

Characterisation of authentic Italian extra-virgin olive oils by stable isotope ratios of C, O and H and mineral composition

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Abstract

The paper shows the isotopic ratios ($^{13}\text{C}/^{12}\text{C}$, D/H, $^{18}\text{O}/^{16}\text{O}$) in oil and extracted glycerol and the mineral composition of authentic PDO and PGI Italian extra-virgin olive oils, officially collected from 2000 to 2005 (N = 539) to establish a national databank. $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ increased from Trentino to Sicily, each year distinguishing Northern Italy from Sicily and Calabria. Significant differences were found among the years and in some cases also between PDOs from the same region. $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ in bulk oil were significantly correlated with those in glycerol. D/H, measured in 2005 for the first time in oil, showed promising geographical discrimination capability. The content of 26 elements - Li, Rb, Cs, La, Ce and Yb rarely reported in the literature - was measured in well settled 2005 oils after ultrasound acid extraction.

1. Introduction

Olive oil is one of the most important commodities produced in Italy, which is the second largest producer in the world (630,000 tons in 2006-2007) and the largest consumer (International Olive Oil Council, <http://www.internationaloliveoil.org>). European law (EEC Reg. No. 2568/91) provides producers with the opportunity of indicating the geographical origin of extra-virgin olive oil using the Protected Denomination of Origin (PDO) or the Protected Geographic Indication (PGI), but it does not indicate specific analytical methods to check the authenticity of these indications.

In the last few years attention has been focused on authentication of the geographical origin of olive oil using in particular the profiles of volatile compounds (Vichi, Pizzale, Conte, Buxaderas & Lopez-Tamames, 2007), sterols (Alves, Cunha, Amaral, Pereira & Oliveira, 2005) or free fatty acids (D'Imperio, Dugo, Alfa, Mannina & Segre, 2007), Nuclear Magnetic Resonance fingerprinting (Rezzi, Axelson, Heberger, Reniero, Mariani & Guillou, 2005) and also stable isotope ratios and mineral content. Moreover, because of its financial importance and role in the Mediterranean diet, olive oil has been investigated for other purposes, such as the identification of defects (e.g. rancid taste, presence of vegetable water or muddy sediment), pollutants (e.g. pesticides or metals) or fraud (e.g. mixing with hazelnut oil) using various analytical approaches, e.g. several chromatographic techniques (Aparicio & Aparicio-Ruiz, 2000), headspace Gas Chromatography -mass spectrometry (Lopez-Feria, Cardenas, Garcia-Mesa, Fernandez-Hernandez & Valcarcel, 2007), Fourier transform infrared spectroscopy (Tay, Sing, Krishnan & Gore, 2002), X-ray scattering (Bortoleto, Pataca & Bueno, 2005), potentiometric stripping analysis (Lo Coco, Ceccon, Ciralo & Novelli, 2003) or inductively coupled plasma optical emission spectrometry (ICP-OES) (De Souza, Mathias, Da Silveira & Aucelio, 2005).

With regard to stable isotope ratio analysis, the $^{13}\text{C}/^{12}\text{C}$ measured using Elemental Analyser – Isotopic Ratio Mass Spectrometry (EA-IRMS) or Gas Chromatography/Combustion/Isotopic Ratio Mass Spectrometry (GC/C/IRMS) in bulk olive oil or in some sub-components (individual fatty acids or aliphatic alcohol and sterols) has been shown to be useful for detecting the

1 adulteration of olive oil with cheaper pomace olive oil or with other vegetable oils (Angerosa,
2 Camera, Cumitini, Gleixner & Reniero, 1997; Spangenberg, Macko & Hunziker, 1998). Moreover
3 $^{13}\text{C}/^{12}\text{C}$, especially in combination with the $^{18}\text{O}/^{16}\text{O}$ of bulk oil, proved to be a good tool for
4 characterising geographical origin. Royer and co-workers (Royer, Gerard, Naulet, Lees & Martin,
5 1999) studied the $^{13}\text{C}/^{12}\text{C}$ of the palmitic, oleic and linoleic fatty acids of olive oils, observing
6 differences between French and Italian olive oils as compared to Greek ones and achieved regional
7 classification of the Greek olive oils. Some authors (Bréas, Guillou, Reniero, Sada & Angerosa,
8 1998; Angerosa, Bréas, Contento, Guillou, Reniero & Sada, 1999) found that both the $^{13}\text{C}/^{12}\text{C}$ and
9 $^{18}\text{O}/^{16}\text{O}$ of olive oils from Italy, Greece, Spain, Tunisia, Morocco and Turkey change according to
10 the latitude, suggesting as co-factors of variability the distance from the sea and environmental
11 conditions during the growing of plants (water stress, atmospheric moisture and temperature).
12 Finally, Aramendia et al. (2007) observed that the $^{18}\text{O}/^{16}\text{O}$ values of bulk olive oils were influenced
13 by the variety of the olives and by their geographical origin, but not by the altitude, ripening degree
14 and harvesting date of olives. To our knowledge, no papers are available in the literature regarding
15 the isotopic ratio of deuterium/hydrogen (D/H) in olive oil.

16 With regard to the mineral content of olive oil, interest initially focused on the presence of
17 toxic lead or cadmium elements and of copper and nickel as catalysts for oxidative reactions
18 affecting the flavour and stability of oils (de Souza et al., 2005). In the last few years, studies have
19 also been carried out to verify whether the mineral profile could be a useful marker of geographical
20 origin, cultivar, harvesting period and adulteration with cheaper vegetable oils (Dugo, La Pera,
21 Giuffrida, Salvo & Lo Turco, 2004; Benincasa, Lewis, Perri, Sindona & Tagarelli, 2007; Cindric,
22 Zeiner & Steffan, 2007). The high organic load and viscosity and very small mineral content of
23 olive oils were a handicap to the development of a simple and effective preparation method and
24 subsequent analysis. Several approaches were used, such as emulsion in water with the aid of some
25 surfactant or solvent (Anthemidis, Arvanitidis & Stratis, 2005; de Souza et al., 2005; Jimenez,
26 Velarte & Castillo, 2003; Castillo, Jimenez & Ebdon, 1999), liquid-liquid extraction (Dugo et al.,
27 2004), wet ashing (Lo Coco et al., 2003) and total microwave digestion (Zeiner, Steffan & Cindric,
28 2005, Benincasa et al., 2007; Cindric et al., 2007). Of the analytical techniques, the following were
29 more frequently applied: electrothermal and graphite furnace atomic absorption spectrophotometry
30 (Dugo et al., 2004; Cindric et al., 2007), derivative potentiometric stripping (Dugo et al., 2004; Lo
31 Coco et al., 2003) and, increasingly in the last few years, inductively coupled plasma–optical
32 emission spectrometry (Anthemidis et al., 2005; Zeiner et al., 2005; Cindric et al., 2007) and –mass
33 spectrometry (ICP-MS) (Castillo et al., 1999; Jimenez et al., 2003; Benincasa et al., 2007).

