stability and the sensory and healthy characteristics of the oil. As water-soluble components, phenols play an important role since they have a wide range of biochemical and pharmaceutical effects, including anti-carcinogenic, antiatherogenic, antimicrobial and antioxidant activities (Kohyama *et al.*, 1997; Visioli and Galli, 1998).

Due to their antioxidant activity, the amount of phenols is correlated with the resistance of oil to oxidation and thus to oil stability (Boskov, 1996). Investigations have been carried out to evaluate and compare the antioxidant action of Se and vitamin E. It has been found that the mechanism of the action of Se as an antioxidant in vivo is different from that of vitamin E. In particular, it was demonstrated that vitamin E prevents lipid peroxidation more effectively than Se, whereas Se prevents the production of free radicals more effectively than vitamin E (Bettger, 1993; Zhu *et al.*, 1992). Therefore, the simultaneous presence of Se, vitamin E and phenols in a food, such as EVOO, should substantially increase its antioxidant activity. Many studies have investigated the biological properties of antioxidant and radical-scavenging micro-constituents of olive oil (Tuck and Hayball, 2002), but there is little available data concerning the amount of Se and its effect on oil stability (Bratakos *et al.*, 1987; Simonoff *et al.*, 1988; Dugo *et al.*, 2004).

The aim of this study was to evaluate the possibility of increasing the Se content in EVOO by spraying the olive tree canopy with sodium selenate and the effect of the increase in Se content on the fatty acid composition, phenol and pigment content, oxidation stability and sensory characteristics of the oils. The effect of Se on fruit characteristics was also studied. Well irrigated and water-stressed trees were treated to investigate Se absorption by trees and if the effects of Se on the oil were influenced by soil water availability.

2. MATERIALS AND METHODS

2.1. Plant material and Se treatments

The research was carried out in Central Italy (Perugia, about 400 m a.s.l., 12°23′E longitude, 43°5′N latitude). Climatic conditions of the experimental site were monitored by a meteorological station. The rainfall pattern in the trial year (2011) was characterized by a scant rainfall in spring, summer and autumn (187 mm, 61 mm and 139 mm of rain from March to May, from June to August and from September to November, respectively).

During the experimental period, the highest average of the maximum daily temperatures was in August (33.6 °C), whereas the lowest one was at the beginning of November (15.7 °C).

The experiment used six-year-old potted olive trees of the Maurino cultivar, trained according to the open-center training system and spaced 2.0×1.5 m between and along the rows (North-South). The trees were about 2.0 m tall and the canopy had a diameter of approximately 1.0 m and a height of about 0.9 m. The soil in the pot (approximately 70 kg) was medium textured and had a pH of 7.7 and a content of 0.2% of total nitrogen, 55 and 190 mg kg^{-1} of assimilable phosphorus and exchangeable potassium, respectively. Each olive was fertilized every year in mid April with the controlled-release fertilizer "Osmocote" ("Scott Italia" 16:8:12 N:P:K) at 100 g per pot. From mid-May to mid-September 2011, 27 trees were irrigated (well irrigated trees: WI) to 80% of the substrate available water (substrate water content approximately 24% on a dry weight basis), whereas the other 27 trees (water stressed trees: WS) were irrigated to 25% of the substrate available water (substrate water content approximately 15% on a dry weight basis). Irrigation was carried out every day in the morning using drip lines (two drippers per pot, each with a flow rate of 4 L h^{-1}). The pots were covered with aluminium foil in order to prevent both overheating and the supply of rain water.

At the beginning of July, the trees (both WI and WS) were sprayed with a solution containing two different Se concentrations, i.e. 50 and 150 mg L^{-1} (respectively 50 Se and 150 Se), obtained by dissolving the correct amount of sodium selenate in water. ("Sigma-Aldrich" cod. S0882-25g). For each treatment, 0.5% of the wetting agent "Albamilagro" was added. The controls included in the experiment were sprayed with a solution containing only the wetting agent.

