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Influence of the olive variety and the zone of provenience on selenium content determined by cathodic stripping potentiometry (CSP) in virgin olive oils

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Abstract

The purpose of this paper was to determine the content of selenium in 50 samples of Sicilian virgin olive oils produced in six olive-growing zones from 10 different varieties of olives, and to investigate the possible connection between the olives varieties (cultivar), the zone of provenience and the selenium content in the oils. Cathodic stripping potentiometric analysis was used as a rapid, sensitive and reproducible method to determine selenium levels in acid extracts of olive oils. A precision of 2.8%, expressed as relative standard deviation of the measurements, and a detection limit of 0.6 $\mu g\,kg^{-1}$ were obtained. The content of selenium found in the studied Sicilian virgin olive oils, ranged from 3.0 to 122.9 $\mu g\,kg^{-1}$. The obtained results evidenced that olive oil samples from the same cultivar had a similar content of selenium, whereas some differences among the cultivars from the six studied zones were observed. These data provided evidence that both genetic factors (cultivar) and geographic factors (olive-growing zone), may influence the content of selenium in the studied Sicilian olive oils.

Keywords: Cathodic stripping potentiometry; Cultivar; Olive-growing zone; Olive oil; Selenium

1. Introduction

Olive oil is one of the major constituent of diet in the Mediterranean area. Spain is the major producer, followed by Italy and Greece (Dugo, 2000). In Europe two regulations for typical products were introduced: the Protected Designation of Origin (PDO) that defines product types, included olive oil (Council Regulation EEC No. 2082/92), and the Protected Geographical Indication (PGI) that protects traditional products (Council Regulation EEC No. 2081/92). In Italy twenty-seven PDO virgin olive oils from different regions are produced and Sicily is one of the major olive oil producer. Epidemiological studies provided evidences that a high consumption of virgin olive oil contributes to the lower incidence of cancer (colon, breast, skin), coronary heart disease and aging (Gerber, 1994; Visioli & Galli, 1995). Olive oil healthy properties, are mainly due to the presence of some antioxidant micro-nutrient: phenolic compounds and tocopherols that are the source of vitamin E. In the last years many studies have investigated the biological properties of the antioxidant and radical-scavenging microconstituents of olive oil (Tuck & Hayball, 2002) but there are few available data about the possible presence of selenium (Bratakos, Zapiropoulos, Siskos, & Ioannou, 1987; Simonoff, Hamon, Moretto, Labador, & Simonoff, 1988), a trace mineral that acts as an antioxidant. Selenoenzymes, particularly glutathione peroxidase, play an important role in protecting cells from the oxidative damage, thus preventing from cardiovascular diseases and other pathologies brought on by oxidative stress (Stadtman, 1991). Recent studies indicate that supplemental Se in the human diet may also reduce cancer risk (Combs & Gray, 1998). Selenium is also involved in the production of the active thyroid hormone, in the muscle function, in the reproduction process and in the immune response to some infection (Dhur, Galan, & Hercberg, 1990). For an adult the RDA is 50–70 μg/day, however concentrations

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higher than 400 μg/day may become toxic (Rayman, 2000). Selenium is present in food matrices in many biological forms as selenite, selenate, elemental selenium, selenocysteine, selenomethionine, selenoproteins (Ganther & Lawrence, 1997); it enters the food chain through plants that take it up from the soil, so the availability of selenium strictly depends on location and season. As a consequence, human dietary intake is very variable, according to geography (Inam & Somer, 1999; McLaughlin, Parker, & Clarke, 1999) and selenium deficiency diseases were identified in parts of the world characterized by low selenium content soils, as volcanic regions (Rayman, 2000). Some authors studied the correlation between the presence of selenium in different food matrices as garlic, nuts and cereals and their region of provenience (Chang, Gutenmann, Raid, & Lisk, 1995; Stadlober, Sager, & Irgolic, 2001). The present work studies the possible correlation among the presence of selenium in Sicilian olive oils, the olives variety and the zone of provenience. In Sicily there are three PDO olive-growing zones: ''Monti Iblei'' (Reg. CEE 2523/97, UG 25/11/97), ''Valli Trapanasi'' (Reg. 2523/97 UG CEE 25/11/97), ''Val di Mazara'' (Reg. CEE 138/01, UG 24/01/01); whereas ''Valdemone'', ''Monte Etna'' and ''Valle del Belice'' zones are obtaining the mark. Moreover the Sicilian olive tree germplasm is very rich and different, it comprises several olive varieties characterized by very interesting physiological and nutritional properties; the environmental and genetic factors are those that basically influence the quality of the product (Dugo, 2000; Lo Curto, Mondello, Errante, & Russo, 2001).The low concentration of selenium in vegetable oil (Dugo, La Pera, Pollicino, & Saitta, 2003b) and the volatility of its compounds (Dugo, La Pera, Lo Turco, Mavrogeni, & Alfa, 2003a), requires a high sensitive analytical method as cathodic stripping potentiometry (CSP) (Adeloju, Jagner, & Renman, 1997), and a sample preparation procedure that avoids laborious steps based on sample carbonization. As previous papers about trace metal determination in vegetable oils reported (Dugo et al., 2003b; La Pera, Lo Curto, Visco, La Torre, & Dugo, 2002b; La Pera, Lo Coco, Mavrogeni, Giuffrida, & Dugo, 2002a), selenium was extracted from olive oil samples by concentrated hydrogen peroxide and hydrochloric acid treatment, then the cathodic stripping potentiometric determination of selenium was carried out.