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35 The present work focuses on the $^{13}\text{C}/^{12}\text{C}$ in bulk oil and extracted glycerol and $^{18}\text{O}/^{16}\text{O}$ in
36 glycerol of 539 authentic PDO and PGI extra-virgin olive oils produced from 2000 to 2005
37 throughout Italy, sampled by the Ministry of Agricultural, Food and Forestry Policy and analysed to
38 establish an yearly databank of isotopic reference values. This was done to evaluate the geographic
39 authenticity of commercial samples, as has been done since 1987 for wine (EEC Reg. No.
40 2676/2000). Moreover, the mineral composition, the $^{18}\text{O}/^{16}\text{O}$ and, for the first time, the D/H in bulk
41 oil of around one hundred 2005 extra-virgin olive oils are shown and discussed for their variability.

42 43 **2. Materials and methods**

44 45 *2.1. Sampling*

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47 Authentic and well settled extra-virgin olive oils (N = 539) were officially collected by the Ministry
48 of Agricultural, Food and Forestry Policy from 2000 to 2005 in the production regions of the only
49 one PGI and the 34 out of 37 PDOs recognised at the present in Italy, according to the EC Reg. No.
50 510/2006. Traditionally, each PDO defines multi-varietal oils (*e.g.* PDO Chianti can include up to
51 76 varieties). The sampling tried to cover all the harvest time, the variability of the multi-varietal

1 blends and the production area. $^{13}\text{C}/^{12}\text{C}$ in bulk olive oil and the extracted glycerol, as well as
2 $^{18}\text{O}/^{16}\text{O}$ in the glycerol, were measured in 2000 (N = 82), 2001 (102), 2002 (66), 2003 (95), 2004
3 (58) and 2005 (136) samples. In 2005, measurement of the $^{18}\text{O}/^{16}\text{O}$ and D/H of bulk olive oil also
4 took place. Finally, the mineral content of a selection of 99 samples of 2005 was measured.

5 6 2.2. Chemicals

7 *Isotopes*

8 All the solutions were prepared with Milli-Q water (18 M Ω cm resistivity; Millipore, Bedford, MA).
9 Sodium hydroxide 2N (RP grade; Carlo Erba Reagents, Milan, Italy), hydrochloric acid at 37%
10 (RP; Carlo Erba Reagents), diethyl ether (Normapur; VWR International, Leuven, Belgium),
11 ethanol at 96% (Sigma Aldrich GmbH, Steinheim, Germany), tin and silver capsules (Säntis
12 analytical AG, Teufen, Switzerland), P₂O₅ at 97% (Sigma Aldrich GmbH) and nitrogen gas at
13 99.999 % (Linde Gas, Milan, Italy) were used.

14 The isotopic values (expressed in $\delta\%$, as described below) were calculated against working in-
15 house standards (commercial olive oil and glycerol), calibrated against international reference
16 materials: fuel oil NBS-22 (IAEA-International Atomic Energy Agency, Vienna, Austria) and sugar
17 IAEA-CH-6 (IAEA) for $^{13}\text{C}/^{12}\text{C}$ measurement; IAEA-CH-6 (IAEA) for $^{18}\text{O}/^{16}\text{O}$ and NBS-22 for
18 D/H. Whereas in the past the data regarding $^{18}\text{O}/^{16}\text{O}$ in glycerol were usually calibrated against
19 glycerol used in the European project SMT4-CT98-2236 (Camin, Wietzerbin, Cortes, Haberhauer,
20 Lees & Versini, 2004), in this work they were calibrated against the IAEA-CH6 value ($\delta^{18}\text{O} =$
21 $+36.4\%$ vs V-SMOW) assigned since 2005 (Boschetti & Iacumin, 2005) and accepted in the
22 European TRACE project (proposal contract N^oFP6-2003-FOOD-2-A 006942).

23 The isotopic values of the aforementioned international reference materials and therefore also of the
24 samples were expressed in $\delta\%$, versus V-PDB (Vienna - Pee Dee Belemnite) for $\delta^{13}\text{C}$ and V-
25 SMOW (Vienna – Standard Mean Ocean Water) for $\delta^{18}\text{O}$ and δD , according to the following
26 formula: $[(\text{Rs}-\text{Rstd})/\text{Rstd}] \times 1000$, where Rs is the isotope ratio measured for the sample and Rstd is
27 the isotope ratio of the international standard.

28 29 *Elements*

30 Nitric acid at 69.5% (Superpure; Merck, Darmstadt, Germany), hydrochloric acid at 37% (ACS;
31 Riedel-deHaën, Seelze, Germany), ICP Multielement Standard Solution VI (Merck), Multielement
32 Calibration Standard 1 (Agilent Technologies, Santa Clara, CA, USA), and Cesium 1000ug/ml
33 (Ultra Scientific, Bologna, Italy) were used. Standard solutions were diluted and stabilized with the
34 addition of a 1% HNO₃ and 0.2% HCl solution. SRM 2387 ‘Peanut butter’ (National Institute of
35 Standard and Technologies, Gaithersburg, MD, USA) was used as standard reference material to
36 check the accuracy of the method. An on-line solution of Sc 3 mg/L, Rh 3 mg/L and Tb 3 mg/L was
37 used as the internal standard. All the glassware was rinsed with nitric acid (5% v/v) and twice with
38 milli-Q water before use.

39 40 2.3. Apparatus

41 *Isotopes*

42 The analysis was performed using an Isotopic Ratio Mass Spectrometer (IRMS) (Finnigan DELTA
43 XP, Thermo Scientific, Bremen, Germany) coupled with an Elemental Analyser (Flash EATM1112,
44 Thermo Scientific,) for $^{13}\text{C}/^{12}\text{C}$ measurement and with a Pyrolyser (FinniganTM TC/EA, High
45 Temperature Conversion Elemental Analyzer, Thermo Scientific,) for D/H and $^{18}\text{O}/^{16}\text{O}$
46 measurement. To separate the gases, the Elemental Analyser was supplied with a Porapack QS (3
47 m; 6 x 4mm, OD/ID) GC column and the Pyrolyser with a Molecular Sieve 5A (0.6 m) GC
48 column. The devices were equipped with an autosampler (Finnigan AS 200, Thermo Scientific) and
49 interfaced with the IRMS through a dilutor (Conflo III, Thermo Scientific) dosing the sample and
50 reference gases.

