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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 ($^{13}C/^{12}C$, D/H, $^{18}O/^{16}O$) 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. $^{13}C/^{12}C$ and $^{18}O/^{16}O$ 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. $^{13}C/^{12}C$ and $^{18}O/^{16}O$ 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 et al., 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, Singh, Krishnan, & Gore, 2002), X-ray scattering (Bortoleto, Pataca, & Bueno, 2005), potentiometric stripping analysis (Lo Coco, Ceccon, Circolo, & 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 $^{13}C/^{12}C$ 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 adulteration of olive oil with cheaper pomace olive oil or with other vegetable oils (Angerosa, Camera, Cumitini, Gleixner, & Reniero, 1997; Spangenberg, Macko, & Hunziker, 1998). Moreover $^{13}C/^{12}C$, especially in combination with the $^{18}O/^{16}O$ of bulk oil, proved to be a good tool for characterising geographical origin. Royer and co-workers (Royer, Gerard, Naulet, Lees, & Martin, 1999) studied the $^{13}C/^{12}C$ of the palmitic, oleic and linoleic fatty acids of olive oils, observing differences between French and Italian olive oils as compared to Greek ones and achieved regional classification of the Greek olive oils. Some authors (Bréas, Guillou, Reniero, Sada, & Angerosa, 1998;

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Angerosa et al., 1999) found that both the $^{13}C/^{12}C$ and $^{18}O/^{16}O$ of olive oils from Italy, Greece, Spain, Tunisia, Morocco and Turkey change according to the latitude, suggesting as co-factors of variability the distance from the sea and environmental conditions during the growing of plants (water stress, atmospheric moisture and temperature). Finally, Aramendia et al. (2007) observed that the $^{18}O/^{16}O$ values of bulk olive oils were influenced by the variety of the olives and by their geographical origin, but not by the altitude, ripening degree and harvesting date of olives. To our knowledge, no papers are available in the literature regarding the isotopic ratio of deuterium/hydrogen (D/H) in olive oil.

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

The present work focuses on the $^{13}C/^{12}C$ in bulk oil and extracted glycerol and $^{18}O/^{16}O$ in glycerol of 539 authentic PDO and PGI extra-virgin olive oils produced from 2000 to 2005 throughout Italy, sampled by the Ministry of Agricultural, Food and Forestry Policy and analysed to establish an yearly databank of isotopic reference values. This was done to evaluate the geographic authenticity of commercial samples, as has been done since 1987 for wine (EEC Reg. No. 2729/2000). Moreover, the mineral composition, the $^{18}O/^{16}O$ and, for the first time, the D/H in bulk oil of around one hundred 2005 extra-virgin olive oils are shown and discussed for their variability.

2. Materials and methods

2.1. Sampling

Authentic and well settled extra-virgin olive oils ($N = 539$) were officially collected by the Ministry of Agricultural, Food and Forestry Policy from 2000 to 2005 in the production regions of the only one PGI and the 34 out of 37 PDOs recognised at the present in Italy, according to the EC Reg. No. 510/2006. Traditionally, each PDO defines multi-varietal oils (e.g. PDO Chianti can include up to 76 varieties). The sampling tried to cover all the harvest time, the variability of the multi-varietal blends and the production area. $^{13}C/^{12}C$ in bulk olive oil and the extracted glycerol, as well as $^{18}O/^{16}O$ in the glycerol, were measured in 2000 ($N = 82$), 2001 (102), 2002 (66), 2003 (95), 2004 (58) and 2005 (136) samples. In 2005, measurement of the $^{18}O/^{16}O$ and D/H of bulk olive oil also took place. Finally, the mineral content of a selection of 99 samples of 2005 was measured.

2.2. Chemicals

2.2.1. Isotopes

All the solutions were prepared with Milli-Q water (18M Ωcm resistivity; Millipore, Bedford, MA). Sodium hydroxide 2N (RP grade; Carlo Erba Reagents, Milan, Italy), hydrochloric acid at 37% (RP; Carlo Erba Reagents), diethyl ether (Normapur; VWR International, Leuven, Belgium), ethanol at 96% (Sigma–Aldrich GmbH, Steinheim, Germany), tin and silver capsules (Säntis analytical AG, Teufen, Switzerland), P_2O_5 at 97% (Sigma–Aldrich GmbH) and nitrogen gas at 99.999% (Linde Gas, Milan, Italy) were used.