2. Experimental procedures

2.1. Reagents

All the reagents used were of analytical grade. Hydrochloric acid (34–37%) and Se (IV) (1000 $\mu g\,ml^{-1}$, 0.5 N in HNO_3) standard solutions were purchased from Panreac (Barcelona, Spain). The Se (IV) standard solutions was diluted with ultra-pure water to obtain 5.0 and 10.0 $\mu g\,ml^{-1}$ Se (IV) solutions. 35% H_2O_2, used in the extraction procedure, was purchased from Carlo Erba Reagenti (Milano, Italia). Anhydrous $CaCl_2$ (Baker J.T., Deventer, Holland) was used to prepare a 2 M aqueous solution of $CaCl_2$solution, 4 M HCl. The oils extracts were filtrated on a carbon column Supelclean ENVI-Carb SPE (0.5 g, 6 ml), Supelco, Bellefonte, PA, USA. The carbon columns were activated by methanol (Carlo Erba Reagenti, Milan, Italy). Ultra-pure water (18.2 $M\Omega\,cm^{-1}$) was prepared at the Department of Organic and Biological Chemistry, University of Messina.

2.2. Apparatus

Selenium analysis was carried out on a PSA ION 3 potentiometric stripping analyzer (Steroglass, S. Martino in Campo, Perugia, Italy), already described in a previous paper (La Pera et al., 2002a, 2002b). The determination was executed in a conventional three-electrodes cell. The working electrode was a glassy carbon one coated with a thin mercury film; the reference electrode was an Ag/AgCl electrode (3 M KCl), and a platinum wire auxiliary electrode was used. To confirm the analytical results of the potentiometric method, the oily extracts were subjected to AAS, using a Shimadzu 800 series graphite furnace atomic absorption spectrometer, equipped with auto sampler ASC-6100.

2.3. Samples

All the olive oil samples were stored in dark glassy bottles with corks at 4 °C until the analysis. Fifty olive oil samples from the crop year 2001–2002, produced in different zones of Sicily were analyzed. The samples were grouped according to their zone of provenience (Fig. 1) and further divided according to the olive variety (cultivar); five samples from each variety were analysed. Ten samples came from ''Valli Trapanesi'' zone, produced from Cerasuola (Paceco–Trapani) and Crastu (Collesano–Palermo) cultivars. Ten samples came from ''Val di Mazara'' zone, produced from Biancolilla–Sciacca variety (Agrigento) and Biancolilla–Misilmeri (Palermo). Five samples came from ''Valle del Belice'' zone, obtained from Nocellara del Belice cultivar (Castelvetrano–Trapani). Ten samples came from ''Valdemone'' zone obtained from Santagatese (Mistretta–Messina) and Minuta (Castell'Umberto–Messina) cultivars. Ten samples were produced in ''Monti Iblei'' zone from Tonda Iblea (Ragusa) and Moresca (Leonforte–Enna) cultivars. Five samples were produced from ''Monte Etna'' zone from Nocellara Etnea cultivar (Paternò–Catania).