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The analysis was performed using an Agilent 7500ce ICP-MS (Agilent Technologies, Tokyo, Japan) equipped with an autosampler ASX-520 (Cetac Technologies Inc., Omaha, NE, USA). After extraction, the samples were introduced into a Scott spray chamber using a MicroMist nebulizer and then into a Fassel type torch. An Octopole Reaction System (ORS) using He and H₂ as collision and reaction gases respectively, was used to remove polyatomic interferences.

2.4. Sample preparation and analysis

Isotopes

Glycerol was obtained through hydrolysis of 20 ml of oil in NaOH, acidification of the solution, extraction of fatty acids and purification by under vacuum distillation, according to the method described for fat from cheese in Camin et al., 2004.

Aliquots of 0.3 mg of sample were weighed in tin capsules for determination of ¹³C/¹²C and silver capsules for quantification of ¹⁸O/¹⁶O and ²H/¹H.

For ¹³C/¹²C, the precision of measurement, expressed as standard deviation when measuring the same sample 10 times, was 0.1‰.

For ¹⁸O/¹⁶O and D/H analysis, the samples were stored in a desiccator above P₂O₅ for at least 24 hours, then weighed into silver capsules and put into the auto-sampler equipped with a suitable cover. During measurement, dryness was guaranteed by flushing nitrogen continuously over the samples. The pyrolyser temperature was 1450°C. The D/H and ¹⁸O/¹⁶O ratios of bulk olive oils were measured simultaneously in one run. The IRMS measured first D/H and then, following the magnet jump, ¹⁸O/¹⁶O, taking about 10 minutes for each sample. Before measuring D/H, the H3 factor, which allows correction of the contribution of [H3]⁺ to the m/z 3 signal (Sessions, Burgoyne & Hayes, 2001), was shown to be lower than 9. The precision of measurement, expressed as standard deviation when measuring the same sample 10 times, was 0.3‰ for ¹⁸O/¹⁶O and 2‰ for D/H.

Elements

15g of sample were weighed into a 50 ml conical vial of polypropylene (PP) and 15 mL of 1% HNO₃/0.2% HCl water solution was added. The mixture was thoroughly shaken for 30 s using a vortex mixer and immediately placed in an ultrasonic bath (170 W x 5 min) to extract the trace elements from the oil to the acid solution. The mixture was centrifuged (4000 rpm x 5 min) to separate the two phases. The upper oil phase was accurately removed by aspiration and the lower aqueous phase transferred into a clean PP vial and subjected to ICP-MS analysis of Li, B, Na, Mg, K, Ca, Mn, Co, Cu, Ga, Se, Rb, Sr, Mo, Cd, Cs, Ba, La, Ce, Nd, Sm, Eu, Yb, Tl, Pb, and U. Isotopes and ORS gases are shown in Table 1. Extraction and analysis was carried out in duplicate. The accuracy of the extraction method was evaluated in a natural oil sample spiked with a defined aliquot of the reference material (0.6 g of ‘semi-solid’ peanut butter mixed into 15 g of oil until thoroughly combined). The oil and fortified mixture were both extracted and analysed ten times. Recoveries were calculated on the difference of the mean content of the spiked and the un-spiked samples. The detection limit (DL) of each element was calculated as 3 times the standard deviation of the signal of the blank sample, extracted and analysed ten times, whereas the blank sample was prepared using Milli-Q water to substitute the oil sample in the extraction step. Precision (RSD%) was evaluated by preparing and analyzing an oil sample ten times. DL and RSD% are shown in Table 1.

2.5 Statistical analysis

The data were statistically evaluated according to the procedures of the software Statistica 7.1 (StatSoft Italia srl, Padua, Italy). Non parametric tests (Kruskall-Wallis and multiple bilateral

1 comparison) were applied because of the low and unequal numbers of samples per group and the
2 not always normal distribution (Soliani, 2003).

3 4 **3. Results and discussion**

5 **Stable isotope ratios**

6 In Table 2 the median, minimum and maximum values of bulk and glycerol $\delta^{13}\text{C}$ and of glycerol
7 $\delta^{18}\text{O}$ were summarised for 2000-2004 production and the different Italian regions listed according
8 to the latitude. $\delta^{18}\text{O}$ was measured in the glycerol instead of in the bulk oil because the former was
9 assumed to be more closely related to the isotopic characteristics of ground water (Schmidt, Werner
10 & Rossmann, 2001).

11 The $\delta^{13}\text{C}$ values measured in glycerol were always lower than in bulk olive oil, with a mean
12 difference \pm std. dev. of 1.87 ± 0.67 , confirming previous results (Zhang, Buddrus, Trierweiler &
13 Martin, 1998). Moreover, $\delta^{13}\text{C}_{\text{glycerol}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ of the 403 samples were significantly correlated
14 ($\delta^{13}\text{C}_{\text{glycerol}} = 1.1114 \times \delta^{13}\text{C}_{\text{bulk}} + 1.4057$; $p < 0.001$). If we consider 2000, 2001, 2002 and 2004
15 individually, the intercepts ranged from -0.3075 to +6.3585 and the slopes from 1.0435 to 1.2841.
16 The correlation for 2003, also significant, showed particular and extreme values, both as regards
17 intercept (-7.0134) and slope (0.8162).

18 Significant differences ($p < 0.001$) were found among the years of harvest (Table 2). In particular
19 2003, well-known as one of the hottest years in the last few decades in Italy, was consistently
20 different ($p < 0.001$) as compared to at least one isotopic parameter from all the other years, showing
21 enrichment in the heavier isotopomers.