2.2. Yield per tree and fruit characteristics

At harvest (beginning of November) the yield per tree, the fruit ripeness indexes (detachment force, flesh firmness and fruit pigmentation) of the fresh fruit and dry weight and oil content were determined.

The yield per tree was determined by weighing the harvested olives for each tree (9 trees per treatment).

Detachment force was measured using the "Carpo" hand dynamometer, and flesh firmness was determined by means of an "Effe.gi" dynamometer DT 05 (with 1.0 mm diameter tip), on about 20 olives per tree. Fruit pigmentation was evaluated using the maturity index (MI) according to the method developed by the Agronomic Station of Jaén (Uceda and Hermoso, 1998) based on the evaluation of the olive skin and pulp colors. MI values range from 0 (100% intense green skin) to 7 (100% purple flesh and black skin).

The fresh fruit fresh and dry weight (the latter determined by drying three samples of fresh olives per thesis at 90 °C) and the oil content (using the "SpectraAlyzer ZEUTEC" – NIR: Near Infra Red on two samples of olives per thesis) were determined.

2.3. Oil extraction

The oil samples from approximately 2.5 kg of olive for each sample (two per thesis) were extracted using a mini olive-mill one day after harvesting (beginning of November), and carried out in the following stages: hammer crushing, 25 minutes rippling at room temperature (about 22 °C), centrifugation of the paste to separate the oil with a centrifugation basket without using water, oil filtration with cotton wool and sodium sulphate anhydrous to remove the water and impurities. The oil samples were stored in closed, dark glass bottles in a fridge at 4 °C until analysis (a week after extraction).

2.4. Analytical Methods

The Se content in the oil samples was determined as follows (US EPA Method 3031, 1996). 0.5 g of oil was mixed with 0.5 g of finely ground $KMnO_4$, and then 1.0 mL of concentrated H_2SO_4 was added while stirring. A strong, exothermic reaction occurred, and so the sample was ice cooled. The sample was then treated with 2 mL of concentrated HNO_3. 10 mL of concentrated HCl were added to the sample, which was heated until the reaction was complete and then filtered. The filter was washed with hot concentrated HCl (temperature). The filter paper was transferred to a digestion flask, treated with 5 mL of concentrated HCl.

Taking into account the low concentration of Se in vegetable oils, we used a highly sensitive, analytical method, which adopted a graphite furnace for use with the atomic absorption spectrophotometer, Shimadzu AA-6800 (GF-AAS; GFA-EX7, Shimadzu Corp., Tokyo, Japan), with a deuterium lamp background correction and a matrix modifier ($Pd(NO_3)_2$, 0.5 mol·L^{-1} in HNO_3). The accuracy of the analytical procedure was obtained by spiking a suitable, known amount of the analyte into a test

portion of the oil sample, having a known concentration of the added analyte (100 μg·L^{-1}), and by analyzing the spiked test portion along with the original sample. The mean percent recovery of Se for 4 samples of spiked olive oil was 92.4%.

The Fatty Acid Methyl Ester (FAME) composition of the oils was determined by GC as FAME. FAME were prepared by saponification/methylation with sodium methylate according to the Commission Regulation (EU) N°61/2011. A chromatographic analysis was performed in a Hewlett-Packard 5890 Series II gas chromatograph using a capillary column (SP 2330; Supelco, Bellefonte, PA). The column temperature was kept at 190 °C, and the injector and detector temperatures were 220 °C. The fatty acids (FA) were identified by comparing retention times with standard compounds. The contents of 11 FA were determined and expressed as percentages of FAME (Table 3).

Phenol content was determined as described by Vazquez Roncero *et al.* (1974) and the results were expressed as μg·g^{-1} of gallic acid.