The isotopic values (expressed in $\delta‰$, as described below) were calculated against working in-house standards (commercial olive oil and glycerol), calibrated against international reference materials: fuel oil NBS-22 (IAEA-International Atomic Energy Agency, Vienna, Austria) and sugar IAEA-CH-6 (IAEA) for $^{13}C/^{12}C$ measurement; IAEA-CH-6 (IAEA) for $^{18}O/^{16}O$ and NBS-22 for D/H. Whereas in the past the data regarding $^{18}O/^{16}O$ in glycerol were usually calibrated against glycerol used in the European project SMT4-CT98-2236 (Camin et al., 2004), in this work they were calibrated against the IAEA-CH6 value ($\delta^{18}O = +36.4‰$ vs. V-SMOW) assigned since 2005 (Boschetti & Iacumin, 2005) and accepted in the European TRACE project (contract No. FP6-2003-FOOD-2-A 006942).

The isotopic values of the aforementioned international reference materials and therefore also of the samples were expressed in $\delta‰$ vs. V-PDB (Vienna - Pee Dee Belemnitella) for $\delta^{13}C$ and V-SMOW (Vienna – standard mean ocean water) for $\delta^{18}O$ and δD, according to the following formula: $[(Rs-Rstd)/Rstd] \times 1000$, where Rs is the isotope ratio measured for the sample and Rstd is the isotope ratio of the international standard.

2.2.2. Elements

Nitric acid at 69.5% (Superpure; Merck, Darmastadt, Germany), hydrochloric acid at 37% (ACS; Riedel-deHaën, Seelze, Germany), ICP Multielement Standard Solution VI (Merck), Multielement Calibration Standard 1 (Agilent Technologies, Santa Clara, CA, USA), and Cesium 1000 ug/ml (Ultra Scientific, Bologna, Italy) were used. Standard solutions were diluted and stabilized with the addition of a 1% HNO_3 and 0.2% HCl solution. SRM 2387 'Peanut butter' (National Institute of Standard and Technologies, Gaithersburg, MD, USA) was used as standard reference material to check the accuracy of the method. Sc 0.1 mg/L, Rh 0.1 mg/L and Tb 0.1 mg/L were used as internal standards. All the glassware was rinsed with nitric acid (5% v/v) and twice with milli-Q water before use.

2.3. Apparatus

2.3.1. Isotopes

The analysis was performed using an isotopic ratio mass spectrometer (IRMS) (Finnigan DELTA XP, Thermo Scientific, Bremen, Germany) coupled with an Elemental Analyser (Flash EA^{TM}1112, Thermo Scientific,) for $^{13}C/^{12}C$ measurement and with a Pyrolyser (Finnigan^{TM}TC/EA, high temperature conversion elemental analyzer, Thermo Scientific,) for D/H and $^{18}O/^{16}O$ measurement. To separate the gases, the Elemental Analyser was supplied with a Porapack QS (3 m; 6 × 4 mm, OD/ID) GC column and the Pyrolyser with a Molecular Sieve 5A (0.6 m) GC column. The devices were equipped with an autosampler (Finnigan AS 200, Thermo Scientific)

and interfaced with the IRMS through a dilutor (Conflo III, Thermo Scientific) dosing the sample and reference gases.

2.3.2. Elements

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 H_2 as collision and reaction gases, respectively, was used to remove polyatomic interferences.

2.4. Sample preparation and analysis

2.4.1. 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 $^{13}C/^{12}C$ and silver capsules for quantification of $^{18}O/^{16}O$ and $^{2}H/^{1}H$.

For $^{13}C/^{12}C$, the precision of measurement, expressed as standard deviation when measuring an oil sample 10 times, was 0.1‰.