22 All the isotopic parameters showed a trend for the values to increase from Trentino to Sicily in
23 all years (Table 2), with this being less evident in 2003. This trend, already observed in wine
24 (Rossmann, Schmidt, Reniero, Versini, Moussa & Merle, 1996; Rossmann, Reniero, Moussa,
25 Schmidt, Versini & Merle, 1999), is probably positively related to vicinity to the sea and dryness of
26 the climate and negatively to latitude, as suggested by some authors (Bréas, Guillou, Reniero, Sada
27 & Angerosa, 1998; Angerosa, Bréas, Contento, Guillou, Reniero & Sada, 1999). Indeed, the $\delta^{13}\text{C}$
28 values of plant compounds are influenced by the availability of water, relative humidity and
29 temperature, which control stomatal aperture and the internal CO_2 concentration in the leaf
30 (O'Leary, 1995). The $\delta^{18}\text{O}$ of carbohydrates and their immediate descendants, such as glycerol, is
31 correlated to the $\delta^{18}\text{O}$ of leaf water (Schmidt et al., 2001), which reflects the isotopic composition
32 of groundwater and average precipitation in the region - mainly related to latitude, distance from the
33 sea and altitude (Clark & Fritz, 1997) - and the extent of evapotranspiration, mainly influenced by
34 humidity and temperature (Rossmann et al., 1999).

35 For statistical evaluation, we grouped the regions into four clusters on the basis of their latitude
36 and the similarity of their isotopic ratios: North (Trentino, Veneto, Lombardia, Emilia Romagna),
37 Centre (Liguria, Tuscany, Umbria, Abruzzo, Lazio), South-1 (Campania, Apulia) and South-2
38 (Calabria, Sicily). Applying the non parametric test of Kruskal-Wallis (Figure 1), olive oil from
39 Northern Italy showed isotopic values for at least one parameter significantly lower ($p < 0.01$) than
40 for the South-2, Centre and South-1 in five, three and two years respectively, out of the five years.
41 Central Italy was never separated from South-1, whereas it was different from South-2 in two out of
42 five years. The two southern macro areas could be distinguished from one another in three out of
43 five years.

44 Comparing the few PDOs with at least 5 samples within each region and each year, we observed
45 some significant differences ($p < 0.01$). In Lazio, the two 'Canino' and 'Sabina' PDOs were
46 significantly different in 2000, 2002 and 2003 for $\delta^{13}\text{C}$ and in 2001 for $\delta^{18}\text{O}$. This could be ascribed
47 to the inland location of the production area of 'Sabina', nearer Rieti than the 'Canino' area, closer
48 to the sea near Tuscany, as well as to the possible effect of the prevailing olive cultivar (Aramendia
49 et al., 2007). In Abruzzo and Apulia the comparison was only possible for one year (2000 and 2001,
50 respectively). Significant differences in the $\delta^{13}\text{C}$ values were observed between 'Aprutino
51

1 Pescaraese' and 'Colline Teatine' in Abruzzo and between 'Dauno' and 'Collina di Brindisi' in
2 Apulia.

3 For the 2005 samples, along with the aforementioned parameters, the $\delta^{18}\text{O}$ and δD in bulk oil were
4 also measured (Table 3). δD was investigated as a possible additional parameter for the
5 characterisation of geographical origin, being influenced in plant products by the isotopic
6 composition of the primary hydrogen source (source water through the leaf water) and by the
7 geographical and climatic factors mentioned above for $\delta^{18}\text{O}$, together with their biosynthetic
8 pathways (Schmidt, Werner & Eisenreich, 2003).

9 As compared to previous years (Figure 1), the median values of the isotopic parameters were lower,
10 as a consequence of the rainy and cold climate characterising 2005.

11 The $\delta^{18}\text{O}$ values measured in bulk were significantly correlated with those in glycerol
12 ($p < 0.001$): $\delta^{18}\text{O}_{\text{bulk}} = 0.837 * \delta^{18}\text{O}_{\text{glycerol}} - 3.2213$ ($R^2 = 0.8772$), the first always being lower, with a
13 mean difference of \pm std. dev. of $8.15\text{‰} \pm 0.66$.

14 The $\delta^{18}\text{O}$ and δD values in bulk olive oil were also correlated ($p < 0,001$), as happens in water (Clark
15 et al., 1997); the correlation equation is $\delta^2\text{H}_{\text{bulk}} = -208,1 + 2,5091 * \delta^{18}\text{O}_{\text{bulk}}$, but the R^2 value
16 (0.4582) is low.

17 The $\delta^{18}\text{O}$ values of bulk olive oil showed the same capability as the $\delta^{18}\text{O}$ of glycerol in terms of
18 distinguishing the four regional groups. The δD values showed similar capability to that of $\delta^{13}\text{C}$ and
19 $\delta^{18}\text{O}$ to differentiate the four groups,
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21 **Mineral composition**

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23 The recoveries of the extraction method evaluated with the NIST sample were generally satisfactory
24 for all the certified elements, being 82 % for Zn, 84% for Mn, 90% for Ca, 92% for Mg, 95% for K,
25 and 101 for Na. The precision of the analytical method ranged from 13 to 27% for the different
26 elements (Table 1). Such values can be deemed satisfactory, considering the very low content of
27 elements in olive oil.

28 Tables 1 and 4 show the concentration of the elements quantifiable over the DL in at least 10
29 samples. The content of Mo, Cd and Tl were below the respective DLs (0.18, 0.02 and 0.005 $\mu\text{g}/\text{kg}$
30 respectively) in all the samples. Ga was found in quantifiable amounts (DL = 0.004 $\mu\text{g}/\text{kg}$) only in
31 one Terra di Bari PDO oil (0.023 $\mu\text{g}/\text{kg}$). B was found in measurable amounts (DL = 1 $\mu\text{g}/\text{kg}$) only
32 in 5 samples, with a maximum of 12.2 $\mu\text{g}/\text{kg}$ for a Monte Etna PDO oil, in agreement with the
33 results shown by Eschnauer for wines from grapes grown in volcanic areas (Eschnauer, 1982). Se
34 was only detectable (DL= 0.014 $\mu\text{g}/\text{kg}$) in 7 samples, with a maximum of 0.021 $\mu\text{g}/\text{kg}$, far below
35 the content reported by Dugo et al., 2004, for 50 Sicilian oils analysed using cathodic stripping
36 potentiometry and by Benincasa et al., (2007) for 36 oils from 4 Central-Southern Italian regions
37 digested by microwave and analysed using ICP-MS. Nd was only quantifiable (DL= 0.023 $\mu\text{g}/\text{kg}$)
38 in 9 samples, with a maximum value of 0.932 $\mu\text{g}/\text{kg}$ in a Terra di Bari PDO oil.