Pigments (chlorophyll a and b, and total carotenoids) were determined in 0.5 g oil samples dissolved in 25 mL of 95% diethyl ether. The solution was filtered through a double layer of cheese cloths and absorbances at 662, 646 and 470 nm were determined using a Genesys 10 BIO spectrophotometer (Wellburn, 1994). The pigments were calculated using the following formulas:

$$C_a \ (\text{mg·L}^{-1}) = (10.05 \times A_{662}) - 0.766 \times A_{644}$$

$$C_b \ (\text{mg·L}^{-1}) = (16.37 \times A_{644}) - 3.140 \times A_{662}$$

$$C_{x+c} \ (\text{mg·L}^{-1}) = (1000 \times A_{470}) - (1.280 \times C_a) \times C \ (56.7 \times C_b / 230)$$

Where C_a=Chlorophyll a, C_b=Chlorophyll b, C_{x+c}=Total carotenoids.

The acidity and peroxide number of the oil were determined according to the EEC 2568/91 (1991).

Oxidation induction time (h) was determined using a Rancimat 679 apparatus (Metrohm Co., Herisau, Switzerland) according to Mateos *et al.* (2006).

A flow of air (20 mL·h^{-1}) was bubbled through 5.0 g of oil heated to 110 °C, 120 and 130 °C. The volatile oxidation products were stripped from the oil and dissolved in cold water, thus increasing its conductivity. The time taken to reach an inflection point at the induction curve was measured.

2.5. Sensory evaluation

Sensory evaluation of the oil was carried out by nine panel tasters. The tasters were fully trained in the evaluation of virgin olive oil according to the EEC 2568/91 (1991). Panellists completed a profile sheet, giving a score ranging from 0 to 5 for fruity, bitter, pungent and color (the latter was visually determined and values range from 0=yellow to 5=intense green), and then gave an overall evaluation, assigning scores ranging from 1 to 9. The results obtained were averaged over all tasters.

2.6. Statistical Analysis

The experimental design was a completely randomized 2-factor factorial with 9 trees per treatment, though samples were collected at harvest time from three trees and mixed together for the analyses, so that the final number of true-replicates was three.

All data, with the exception of those of the sensorial evaluation, were submitted to 2-way ANOVA and the means were compared using the Fisher Least Significant Difference at p=0.05. A graphical analysis of residuals was used to make sure that the basic assumptions for ANOVA were met and, whenever necessary, data was square-root transformed prior to analyses.

3. RESULTS AND DISCUSSION

In general, as expected, yield per tree was higher in WI than in WS (Table 1). Even though they did not completely counteract the effect of drought, Se treatments increased yield per tree. According to Proietti *et al.* (2013), these results seem to show a positive effect in WS of Se treatments on reducing the negative effects of water stress. In WI trees no differences due to Se treatments were found. The increase in the production of WI compared to WS, and of WS Se-treated trees compared to untreated ones, was due to the increase in fruit fresh weight; no differences in fruit number per node were found (data not shown). Without differences due to Se treatments, the water content percentage of fruit was higher in WI than in WS. However, the differences were not very high (water content about 60% and 55% in WI and WS, respectively) and this was probably due to the fact that, in the last period of fruit growth, the fruit water status can recover. The percentage of oil in the fruit, on a dry matter basis, did not significantly differ between WI and WS and Se-treated and untreated trees (data not shown).

The fruits of the WI trees showed a lower pigmentation and higher detachment force and flesh firmness than those of stressed trees. These effects indicate, as expected, that in trees with a higher crop load ripening is slowed down. No differences due to Se treatments were found.