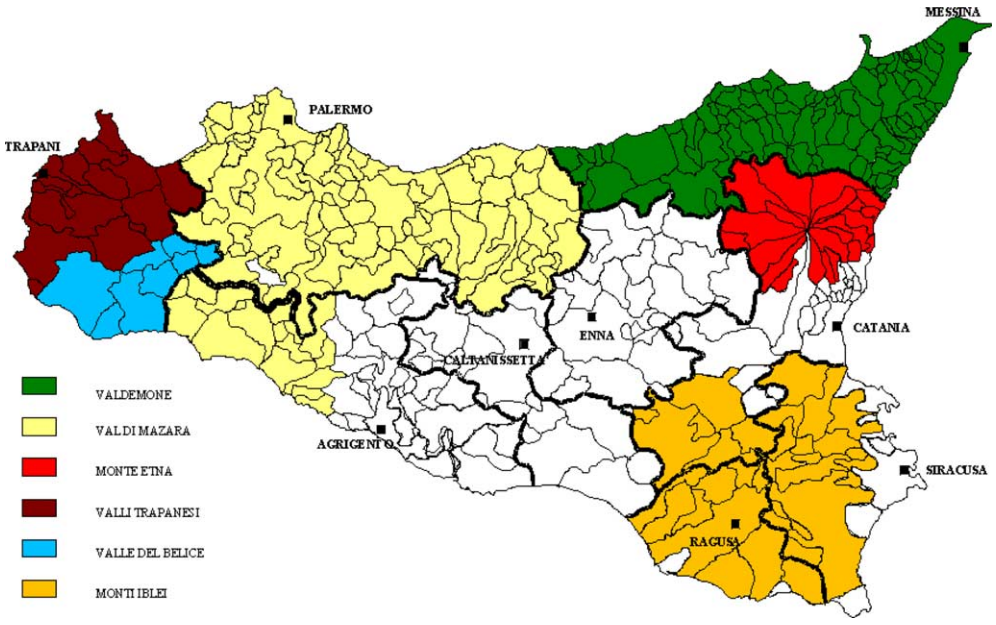

Fig. 1. Map of Sicilian olive-growing zones.

2.4. Procedure

2.4.1. Sample preparation

Dugo (2003) described the hydrogen peroxide and hydrochloric acid extraction procedure in a previous paper concerning selenium determination in vegetable oils. A 5.0 g aliquot of olive oil, 2.0 ml of 35% H_2O_2 and 5.0 ml of 36% ultrapure-hydrochloric acid were placed in a Teflon beaker. The extraction was carried out for about 30 min under magnetic stirring at 90 °C. The mixture was transferred in a separating funnel: the acid phase was taken apart in a 10.0 ml flask while the organic layer was extracted twice with 2.5 ml of concentrated hydrochloric acid for 10 min in the conditions described earlier. The treatment with concentrated HCl allowed the conversion of all selenium to the electro positive Se (IV) species (Adeloju et al., 1997). The collected acid phases were filtered on a carbon column previously activated by 2.0 ml of methanol followed by 2.0 ml of ultra-pure water.

2.4.2. Cathodic stripping potentiometric analysis

All the information concerning to the electrodes storage and the plating procedure of the working electrode were reported in previous papers (Dugo et al., 2003a, 2003b; La Pera et al., 2002a, 2002b). Then 3.0 ml of olive oils acid extracts and 8.0 ml of the HCl 4 M/$CaCl_2$ 2 M solution were placed into the electrochemical cell (Dugo et al., 2003a, 2003b)

The pre-concentration of selenium (IV) occurred at −150 mV vs Ag/AgCl reference electrode, the electrolysis time was 60 s. The deposited Hg–Se was further reduced to Se (II) during the stripping step by a −30 μA cathodic constant current while the potential decreased to −750 mV. The peak of selenium appeared at −580 mV (Fig. 2) and the final acquisition potential was −780 mV. The quantitative analysis was executed by the multiple points standard additions method: optimum precision and accuracy was obtained by executing two 0.02 or 0.05 ml standard additions of 5.00 $\mu g\,ml^{-1}$ of Se (IV) standard solution and performing each measure four times. In the electrochemical conditions described earlier the linearity concentration range was 0–500.0 $\mu g\,kg^{-1}$.