39 Tables 1 and 4 show the distribution of the mineral element content in the total sampling and for
40 each PDO. As regards the content of Li, Rb, Cs, La, Ce and Yb shown in Table 1, we could not find
41 other data for extra-virgin olive oils in the literature, while Mg, Ca, Mn, Sr, Sm, Eu and U were
42 found in the concentration ranges reported in the literature. Na and K were measured in notably
43 lower and higher amounts respectively, as compared to olive oils from Croatia analysed by Zeiner
44 et al., 2005, and Cindric et al., 2007. The Co and Cu content were also in the ranges reported in
45 literature, with the exclusion of the maximum values found for some of the aforementioned
46 Croatian oils. The Ba content agreed with that found by Jimenez et al., 2003, and Anthemidis et al.,
47 2005, but was far below the maximum content (700 $\mu\text{g}/\text{kg}$) measured by Castillo et al., 1999, using
48 a semi-quantitative ICP-MS approach with direct emulsion nebulisation of the oil sample. The Pb
49 content was low and always below the legal limits (0.1 mg/kg, EEC Reg. No. 466/2001), probably
50 as a consequence of the increasingly widespread use of adequate equipment throughout the olive oil
51 processing chain.

1 Comparison between the mineral content of the individual PDOs was not an aim of this first work
2 carried out on Italian extra-virgin olive oils in agreement with the Italian Ministry of Agricultural,
3 Food and Forestry Policy. At all events - taking into account only the 3 PDOs with at least 10
4 samples each, namely Garda, Umbria and Terra di Bari - a trend toward higher Mg, Ca, Mn and Sr
5 content would seem to characterise the Garda oils, especially if compared to the Umbrian oils,
6 possibly related to the soil of the region, mainly originating from dolomitic limestone rock.

8 **Conclusions**

9 This paper shows the results of the largest investigation ever carried out on multi-element stable
10 isotope ratio and mineral composition using IRMS and ICP-MS in authentic PDO and PGI Italian
11 extra-virgin olive oils. The study was done in collaboration with the Ministry of Agricultural, Food
12 and Forestry Policy to establish a national databank for olive oils.

13 The stable isotope ratios of carbon, oxygen and hydrogen in olive oil were shown to increase from
14 Trentino to Sicily, making it possible to distinguish Northern Italy from Sicily and Calabria each
15 year and confirming the trend observed for other commodities such as wine. Significant differences
16 were found among the years of harvest and in some cases also between PDOs from the same region.
17 The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in bulk oil were significantly correlated with those in glycerol. The $\delta^{18}\text{O}$
18 in glycerol showed the same capability to differentiate the geographic origin as $\delta^{18}\text{O}$ in bulk,
19 whereas in some years the $\delta^{13}\text{C}$ of glycerol showed a better capability to discriminate as compared
20 to bulk. Because the discriminating capability achievable using $\delta^{13}\text{C}$ in glycerol is the same as that
21 of $\delta^{18}\text{O}$ in bulk, it would seem preferable to directly measure the isotopic ratios in bulk, making
22 savings in terms of time and costs. The δD values, measured in 2005 for the first time in oil, showed
23 promising geographical discrimination capability.

24 The content of each mineral element in well settled olive oil, measured after ultrasound acid
25 extraction, was basically low and similar to that seen in the literature. The capability to discriminate
26 on the basis of minerals in PDOs could only be checked for few oils produced in specific areas with
27 different geology and requires further research in order to be confirmed.

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Table 1: Instrumental conditions and mineral content distribution of well settled extra-virgin Italian olive oils (ORS: collision cell Octopole Reaction System)

Element	Iso- tope	ORS mode	unit	DL	N. of samples > DL	RSD %	25° perc.	Median	75° perc.	90° perc.	Max	range (min-max)	Literature
Li	7	--	µg/kg	0.005	56	18		0.007	0.013	0.023	0.208	-	-
Na	23	He	mg/kg	0.04	42	27			0.100	0.211	1.105	28.8-38.0	d,n
Mg	26	He	mg/kg	0.014	62	20		0.019	0.055	0.109	0.495	0.056-3.8	d, f, g
K	39	He	mg/kg	0.06	68	20		0.163	0.645	1.702	9.94	<0.001- 0.19	d, n
Ca	40	H ₂	mg/kg	0.03	12	16				0.380	0.950	<0.05-26.9	a, b, d, n
Mn	55	--	µg/kg	0.01	60	21		0.211	0.630	1.43	10.0	<1-200	a, b, c, d, f, m, n
Co	59	--	µg/kg	0.004	20	13				0.012	0.033	0.023-5450	a, b, c, d, f, m, n
Cu	63	He	µg/kg	0.13	88	21	0,237	0.360	0.689	1.45	26.3	<1-4510	a, c, d, f, g, h, l, m, n
Rb	85	--	µg/kg	0.03	83	21	0,041	0.110	0.375	1.03	13.4	-	-
Sr	88	--	µg/kg	0.04	15	17				0.483	3.85	1.52-48.9	b
Cs	133	--	µg/kg	0.003	67	20		0.004	0.005	0.012	0.819	-	-
Ba	137	--	µg/kg	0.29	22	21				0.543	2.49	<0.15-700	a, c, g
La	139	--	µg/kg	0.0017	46	24			0.006	0.040	2.94	-	-
Ce	140	--	µg/kg	0.0027	50	24		0.003	0.008	0.046	4.72	-	-
Sm	147	--	µg/kg	0.0009	27	24			0.001	0.004	0.111	0.004-0.226	i
Eu	151	He	µg/kg	0.0002	39	20			0.001	0.002	0.023	<0.009- 0.021	b
Yb	171	--	µg/kg	0.0004	61	20		0.001	0.001	0.002	0.041	-	-
Pb	208	--	µg/kg	0.02	80	21	0,195	0.372	0.725	1.50	8.46	<0.42-79.9	a, c, d, f, g, h, l, m, n
U	238	--	µg/kg	0.001	67	18		0.001	0.007	0.015	0.119	<0.25	c

a: Anthemidis et al., 2005; b: Benincasa et al., 2007; c: Castillo et al., 1999; d: Cindric et al., 2007; e: Dugo et al., 2004; f: Jimenez et al., 2003; g: La Pera et al., 2002; h: Lo Coco et al., 2000; i: Lo Coco et al., 2003; l: Martín-Polvillo et al., 1994; m: Solinas et al., 1987; n: Zeiner et al., 2005.