Table 2 reports the Se concentration in EVOO coming from the the trees in the experiment. There was a sharp increase in the Se content due to the treatments. This ranged from 15.0 (WI) to 22.3 (WS) μg kg^{-1} in oils from Se-untreated

TABLE 1. Yield per tree and fruit characteristics in olives treated with Se or not, water stressed (WS) or not (WI)

Sample	Yield per tree (g)	Fruit fresh weight (g)	Fruit dry weight (g)	Maturity index (MI)	Fruit detachment force (N)	Flesh firmness (g)
WI	610±16[d]	1.91±0.15[b]	0.76±0.08[b]	2.98±0.45[a]	3.39±0.40[a]	386±16[d]
WS	461±11[a]	1.38±0.17[a]	0.61±0.11[a]	4.96±0.32[b]	3.25±0.42[a]	300±11[a]
50 Se WI	606±15[d]	2.06±0.15[bc]	0.85±0.08[bc]	3.07±0.25[a]	3.52±0.52[a]	380±18[d]
50 Se WS	581±15[c]	1.55±0.16[a]	0.68±0.07[a]	4.13±0.25[b]	3.44±0.48[a]	363±13[c]
150 Se WI	620±16[d]	2.22±0.16[c]	0.90±0.05[c]	2.25±0.52[a]	3.53±0.49[a]	400±16[d]
150 Se WS	501±12[b]	1.84±0.16[b]	0.83±0.16[b]	5.04±0.65[b]	3.43±0.43[a]	340±13[b]

[a]In each column, means followed by different letters are significantly different ($P<0.05$). The values are expressed as mean values±SD.

TABLE 2. Se concentration in extra virgin olive oil from treated with Se or not, water stressed (WS) or not (WI) olives

Sample	Mean Se content ([a]$\mu g\ kg^{-1}$)
WI	15.0±1.5[a]
WS	22.3±2.1[b]
50 Se WI	430.8±7.6[c]
50 Se WS	458.6±47[d]
150 Se WI	850.3±20.2[e]
150 Se WS	956.6±8.5[f]

[a]Mean and standard deviation are based on three replicate analyses. Anova and multiple comparising testing were performed on square root transformed data.

trees (controls) to values of 430.8 (WI) to 458.6 (WS) $\mu g \cdot kg^{-1}$ (50 Se oil samples) and 850.3 (WI) to 956.6 (WS) $\mu g \cdot kg^{-1}$ (150 Se oil samples). In the oil samples coming from Se-treated trees, the Se concentration in WS samples was significantly higher than ($P<0.05$) in the WI samples, with the exception of the Se dose.

The U.S. Recommended Dietary Allowance (RDA) (National Research Council, 1980) for Se ranges from 75 to 60 $\mu g \cdot day^{-1}$ for adult men and women, whereas the tolerable dose is set at 400 $\mu g\ day^{-1}$. The daily Se requirement in human daily nourishment is not often reached (Pifferi and Poggi, 1998). Considering that a person usually eats about 40 g per day of EVOO (www.piramideitaliana.it, 2005), the contribution of oil from treated trees to the daily Se RDA could be very important since it is 17.2 (WI) and 18.3 (WS) μg Se (50 Se oil samples), and 34.0 (WI) and 38.6 (WS) μg Se (150 Se oil samples). These Se intakes due to the oils are lower than the RDA and very far from the toxic dose (400 $\mu g \cdot day^{-1}$). Also taking into account that a person can find Se in other foods, the Se concentration in all the WI and WS oil samples can be considered adequate and useful to provide the human diet with the correct dose of Se. In any case, it is always possible to change the Se concentration of the solution sprayed, if the Se concentration in EVOO is too high.

Acidity, peroxide number, K_{232} and K_{270} were not influenced either by the irrigation regime or by the Se treatments, and they were at an optimal level and considerably lower than the limits established by the Commission Regulation (EU) N°61/2011 (Table 3). These results agree with those reported by Dettori and Russo (1993) and Patumi et al., (1999) who found that the irrigation regime had no effect on these parameters.