For $^{18}O/^{16}O$ and D/H analysis, the samples were stored in a desiccator above P_2O_5 for at least 24 h, 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 $^{18}O/^{16}O$ ratios of bulk olive oils were measured simultaneously in one run. The IRMS measured first D/H and then, following the magnet jump, $^{18}O/^{16}O$, taking about 10 min 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 verified to be lower than 9. The precision of measurement, expressed as standard deviation when measuring an oil sample 10 times, was 0.3‰ for $^{18}O/^{16}O$ and 2‰ for D/H.

2.4.2. Elements

About 15 g of sample were weighed into a 50 ml conical vial of polypropylene (PP) and 15 ml of 1% HNO_3/0.2% 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 × 5 min) to extract the trace elements from the oil to the acid solution. The mixture was centrifuged (4000 rpm × 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 10 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 three 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 10 times. DL and RSD% are shown in Table 1.

Table 1
Instrumental conditions and mineral content distribution of well settled extra-virgin Italian olive oils (ORS: collision cell octopole reaction system)

Element	Isotope	ORS mode	Unit	DL	Number of Samples > DL	RSD (%)	25° Percentage	Median	75° Percentage	90° Percentage	Maximum	Range (Minimum–Maximum)	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: Martin-Polvillo et al. (1994); m: Solinas et al. (1987); n: Zeiner et al. (2005).

Table 2
Median, minimum and maximum values of $\delta^{13}C$ (bulk olive oil and glycerol) and of $\delta^{18}O$ 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

Region	N_{tot}	Parameter	2000				2001				2002				2003				2004			
			N	Median	Minimum	Maximum	*N*	Median	Minimum	Maximum	*N*	Median	Minimum	Maximum	*N*	Median	Minimum	Maximum	*N*	Median	Minimum	Maximum
Trentino	37	$\delta^{13}C$ 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
		$\delta^{13}C$ 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
		$\delta^{18}O$ 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	$\delta^{13}C$ 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
		$\delta^{13}C$ 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
		$\delta^{18}O$ 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	$\delta^{13}C$ 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
		$\delta^{13}C$ 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
		$\delta^{18}O$ 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-	13	$\delta^{13}C$ bulk	2	−29.2			3	−29.6	−29.8	−29.4	2	−30.0			6	−29.1	−29.3	−28.7				
Romagna		$\delta^{13}C$ glycerol		−31.6				−31.6	−31.6	−31.3		−32.3				−31.2	−31.7	−30.7				
		$\delta^{18}O$ glycerol		29.7				29.8	29.1	30.1		29.7			31.1	30.9	32.4					
Liguria	18	$\delta^{13}C$ 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
		$\delta^{13}C$ 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
		$\delta^{18}O$ 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	$\delta^{13}C$ 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				
		$\delta^{13}C$ 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				
		$\delta^{18}O$ 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	$\delta^{13}C$ bulk	4	−29.3	−29.5	−29.2	15	−29.4	−30.0	−28.1					11	−28.9	−29.5	−28.3				
		$\delta^{13}C$ glycerol		−30.1	−31.4	−29.7		−30.9	−32.0	−29.6						−30.6	−31.7	−29.8				
		$\delta^{18}O$ glycerol		31.8	30.5	33.7		32.1	30.0	33.4						32.6	31.4	33.4				
Abruzzo	30	$\delta^{13}C$ 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
		$\delta^{13}C$ 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
		$\delta^{18}O$ 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	$\delta^{13}C$ 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
		$\delta^{13}C$ 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
		$\delta^{18}O$ 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	$\delta^{13}C$ 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
		$\delta^{13}C$ 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
		$\delta^{18}O$ 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	$\delta^{13}C$ 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
		$\delta^{13}C$ glycerol		−31.4	−32.3	−29.9		−30.8	−31.8	−29.3		−32.1	−34.4	−32.0	00					−31.8	−33.0	−30.7
		$\delta^{18}O$ 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	$\delta^{13}C$ bulk									8	−29.8	−31.3	−28.3	9	−29.4	−30.1	−28.8	7	−29.2	−30.2	−29.0
		$\delta^{13}C$ glycerol										−31.0	−32.0	−29.9		−30.5	−31.1	−30.2		−31.0	−31.3	−30.4
		$\delta^{18}O$ glycerol										32.9	30.7	34.1		33.5	31.3	34.8		34.8	32.5	35.6
Sicily	52	$\delta^{13}C$ 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
		$\delta^{13}C$ 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
		$\delta^{18}O$ 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	$\delta^{13}C$ bulk	82	-29.3^{ab}	−31.8	−27.5	102	-29.2^{ab}	−32.4	−26.6	66	-30.0^{c}	−31.3	−27.7	95	-28.9^{a}	−30.1	−27.5	58	-29.4^{bc}	−31.4	−28.0
		$\delta^{13}C$ glycerol		-31.2^{b}	−34.7	−28.5		-31.2^{b}	−34.6	−28.4		-31.7^{b}	−34.4	−28.8		-30.6^{a}	−32.4	−28.4		-31.3^{b}	−33.5	−29.1
		$\delta^{18}O$ 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