Table 2: Median, minimum and maximum values of $\delta^{13}\text{C}$ (bulk olive oil and glycerol) and of $\delta^{18}\text{O}$ values of glycerol (‰, vs V-PDB and V-SMOW, respectively) in the Italian olive oil databank (2000-2004) and results of the non-parametric statistical test (Kruskall-Wallis' test) among crops; different letters correspond to significantly different median values ($p<0.001$). N = number of samples

Region	N tot	Parameter	2000			2001			2002			2003			2004							
			N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max				
Trentino	37	$\delta^{13}\text{C}$ bulk	5	-30.7	-31.8	-29.7	23	-30.7	-32.4	-30.1	3	-29.7	-30.4	-29.7	3	-29.5	-29.5	-29.4	3	-30.7	-30.8	-30.4
		$\delta^{13}\text{C}$ glycerol		-33.6	-34.7	-32.7		-33.5	-34.6	-32.7		-32.8	-33.3	-31.8		-32.2	-32.4	-31.9		-33.4	-33.5	-33.2
		$\delta^{18}\text{O}$ glycerol		29.2	27.8	29.2		29.1	28.0	30.5		29.3	28.5	30.2		30.9	30.8	32.4		29.3	29.2	29.3
Veneto	20	$\delta^{13}\text{C}$ bulk	3	-30.3	-30.9	-30.3	3	-30.3	-30.7	-30.3	3	-31.1	-31.2	-30.7	5	-28.8	-29.0	-27.5	6	-30.1	-31.4	-29.0
		$\delta^{13}\text{C}$ glycerol		-32.4	-33.9	-32.3		-32.5	-32.6	-32.1		-32.6	-33.3	-32.0		-31.5	-31.8	-29.2		-32.0	-33.5	-31.0
		$\delta^{18}\text{O}$ glycerol		31.3	29.1	31.7		29.8	29.5	30.8		28.9	28.0	30.3		32.7	30.2	33.1		30.6	29.7	33.1
Lombardia	16	$\delta^{13}\text{C}$ bulk	4	-30.3	-30.6	-29.9	3	-30.4	-30.6	-30.3	-	-	-	5	-29.1	-29.3	-28.6	4	-31.0	-31.4	-29.4	
		$\delta^{13}\text{C}$ glycerol		-32.5	-33.6	-32.1		-32.2	-32.3	-31.9		-	-		-30.7	-31.3	-29.8		-32.8	-33.1	-31.7	
		$\delta^{18}\text{O}$ glycerol		30.2	28.1	31.3		29.8	29.7	30.1		-	-		31.5	31.3	32.1		30.3	29.6	33.2	
Emilia-Romagna	13	$\delta^{13}\text{C}$ bulk	2	-29.2	-	-	3	-29.6	-29.8	-29.4	2	-30.0	-	-	6	-29.1	-29.3	-28.7	-	-	-	
		$\delta^{13}\text{C}$ glycerol		-31.6	-	-		-31.6	-31.6	-31.3		-32.3	-	-		-31.2	-31.7	-30.7		-	-	
		$\delta^{18}\text{O}$ glycerol		29.7	-	-		29.8	29.1	30.1		29.7	-	-		31.1	30.9	32.4		-	-	
Liguria	18	$\delta^{13}\text{C}$ bulk	3	-30.3	-30.6	-30.1	3	-29.7	-30.0	-29.1	3	-30.3	-30.5	-29.6	6	-28.8	-29.1	-28.2	3	-30.0	-30.2	-29.6
		$\delta^{13}\text{C}$ glycerol		-33.3	-33.4	-33.1		-32.2	-32.7	-31.7		-32.7	-33.3	-32.6		-31.1	-31.7	-30.9		-33.1	-33.1	-32.6
		$\delta^{18}\text{O}$ glycerol		31.3	31.1	31.3		31.8	31.6	33.2		29.8	29.5	29.9		32.3	31.5	32.6		31.4	30.4	32.0
Tuscany	23	$\delta^{13}\text{C}$ bulk	5	-29.3	-30.0	-28.3	6	-29.3	-30.3	-29.0	9	-30.4	-30.8	-29.9	3	-28.7	-29.5	-27.9	-	-	-	
		$\delta^{13}\text{C}$ glycerol		-31.0	-32.3	-30.2		-31.3	-32.1	-30.9		-32.5	-33.3	-31.4		-31.4	-31.7	-30.5		-	-	
		$\delta^{18}\text{O}$ glycerol		32.9	30.9	34.2		31.0	30.2	31.8		31.2	28.4	33.1		31.9	31.6	32.8		-	-	
Umbria	30	$\delta^{13}\text{C}$ bulk	4	-29.3	-29.5	-29.2	15	-29.4	-30.0	-28.1	-	-	-	11	-28.9	-29.5	-28.3	-	-	-		
		$\delta^{13}\text{C}$ glycerol		-30.1	-31.4	-29.7		-30.9	-32.0	-29.6		-	-		-30.6	-31.7	-29.8		-	-		
		$\delta^{18}\text{O}$ glycerol		31.8	30.5	33.7		32.1	30.0	33.4		-	-		32.6	31.4	33.4		-	-		
Abruzzo	30	$\delta^{13}\text{C}$ bulk	10	-28.7	-29.7	-28.1	-	-	-	-	6	-29.4	-30.1	-29.0	8	-28.3	-28.5	-28.1	6	-29.1	-30.0	-28.4
		$\delta^{13}\text{C}$ glycerol		-30.4	-31.3	-28.8		-	-	-		-30.8	-31.5	-29.9		-29.8	-30.1	-29.7		-31.0	-31.8	-30.3
		$\delta^{18}\text{O}$ glycerol		32.9	31.1	34.1		-	-	-		31.6	30.6	32.2		33.4	33.2	33.5		32.6	31.2	33.3
Lazio	50	$\delta^{13}\text{C}$ bulk	10	-29.2	-30.3	-28.1	12	-28.8	-29.5	-28.0	12	-30.1	-31.1	-29.6	12	-28.6	-29.3	-27.8	4	-29.3	-29.9	-28.9
		$\delta^{13}\text{C}$ glycerol		-30.7	-32.1	-28.5		-30.3	-31.2	-28.9		-31.1	-32.8	-29.6		-29.8	-31.3	-28.4		-31.0	-31.7	-30.3
		$\delta^{18}\text{O}$ glycerol		33.3	31.8	33.5		32.8	30.6	33.5		31.6	29.9	33.3		33.0	32.4	33.4		32.5	32.2	32.9
Campania	35	$\delta^{13}\text{C}$ bulk	9	-29.4	-29.7	-27.5	9	-28.2	-29.3	-27.4	9	-30.0	-30.8	-28.9	6	-28.5	-29.5	-27.9	2	-28.8	-29.0	-28.6
		$\delta^{13}\text{C}$ glycerol		-31.5	-32.5	-30.0		-30.4	-31.1	-29.8		-31.7	-33.5	-29.7		-30.5	-31.5	-29.7		-30.8	-31.1	-30.5
		$\delta^{18}\text{O}$ glycerol		33.3	31.9	34.4		33.0	31.2	34.3		31.2	27.6	31.9		33.1	31.2	33.9		32.0	31.7	32.3
Apulia	55	$\delta^{13}\text{C}$ bulk	18	-29.2	-30.4	-28.2	22	-28.9	-30.0	-26.6	5	-30.6	-30.8	-30.0	-	-	-	-	10	-29.6	-30.6	-28.5
		$\delta^{13}\text{C}$ glycerol		-31.4	-32.3	-29.9		-30.8	-31.8	-29.3		-32.1	-34.4	-32.0		-	-	-		-31.8	-33.0	-30.7
		$\delta^{18}\text{O}$ glycerol		31.7	30.3	35.7		31.9	31.1	34.2		31.0	29.8	31.7		-	-	-		31.9	30.5	32.6
Calabria	24	$\delta^{13}\text{C}$ bulk	-	-	-	-	-	-	-	-	8	-29.8	-31.3	-28.3	9	-29.4	-30.1	-28.8	7	-29.2	-30.2	-29.0
		$\delta^{13}\text{C}$ glycerol		-	-	-		-	-	-		-31.0	-32.0	-29.9		-30.5	-31.1	-30.2		-31.0	-31.3	-30.4
		$\delta^{18}\text{O}$ glycerol		-	-	-		-	-	-		32.9	30.7	34.1		33.5	31.3	34.8		34.8	32.5	35.6
Sicily	52	$\delta^{13}\text{C}$ bulk	9	-28.4	-28.7	-28.0	3	-28.3	-28.5	-27.0	6	-28.5	-29.4	-27.7	21	-28.8	-30.0	-27.7	13	-29.1	-29.8	-28.0
		$\delta^{13}\text{C}$ glycerol		-30.4	-31.4	-29.3		-29.3	-29.4	-28.4		-29.5	-31.8	-28.8		-30.2	-31.5	-29.0		-30.6	-31.8	-29.1
		$\delta^{18}\text{O}$ glycerol		35.5	32.5	36.2		33.9	33.4	35.1		33.7	33.1	34.8		33.0	30.6	35.0		34.2	32.4	34.6
Total	403	$\delta^{13}\text{C}$ bulk	82	-29.3 ^{ab}	-31.8	-27.5	102	-29.2 ^b	-32.4	-26.6	66	-30.0 ^c	-31.3	-27.7	95	-28.9 ^a	-30.1	-27.5	58	-29.4 ^c	-31.4	-28.0
		$\delta^{13}\text{C}$ glycerol		-31.2 ^b	-34.7	-28.5		-31.2 ^b	-34.6	-28.4		-31.7 ^b	-34.4	-28.8		-30.6 ^b	-32.4	-28.4		-31.3 ^b	-33.5	-29.1
		$\delta^{18}\text{O}$ glycerol		30.0 ^{ab}	25.2	33.6		29.0 ^b	25.4	32.5		28.7 ^b	25.0	32.2		30.1 ^a	27.6	32.4		29.7 ^{ab}	26.7	33.1