2.4.3. Detection limit

The detection limit (LOD) was evaluated using the expression $3\sigma/S$ (The European Pharmacopoeia Forum, 1999): σ indicated the standard deviation of the response (set at 200 mV/s) and S (1000 $mV\,\mu g\,kg^{-1}\,s$) was the sensitivity obtained from the slope of the calibration curve ($R^2 \geqslant 0.995$). A LOD of 0.6 $\mu g\,kg^{-1}$ was obtained.

2.4.4. Precision and reproducibility

The precision and reproducibility of the analytical method were evaluated by executing the extraction procedure three times on an olive oil sample, and quantifying selenium four times in each extract. The instrument precision is indicated as the mean rsd% for each extract, and the method reproducibility is represented by the total mean rsd% for all the extracts (Table 1).

2.4.5. Recovery tests

The possibility of loss or gain of Se amount, due to the extraction procedure, was explored by executing recovery tests both on a $CaCl_2$ 2 M/HCl 4 M solution (blank)

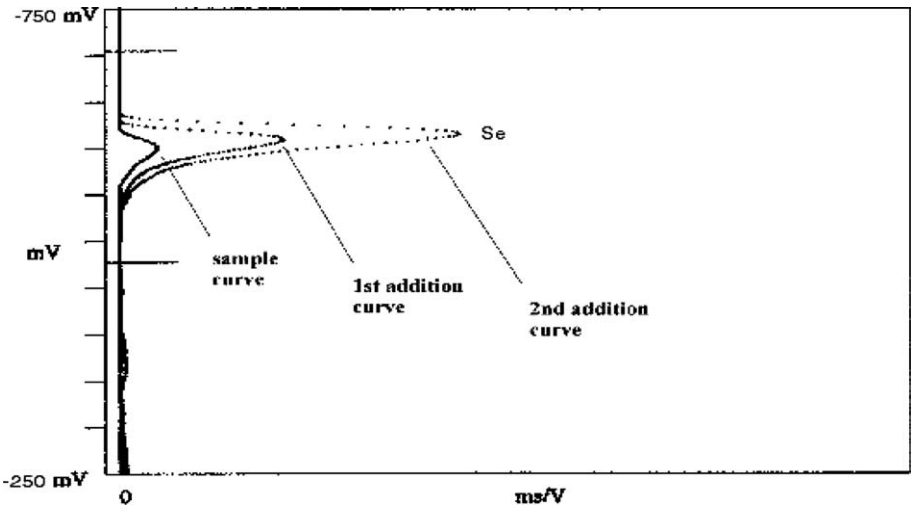

Fig. 2. Determination of selenium in 3 ml of acid olive oil extract using CSP; $E_{electr} = -150$ mV, $T_{electr} = 120$ s, $I_{stripping} = -30$ μA.

Table 1
Precision (rsd%) and method reproducibility (total rsd%), for the determination of Se (IV) in virgin olive oils[a]

	Se (μg kg^{-1})	rsd%
1st extraction	54.0 ± 1.5	2.8
2nd extraction	54.5 ± 1.6	2.8
3rd extraction	51.0 ± 1.4	2.7
Total mean	53.3 ± 2.1	
Total rsd%	3.9	

[a] Hydrochloric acid treatment as selenium extraction procedure was tested. Each sample was extracted three times, and each extract was analyzed four times.

and on a selenium-free olive oil sample. Both the blank solution and 5.0 g of the olive oil sample were spiked with varying volumes of a 5.0 μg ml^{-1} Se (IV) standard solution. The blank solution was directly analyzed, while the oil mixture was homogenized under magnetic stirring over night (Dugo et al., 2003b; La Pera et al., 2002a, 2002b). Then the extraction procedure described earlier was executed. The obtained results demonstrated that selenium quantification remained unaffected by the clean-up steps of the extraction procedure and by the use of the electrolyte solution for the analysis; recoveries of 95.5% and 97.7% were obtained, respectively, from the oil sample and the blank (Table 2).

2.4.6. GFAAS confirmation analysis

The results obtained with the proposed method, were compared with those obtained via graphite furnace atomic absorption spectroscopy (GFAAS).The olive oil samples were analyzed in triplicate by Zeeman graphite furnace atomic absorption spectroscopy for Se determination. The analysis was carried out by adding, for each 20.0 μl injection, 5.0 μl of Pd $(NO_3)_2$ solution (Pd concentration 100 μg ml^{-1}), as matrix modifier. The working wavelength was 196.0 nm. The results of the two methods agreed within the 97.4–104% (Table 3).