Different letters correspond to significantly different median values ($p < 0.001$) *N* = number of samples.

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

3. Results and discussion

3.1. Stable isotope ratios

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

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

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

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

For statistical evaluation, we grouped the regions into four clusters on the basis of their latitude and the similarity of their isotopic ratios: North (Trentino, Veneto, Lombardia, Emilia Romagna), Centre (Liguria, Tuscany, Umbria, Abruzzo, Lazio), South-1 (Campania, Apulia) and South-2 (Calabria, Sicily). Applying the non parametric test of Kruskall–Wallis (Fig. 1), olive oil from Northern Italy showed isotopic values for at least one parameter significantly lower ($p < 0.01$) than for the South-2, Centre and South-1 in 5, 3 and 2 years respectively, out of the 5 years. Central Italy was never separated from South-1, whereas it was different from South-2 in two out of 5 years. The two southern macro areas could be distinguished from one another in three out of 5 years.

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

For the 2005 samples, along with the aforementioned parameters, the $\delta^{18}O$ and δD in bulk oil were also measured (Table 3). δD was investigated as a possible additional parameter for the

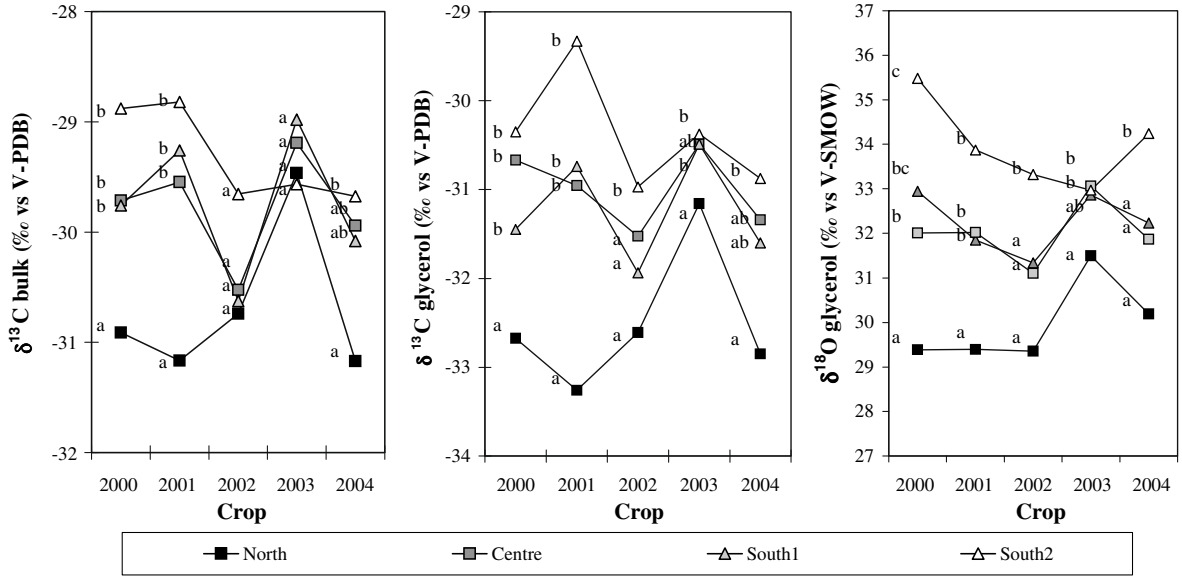

Fig. 1. Median values of the $\delta^{13}C$ (bulk olive oil and glycerol) and the $\delta^{18}O$ (glycerol) for North, Centre, South-1 and South-2 Italian olive oils (2000–2004) and results of the non-parametric statistical test (Kruskall–Wallis' test) among North, Centre, South-1 and South-2 Italian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