The fatty acid composition was similar in all the oils, with percentages of total saturated fatty acid (SFAs), total polyunsaturated fatty acids (PUFAs) and total monounsaturated fatty acids (MUFAs) ranging from 13.34 to 15.60%, from 9.67 to 10.66% and 72.65 to 73.94%, respectively. The unsaturated/saturated acid ratio (ranging from 6.90 to 7.62) and the mono-unsaturated/poly-unsaturated acid ratio (ranging from 5.36 to 5.85) were also uninfluenced by irrigation and the Se treatments (Table 3).

All the EVOO samples obtained from the WS Se-treated and control trees, showed concentrations of phenols significantly higher ($P<0.05$) than those from the WI trees (Table 4). This concurs with several studies, which report an increase in phenol contents in oils as the amount of water availability decreases; in fact, phenols are often associated with abiotic stress defence, signalling disease resistance and are considered a good indicator of the antioxidant capacity of plant tissues (Baidez et al., 2007).

The high phenol content in EVOO could be considered a positive factor, since the higher the phenol content, the greater the antioxidant activity of the oil. Furthermore, they positively influence the oil sensory profile, giving tastes of bitterness and pungency which consumers appreciate at the correct level. In the EVOO samples obtained from the Se-treated trees there was a significant increase of the phenol content compared with the control. The increase in WI was approximately 4 and 24% for 50 Se and 150 Se, respectively, and in WS it was approximately 6 and 10% for 50 Se and 150 Se, respectively. This means that Se-treatments could cause a further increase in phenol contents in the oils from water stressed trees. As reported by

TABLE 3.   Acidity, peroxide value and fatty acid composition of extra virgin olive oil from treated with Se or not, water stressed (WS) or not (WI) olives

	WI	WS	50 Se WI	50 Se WS	150 Se WI	150 Se WS
Acidity (% oleic acid)	0.23±0.03[a]	0.22±0.02	0.19±0.01	0.20±0.02	0.22±0.02	0.19±0.01
Peroxide value (meq O_2 kg^{-1} of oil)	12.1±0.4	18.3±0.5	13.6±0.1	13.2±0.2	11.4±0.4	11.1±0.5
K_{270}	0.12±0.03	0.13±0.05	0.12±0.03	0.11±0.02	0.16±0.01	0.14±0.02
K_{232}	1.89±0.30	1.90±0.21	1.78±0.51	1.95±0.31	1.54±0.44	1.95±0.41
C16:0[b]	12.99±0.33	13.35±0.25	12.89±0.28	12.91±0.22	12.57±0.31	13.31±0.29
C16:1	1.22±0.01	1.26±0.01	1.20±0.02	1.25±0.03	1.14±0.02	1.20±0.01
C17:0	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.04±0.01
C17:1	0.1±0.05	0.09±0.05	0.09±0.02	0.09±0.02	0.08±0.01	0.09±0.02
C18:0	1.37±0.12	1.84±0.09	1.48±0.08	1.65±0.07	1.49±0.05	1.69±0.04
C18:1	71.94±0.34	71.05±0.45	71.83±0.48	72.12±0.21	72.45±0.38	71.79±0.24
C18:2	9.91±0.40	9.7±0.25	9.65±0.33	9.05±0.25	9.34±0.21	9.1±0.21
C18:3	0.75±0.03	0.71±0.02	0.67±0.02	0.62±0.03	0.68±0.02	0.71±0.03
C20:0	0.25±0.04	0.28±0.03	0.24±0.02	0.26±0.04	0.25±0.03	0.27±0.03
C20:1	0.26±0.03	0.25±0.03	0.24±0.03	0.23±0.04	0.27±0.02	0.24±0.02
C22:0	0.07±0.01	0.1±0.01	0.06±0.01	0.07±0.02	0.07±0.03	0.08±0.03
Σ SFAs	13.34±0.51	15.60±0.39	14.7±0.55	14.92±0.37	14.41±0.43	15.39±0.40
Σ PUFAs	10.66±0.43	10.41±0.27	10.32±0.35	9.67±0.28	10.02±0.23	9.81±0.24
Σ MUFAs	73.52±0.43	72.65±0.54	73.36±0.55	73.69±0.30	73.94±0.43	73.32±0.29
Unsat/Sat	5.75	5.36	5.72	5.61	5.85	5.43
MUFAs/PUFAs	6.90	6.98	7.11	7.62	7.38	7.47
C18:1/C18:2	7.26	7.32	7.44	7.97	7.76	7.89

[a]Mean ± standard deviation (n=3).