3. Results and discussions

The proposed method enabled the determination of trace selenium level in olive oil. The method does not require a pre-concentration step and the use of high sample preparation temperature, which are the main causes of selenium losses (Dugo et al., 2003b). The presence of selenium was determined by CSP in fifty

Table 2
Recovery test from the blank ($CaCl_2$ 2 M/ HCl 4 M solution) and from the olive oil[a]

	Se (μg kg^{-1})	Added (μg kg^{-1})	Theoric (μg kg^{-1})	Found[a] (μg kg^{-1})	Recovery (%)
Blank	<0.6	2.5	2.5	2.4	96.0
	<0.6	10.0	10.0	9.8	98.8
	<0.6	50	50	49.5	99.0
Mean ± SD					97.7 ± 1.5
Oil	<0.6	5.0	5.0	4.8 ± 0.1	95.2
	<0.6	10.0	10.0	9.5 ± 0.3	96.7
	<0.6	50.0	50.0	47.1 ± 1.5	94.3
Mean ± SD					95.5 ± 1.2

[a] Each value regarding to the found concentration of Se, is the mean of three determinations.

Table 3
Selenium mean concentration and standard deviation ($n = 4$) determined by CSP and ZGFAAS in virgin olive oil

	GFAAS ($\mu g\,kg^{-1}$)	CSP ($\mu g\,kg^{-1}$)	Reliability (%)
A	49.5 ± 3.5	48.2 ± 1.6	97.4
B	22.2 ± 7.3	22.9 ± 0.8	103.1
C	9.9 ± 1.0	10.3 ± 0.3	104.0

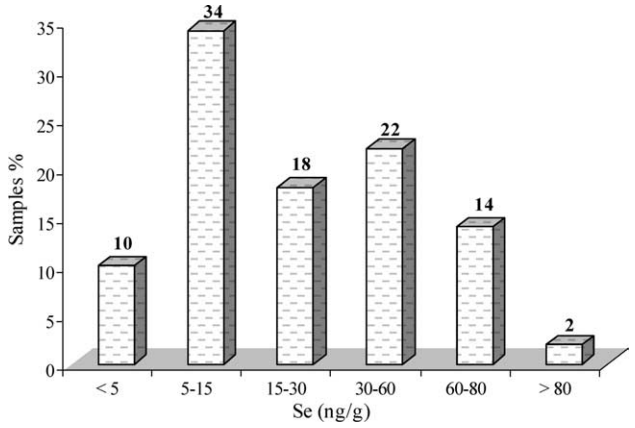

Fig. 3. Groups of selenium concentrations of Sicilian virgin olive oils.

samples of Sicilian olive oil from six different olive growing zone. The sample were grouped according to their zone of origin. For each olive-growing zone, oils samples from one or two olive varieties were studied; five oil samples from each variety were analyzed. The studied Sicilian olive oils contain very low amount of selenium: the 34% of the studied samples had a selenium concentration of 5–15 $\mu g\,kg^{-1}$, only the 2% presented selenium levels higher than 80.0 $\mu g\,kg^{-1}$ (Fig. 3). The selenium mean content was 29.0 $\mu g\,kg^{-1}$ ($n = 50$), the maximum value (122.9 ± 3.1 $\mu g\,kg^{-1}$) was found in a sample from Santagatese cultivar – Valdemone zone, and the minimum (2.4 ± 0.1 $\mu g\,kg^{-1}$) in a sample from Biancolilla–Sciacca variety, Val di Mazara zone. The obtained results (Table 4) provide evidences that olive oil samples from the same cultivar had a similar content of selenium, except oils from Santagatese variety, whereas some differences among the studied cultivars were observed. Particularly olive oil samples from Biancolilla, Moresca and Nocellara Etnea varieties showed the lowest mean selenium amounts (<10.0 $\mu g\,kg^{-1}$) and oils from Nocellara del Belice and Tonda Iblea the highest (>60.0 $\mu g\,kg^{-1}$). Moreover different mean selenium levels were found between the cultivars coming from the six olive-growing zones (Fig. 4), confirming the influence of geographic factors on selenium presence in food. Particularly oils from Val di Mazara and Monte Etna zones presented an average content of selenium lower than 10.0 $\mu g\,kg^{-1}$; samples from Monti Iblei, Valdemone and Valli Trapanesi presented an