Table 3
Median values of $\delta^{13}C$ (bulk olive oil and glycerol), $\delta^{18}O$ (bulk and glycerol) and 8D (bulk oil) for North, Centre, South-1 and South-2 Italian olive oils produced in 2005 and results of the non-parametric statistical test (KruskalWallis' test)

	N	$\delta^{13}C$ bulk (‰ vs. V-PDB)			$\delta^{13}C$ glycerol (‰ vs. V-PDB)			$\delta^{18}O$ glycerol (‰ vs. V-SMOW)			$\delta^{18}O$ bulk (‰ vs. V-SMOW)			$\delta^{2}H$ bulk (‰ vs. V-SMOW)		
		Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum
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
South-1	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
South-2	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

Different letters correspond to significantly different median values $p < 0.01$.

characterisation of geographical origin, being influenced in plant products by the isotopic composition of the primary hydrogen source (source water through the leaf water) and by the geographical and climatic factors mentioned above for $\delta^{18}O$, together with their biosynthetic pathways (Schmidt, Werner, & Eisenreich, 2003).

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

The $\delta^{18}O$ values measured in bulk were significantly correlated with those in glycerol ($p < 0.001$): $\delta^{18}O_{bulk} = 0.837 * \delta^{18}O_{glycerol} - 3.2213$ ($R^2 = 0.8772$), the first always being lower, with a mean difference ± standard deviation of 8.15‰ ± 0.66.

The $\delta^{18}O$ and δD values in bulk olive oil were also correlated ($p < 0{,}001$), as happens in water (Clark & Fritz, 1997); the correlation equation is $\delta^{2}H_{bulk} = (-208{,}1 + 2{,}5091) \times \delta^{18}O_{bulk}$, but the R^2 value (0.4582) is low.