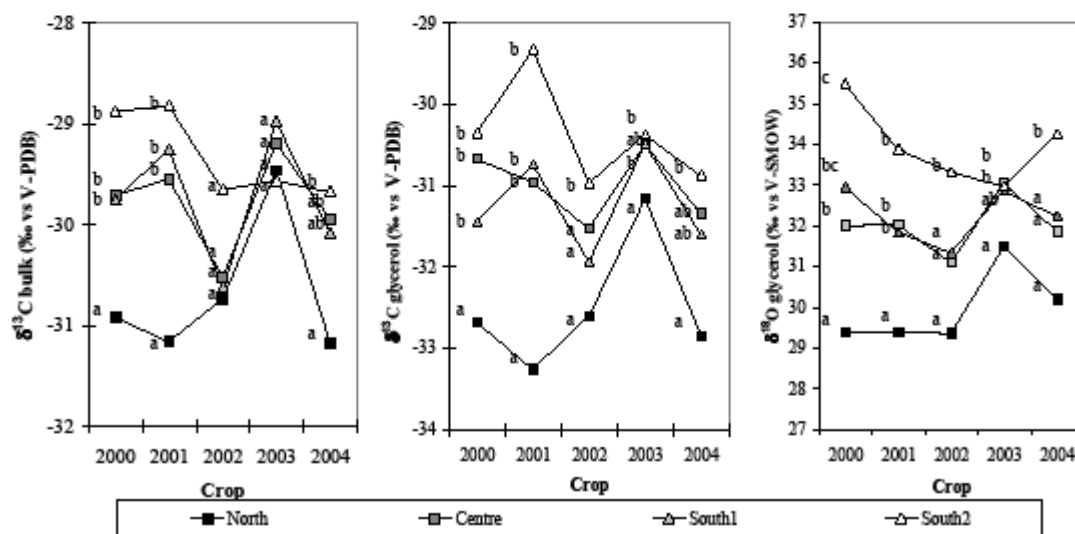
Table 3: Median values of $\delta^{13}\text{C}$ (bulk olive oil and glycerol), $\delta^{18}\text{O}$ (bulk and glycerol) and δD (bulk oil) for North, Centre, South-1 and South-2 Italian olive oils produced in 2005 and results of the non-parametric statistical test (Kruskall-Wallis' test); different letters correspond to significantly different median values ($p<0.01$).