[b]C16:0 palmitic, C16:1 palmitoleic, C17:0 margaric, C17:1 margaroleic, C18:0 stearic, C18:1 oleic, C18:2 linoleic, C18:3 linolenic, C20:0 arachidic, C20:1 gadoleic, C22:0 behenic, SFAs saturated fatty acids, PUFAs polyunsaturated fatty acids, MUFAs monounsaturated fatty acids.

TABLE 4.   Phenol, chlorophyll and carotenoid contents of extra virgin olive oil from treated with Se or not, water stressed (WS) or not (WI) olives

	WI	WS
Total phenolic compounds (expressed as µg of gallic acid g^{-1})[a]		
control	473±12[a]	626±15[c]
50 Se	490±20[a] (3.6)	663±16[c] (5.8)
150 Se	586±19[b] (23.9)	686±21[d] (9.5)
Total carotenoids (µg g^{-1})[a]		
control	8.08±0.32[a]	10.37±0.42[b]
50 Se	12.14±0.43[c] (50.2)	12.68±0.52[c] (22.1)
150 Se	18.64±0.41[e] (129.6)	16.52±0.62[d] (58.6)
Total chlorophylls (µg g^{-1})[a]		
control	12.70±0.41[a]	14.9±0.56[b]
50 Se	20.10±0.48[c] (107.2)	22.60±0.42[c] (51.6)
150 Se	31.57±0.56[e] (225.4)	27.93±0.51[d] (87.4)

[a]Means followed by different letters are significantly different (P<0.05). The values are expressed as mean values ± SD (n=3). Data on brackets are percent of variation from control.

Turlo *et al.* (2010) the higher levels of phenols in oils from Se-treated trees may be due to a secondary effect, caused by the inhibition of enzymatic phenol oxidation by strong, antioxidant-active Se compounds. Since no differences in MI due to Se treatments were found (Table 1), it is possible to exclude that the higher levels of phenols in oils from Se-treated trees can be due to a delay in the ripening of the fruit.

In the EVOO samples from Se-untreated trees, the pigment (chlorophylls and carotenoids) concentrations in the oils from WS plants were significantly higher than in the oils from WI trees (Table 4). In the EVOO samples from both WI and WS Se-treated trees, the pigment concentrations were significantly higher than those of the controls (P<0.05), even though the increase is higher in the samples from the WI trees. The dose of 150 Se induced a significantly higher pigment concentration increase in oils than the 50 Se dose (P<0.05). With regard to the EVOO samples from trees treated with a 50 Se dose, there was no significant difference in pigment

TABLE 5. Induction time (h)[a] determined by the Rancimat method at various temperatures in EVOO from treated with Se or not, water stressed (WS) or not (WI) olives

Sample	Induction time at 110 °C	Induction time at 120 °C	Induction time at 130 °C
WI	21.1±1.2	10.6± 1.1	5.0±0.1
WS	30.7±2.1	13.9±0.5	6.7±0.2
50 Se WI	20.8±1.3	11.1±1.2	5.2±0.2
50 Se WS	28.2±1.2	13.5±1.2	6.4±0.5
150 Se WI	26.1±1.5	12.9±1.0	6.1±0.2
150 Se WS	32.0±2.1	15.2±0.5	6.6±0.5

[a]Each Rancimat value is listed with precision of one tenth of an hour and is the average of 3 determinations; data are expressed as mean values ± standard deviation, Mean ± SD (n=3).

concentrations between the oils from WI and WS trees. On the contrary, in the EVOO samples from trees treated with a 150 Se dose, the chlorophyll and carotenoid concentrations in the WI oil samples were significantly higher than in the WS samples ($P<0.05$).