Table 4
Selenium content of Sicilian olive oils from different olive-growing zones

Zone	Valli Trapanesi		Valle del Belice	Val di Mazara		Monti Iblei		Valdemone		Monte Etna	
Cultivar	Cerasuola	Crastu	Nocellara del Belice	Biancolilla	Biancolilla	Tonda Iblea	Moresca	Santagatese	Minuta	Nocellara Etnea	
Location	Paceco	Collesano	Castelvetrano	Sciacca	Misilmeri	Ragusa	Leonforte	Mistretta	Castell'Umberto	Paternò	
Province	Trapani	Palermo	Trapani	Agrigento	Palermo	–	Enna	Messina	Messina	Catania	
1	30.5 ± 0.9	16.0 ± 0.4	59.9 ± 1.7	4.0 ± 0.1	9.5 ± 0.3	70.0 ± 1.9	10.0 ± 0.3	40.0 ± 1.1	14.7 ± 0.4	8.4 ± 0.2	
2	38.2 ± 1.0	18 ± 0.5	70.0 ± 2.0	3.8 ± 0.1	11.0 ± 0.4	60.0 ± 1.7	12.0 ± 0.3	44.7 ± 1.3	20.2 ± 0.6	10.7 ± 0.4	
3	22.5 ± 0.6	15.7 ± 0.5	53.0 ± 1.6	2.4 ± 0.1	5.3 ± 0.2	72.6 ± 2.3	12.7 ± 0.4	31.0 ± 1.0	20.3 ± 0.6	10.4 ± 0.4	
4	40.3 ± 1.0	22.4 ± 22.4	70.1 ± 2.1	3.0 ± 0.1	10.1 ± 0.3	67.2 ± 2.1	8.4 ± 0.2	122.9 ± 3.4	18.0 ± 0.5	10.0 ± 0.3	
5	49.1 ± 1.3	10.8 ± 0.2	60.5 ± 2.0	4.2 ± 0.2	12.9 ± 0.4	54.3 ± 1.5	7.6 ± 0.2	50.0 ± 1.5	16.5 ± 0.4	9.6 ± 0.2	
Mean	36.1 ± 10.0	16.6 ± 4.0	62.7 ± 7.0	3.5 ± 1.0	9.8 ± 3.0	64.8 ± 8.0	10.1 ± 2.0	57.7 ± 37.0	17.9 ± 2.0	9.8 ± 1.0	

Each value is the mean of three determinations.

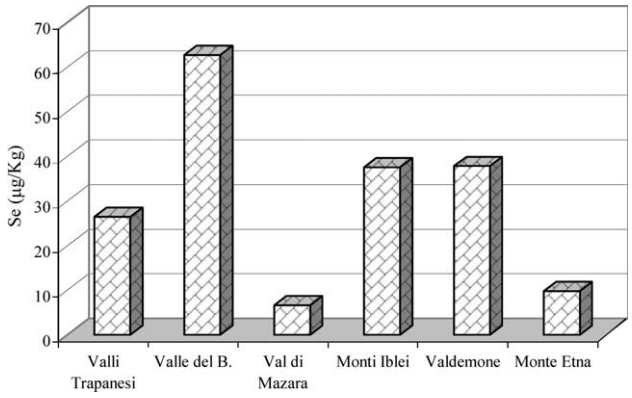

Fig. 4. Mean selenium concentrations found in six Sicilian olive-growing zones.

average concentration of selenium that spanned from 26.0 to 38.0 $\mu g\,kg^{-1}$, while oils from Valle del Belice zone showed the highest mean value ($\sim$63.0 $\mu g\,kg^{-1}$).

4. Conclusion

The overall differences observed among the cultivars and the olive-growing zones, confirm that both genetic factors (cultivar) and geographic factors (olive-growing zone and location of provenience), may influence the content of selenium in the studied Sicilian olive oils. Moreover the average selenium concentration found in this work is in the range of selenium levels found in Greek (2.0 ± 1.0 $\mu g\,kg^{-1}$) and French (220.0 ± 52.0 $\mu g\,kg^{-1}$) olive oils (Bratakos et al., 1987; Simonoff et al., 1988).

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