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

3.2. Mineral composition

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

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

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

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

		Region	South Italy										Centre Italy					North Italy	
			Calabria			Apulia	Sicily						Lazio		Tuscany		Umbria	Veneto	Trentino - Veneto
		PDO/ PGI	Alto Crotonese	Bruzio	Lametia	Terra diBari	Monte Etna	Monti Iblei	Val Demone	Valdi Mazara	Valli del Belice	Valli Trapanesi	Canino	Sabina	Lucca	Toscano PGI	Umbria	Veneto	Garda
Element	Unit	No. Obs.	3	2	3	10	3	3	3	3	3	3	6	6	3	3	12	7	26
Li	μg/kg	median		0.003	0.010	0.007	0.031	0.013		0.011	0.007	0.008	0.004		0.006	0.006		0.016	0.003
		max	0.010	0.006	0.013	0.039	0.208	0.046	0.008	0.013	0.007	0.010	0.018	0.012	0.014	0.007	0.029	0.064	0.091
Na	mg/kg	median			0.049		0.280	0.189	0.066				0.069			0.060		0.154	
		max	0.124		0.100	0.170	1.11	0.492	0.161	0.129		0.052	0.326	0.124		0.133	0.340	0.609	0.312
Mg	mg/kg	median				0.042	0.082	0.087	0.015	0.047	0.016		0.031			0.034		0.050	0.046
		max	0.016		0.017	0.225	0.495	0.139	0.017	0.081	0.017	0.016	0.110	0.056		0.047	0.053	0.104	0.264
K	mg/kg	median		0.038	0.180	0.415	0.726	1.171	0.116	0.418	0.079		0.033			0.292	0.109	0.424	0.516
		max	0.32	0.08	0.22	3.79	9.94	1.70	0.17	0.90	0.10	0.12	0.56	1.13	0.12	0.43	0.64	2.06	3.13
Ca	mg/kg	median																	
		max				0.395	0.447	0.603											0.950
Mn	μg/kg	median			0.408	0.598	0.491	0.351		0.134			0.312	0.058		0.921		0.392	0.507
		max			0.566	10.0	3.42	1.21		0.522			3.61	0.655	0.173	1.17	0.623	1.49	2.87
Co	μg/kg	median				0.003	0.006												
		max				0.033	0.024	0.030		0.009				0.005	0.014		0.030	0.012	0.031
Cu	μg/kg	median	0.160	0.334	0.343	0.404	2.107	0.446	0.360	0.434	0.311	0.171	0.251	0.127	0.416	0.324	0.258	0.583	0.493
	μg/kg	max	0.237	0.363	0.952	2.37	15.8	1.47	0.689	0.472	0.885	0.430	0.663	0.824	0.778	0.355	0.810	1.72	26.3
Rb	μg/kg	median		0.040	0.108	0.394	0.199	0.628	0.040	0.178	0.060	0.036	0.237	0.173		0.214	0.058	0.336	0.183
		max	0.263	0.049	0.175	4.19	13.4	1.59	0.081	0.802	0.065	0.074	2.98	2.69	0.055	0.214	0.584	1.013	1.110
Sr	μg/kg	median																	
	μg/kg	max					1.23	3.85					1.12						1.40
Cs	μg/kg	median	0.003	0.008	0.004	0.004	0.004	0.012	0.004	0.004	0.005	0.004	0.006	0.004	0.003	0.003	0.004	0.004	
		max	0.035	0.012	0.005	0.012	0.038	0.014	0.004	0.013	0.005	0.005	0.819	0.011	0.012	0.004	0.012	0.007	0.012
Ba	μg/kg	median																	
	μg/kg	max				2.05	2.49	0.550					1.13			0.309	0.435	1.34	0.695
La	μg/kg	median			0.064	0.032	0.002						0.016			0.009	0.001	0.002	
		max			0.158	2.94	0.008	0.023		0.004			0.225	0.012		0.035	0.040	0.011	0.005
Ce	μg/kg	median		0.002	0.043	0.058							0.020			0.013		0.004	0.003
	μg/kg	max		0.004	0.111	4.72	0.018	0.045		0.008			0.161	0.024		0.056	0.056	0.013	0.009
Sm	μg/kg	median			0.005	0.003							0.001			0.001			
		max			0.010	0.111		0.002					0.015	0.001		0.004	0.003		0.002
Eu	μg/kg	median		0.0010	0.0017	0.0021	0.0004									0.0004			
	μg/kg	max	0.0020	0.0020	0.0017	0.0226	0.0008	0.0004		0.0020	0.0020	0.0021	0.0032	0.0021		0.0028	0.0025	0.0023	0.0006
Yb	μg/kg	median	0.0012	0.0007	0.0019	0.0022		0.0007		0.0006	0.0011	0.0011	0.0007	0.0012		0.0012	0.0003	0.0007	
		max	0.0017	0.0014	0.0032	0.0412	0.0015	0.0009		0.0017	0.0017	0.0015	0.0032	0.0022		0.0033	0.0030	0.0020	0.0011
Pb	μg/kg	median	0.180	0.262	0.595	0.380	211	0.439	0.815	0.292	0.300		0.329	0.234	0.790	0.345	0.516	0.691	0.260
	μg/kg	max	1.47	0.299	1.50	2.69	3.86	0.972	1.24	0.733	0.409	0.468	1.84	0.464	2.60	0.573	2.76	0.904	8.46
U	μg/kg	median			0.0021	0.0021	0.0104	0.0008	0.0009		0.0030		0.0130	0.0050		0.0067	0.0012	0.0314	0.0023
		max	0.0014		0.0176	0.0401	0.0802	0.0017	0.0016		0.0145	0.0067	0.1190	0.0939		0.0211	0.0044	0.0131	0.0390

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

4. Conclusions

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

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

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

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