The positive effect of Se in increasing pigment content in oil concurs with what is reported by Chen *et al.*, (2008) who found that in *Spirulina platensis*, at a concentration of 150 mg·L^{-1}, Se significantly increased ($P<0.05$) the level of photosynthetic pigments (including lutein, β-carotene, and chlorophyll *a*); by contrast, they found a significant decrease in the content of photosynthetic pigments at higher Se concentrations (≥175 mg·L^{-1}). Similar results were obtained by Hawrylak-Nowak (2009) who found a statistically significant increase in the chlorophyll *a* and *b* and carotenoid content in NaCl-stressed cucumber plants grown with a 5 mM Se addition. The increased chlorophyll and carotenoid contents in Se-treated trees might be attributed to the efficient scavenging of ROS by antioxidant enzymes (Djanaguiraman *et al.*, 2005).

The color of the EVOO, which depends exclusively on biological compounds such as chlorophylls and carotenoids, is very important, since it is one of the factors that influence consumers' choice; moreover, these pigments play an important role in the oxidative stability due to their antioxidant nature

in the dark and pro-oxidant activity in the light (Minguez-Mosquera *et al.*, 1991, Chtourou *et al.*, 2013).

With respect to the EVOO samples from the controls, the induction times in the WS plant oils was higher (30.7 h at 110 °C) than in those from the WI oils (21.1 h at 110 °C), which could be related to the higher content in phenols of the WS oil (Table 5). In fact, the literature reports that the oxidative stability of olive oil is greatly affected by its phenol content, since the phenols are the main agents responsible for resistance to oxidation and photo-oxidation (Patumi *et al.*, 2002, Bendini *et al.*, 2007). The treatment of WI and WS trees with the 150 Se dose caused an increase in the induction time of 23.0% and 4.2%, respectively, with respect to the values of the controls. Consequently, the treatment was useful in producing an increase in the oxidative stability in oils from WI trees, whereas in oil samples from WS trees the effect was not statistically significant. WI and WS trees, treated with a 50 Se dose, did not show any effect which increased the induction time values.

The sensory oil characteristics did not substantially differ between WI and WS and Se treated and untreated trees; the only exception was the bitter and pungent sensations that tend to be higher in WS compared with WI and in Se treated, especially at 150 Se, compared to untreated trees (Table 6). The slight increase in the sensations of bitterness and pungency is attributable to the increase in the phenol content. All oil samples were well-balanced with medium-high fruity values. The overall effects on the sensory characteristics of the oil due to treatment indicated that Se did not cause any negative effects on the sensory quality of the oil.

In conclusion, the technique of fortifying olive tree canopies with sodium selenate, in addition to not adversely affecting the fruit characteristics, is a good way to increase the content of Se in EVOO, adding a beneficial element to this important food in the Mediterranean diet which affects human health. Another advantage is the fact that increased Se in EVOO positively changes some oil properties, such as color intensity, stability and sensory quality.

TABLE 6. Sensory profile of extra virgin olive oils from treated with Se or not, water stressed (WS) or not (WI) olives

	WI	WS	50 Se WI	50 Se WS	150 Se WI	150 Se WS
Fruity (0÷5)	2.5	2.5	2.0	3.0	2.5	3.0
Bitter (0÷5)	2.0	3.0	2.0	3.5	3.0	3.5
Pungent (0÷5)	2.5	3.0	2.0	3.0	3.0	3.0
Color (0÷5)	2.0	1.5	2.5	2.5	2.0	2.5
Note (0÷5)	green	green/mature	mature	green	green	green
Score (1÷9)	8.0	8.0	7.5	8.5	8.0	8.5

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