N	$\delta^{13}\text{C}$ bulk (‰ vs V-PDB)			$\delta^{13}\text{C}$ glycerol (‰ vs V-PDB)			$\delta^{18}\text{O}$ glycerol (‰ vs V-SMOW)			$\delta^{18}\text{O}$ bulk (‰ vs V-SMOW)			$\delta^2\text{H}$ bulk (‰ vs V-SMOW)			
	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	
North	45	-30.9 ^a	-31.9	-29.8	-33.9 ^a	-35.0	-32.0	28.0 ^a	26.9	31.0	20.2 ^a	19.1	22.4	-159 ^a	-165	-145
Centre	45	-30.1 ^b	-30.9	-29.0	-32.3 ^b	-33.5	-30.6	30.5 ^b	28.9	32.3	22.3 ^b	20.3	23.7	-153 ^b	-162	-142
South1	19	-29.6 ^{bc}	-30.9	-29.2	-32.0 ^b	-33.3	-31.0	30.1 ^b	29.6	31.6	22.2 ^b	21.8	23.1	-149 ^{bc}	-156	-140
South2	27	-29.2 ^c	-30.9	-27.9	-31.0 ^c	-32.5	-28.9	32.8 ^c	30.6	35.7	24.5 ^c	22.8	26.8	-148 ^c	-155	-137

Table 4: Mineral content of well settled 2005 extra-virgin Italian olive oils displayed for Region and for PDO or PGI.

Element	Unit	Region	South Italy										Centre Italy				North Italy	
			Calabria			Apulia	Sicily					Lazio		Tuscany		Umbria	Veneto	
			Alto Crotonese	Bruzio	Lametia	Terra di Bari	Monte Etna	Monti Iblei	Val Dermone	Val di Mazara	Valli del Belice	Valli Trapanesi	Canino	Sabina	Lucca	Toscana PGI	Umbria	Veneto
N. Obs.	3	2	3	10	3	3	3	3	3	3	6	6	3	3	12	7	26	
Li	µg/kg	0.010	0.003 0.006	0.010 0.013	0.007 0.039	0.031 0.028	0.013 0.046	0.008 0.008	0.011 0.013	0.007 0.007	0.008 0.010	0.004 0.018	0.012	0.006 0.006	0.006 0.007	0.029	0.016 0.064	0.003 0.091
Na	mg/kg	0.124		0.049 0.100	0.170	0.280 1.11	0.189 0.492	0.065 0.161	0.129		0.052	0.069 0.326	0.124	0.060 0.133	0.340	0.154 0.609	0.312	
Mg	mg/kg	0.016		0.017	0.042 0.225	0.082 0.495	0.087 0.139	0.015 0.017	0.047 0.081	0.016 0.017	0.016	0.031 0.110	0.056	0.034 0.047	0.053	0.060 0.104	0.264	
K	mg/kg	0.32	0.038 0.08	0.180 0.22	0.415 3.79	0.726 9.94	1.171 1.70	0.116 0.17	0.418 0.90	0.079 0.10	0.12	0.033 0.56	1.13	0.12 0.43	0.292 0.64	0.424 2.06	0.516 3.13	
Ca	mg/kg				0.398	0.447	0.603										0.950	
Mn	µg/kg			0.408 0.566	0.598 10.0	0.491 3.42	0.351 1.21		0.134 0.522			0.312 3.61	0.058 0.655	0.173	0.921 1.17	0.623	0.392 1.49	0.507 2.87
Co	µg/kg				0.008 0.033	0.006 0.024	0.030		0.009			0.005	0.014		0.030	0.012	0.031	
Cu	µg/kg	0.160 0.237	0.334 0.363	0.343 0.952	0.404 2.37	2.107 15.8	0.446 1.47	0.360 0.089	0.434 0.472	0.311 0.885	0.171 0.430	0.251 0.663	0.127 0.824	0.446 0.778	0.324 0.355	0.258 0.810	0.583 1.72	0.493 26.3
Rb	µg/kg	0.263	0.040 0.049	0.108 0.175	0.394 4.19	0.199 13.4	0.628 1.59	0.040 0.081	0.178 0.802	0.060 0.066	0.036 0.074	0.237 2.98	0.173 2.69	0.055	0.214	0.584	1.013	1.110
Sr	µg/kg					1.23	3.85					1.12						1.40
Cs	µg/kg	0.003 0.005	0.008 0.012	0.004 0.005	0.004 0.012	0.004 0.038	0.012 0.014	0.004 0.004	0.004 0.013	0.005 0.005	0.004	0.006 0.819	0.004 0.011	0.008 0.012	0.003 0.004	0.004 0.012	0.004 0.007	0.012
Ba	µg/kg				2.05	2.49	0.550					1.13		0.309	0.435	1.34	0.695	
La	µg/kg			0.064 0.158	0.032 2.94	0.002 0.008	0.023		0.004			0.016 0.225	0.012	0.009 0.035	0.001 0.040	0.002 0.011	0.005	
Ce	µg/kg		0.002 0.004	0.043 0.111	0.058 4.72	0.018	0.045		0.008			0.020 0.161	0.024	0.013 0.056	0.056	0.004 0.013	0.003 0.009	
Sm	µg/kg		0.005 0.010	0.005 0.010	0.008 0.111	0.002	0.002					0.001 0.015	0.001	0.001 0.004	0.003	0.002	0.002	
Eu	µg/kg	0.0020	0.0010 0.0020	0.0017 0.0017	0.0021 0.0236	0.0004 0.0008	0.0004		0.0020 0.0020	0.0021	0.0021	0.0032 0.0021	0.0021	0.0004 0.0028	0.0025	0.0023	0.0006	
Yb	µg/kg	0.0012 0.0017	0.0007 0.0014	0.0019 0.0032	0.0022 0.0412	0.0007 0.0015	0.0009		0.0006 0.0017	0.0011 0.0017	0.0011 0.0015	0.0007 0.0032	0.0012 0.0022	0.0012 0.0033	0.0003 0.0030	0.0007 0.0020	0.0011	
Pb	µg/kg	0.180 1.47	0.262 0.299	0.595 1.50	0.380 2.69	2.11 3.86	0.439 0.972	0.815 1.24	0.292 0.733	0.300 0.409	0.468	0.329 1.84	0.234 0.464	0.790 2.60	0.345 0.573	0.516 2.76	0.691 0.904	0.260 8.46
U	µg/kg	0.0014		0.0021 0.0176	0.0021 0.0401	0.0104 0.0802	0.0008 0.0017	0.0009 0.0016		0.0030 0.0145	0.0067	0.0130 0.1190	0.0050 0.0939	0.0067 0.0211	0.0012 0.0044	0.0014 0.0131	0.023 0.0890	

Figure 1: Median values of the $\delta^{13}\text{C}$ (bulk olive oil and glycerol) and the $\delta^{18}\text{O}$ (glycerol) for North, Centre, South-1 and South-2 Italian olive oils (2000-2004) and results of the non-parametric statistical test (Kruskal-Wallis' test) among North, Centre, South-1 and South-2 Italian olive oils; different letters correspond to significantly different median values ($p < 0.01$).



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