

Preliminary Results on the Influence of Carbonic Snow Addition during the Olive Processing: Oil Extraction Yield and Elemental Profile

Zoani C¹, Zappa G¹, Venturi F^{2*}, Sanmartin C², Andrich G² and Zinnai A²

¹ENEA-Italian National Agency for New Technologies, Energy and Sustainable Economic Development - Technical Unit for Sustainable Development and Innovation of Agro-Industrial System (UTAGRI), Casaccia Research Centre - Via Anguillarese, 301- 00123 Roma, Italy

²University of Pisa – Department of Agriculture, Food and Environment (DAFE) - Via del Borghetto 80 - 56124 Pisa, Italy

Abstract

The compositional profile of olive oil is strictly connected to the raw material characteristics, nevertheless technological processes utilized to extract the oil fraction can deeply impact on its final chemical composition.

Trace quantities of elements are naturally present in olive oil. To make objective and measurable food quality and traceability, it is necessary to develop analytical methods useful to detect contaminants potentially coming from any phases of the supply chain and define characteristic markers and patterns for the authenticity verification and origin demonstration.

The objective of the paper is twofold: a) to verify the influence of the addition of cryogen (CO_{2,s}), directly to olives during pre-milling phase, on the yield of the oil production; b) to develop specific analytical methods for evaluating the elemental profile of olive oil and to collect some preliminary data about the possibility to use these methods in order to put in evidence the possible influence of different process conditions on the olive oil elemental profile.

The experimental evidences show as the addition of cryogen directly to the olives during pre-milling phase seem induce a general increase in the oil extraction yield, ranging from 2 to 12,4%. While preliminary results show a rather homogeneous elemental content among all the analyzed samples of olive oil, samples of olive oil obtained by the "traditional" process showed - in general - a higher content of Ca, Cr, Mg, Si and Zn, compared to those obtained by the addition of "carbonic snow". The analytical methods for evaluating the olive oil elemental profile by ICP-AES and ICP-MS were set up and the methodologies for data processing and statistical analysis were developed, so they could be applied during the next crop season to study in depth the influence of different process conditions on the olive oil elemental profile.

Keywords: Olive oil; Cryogen; Elemental profile; Oil extraction yield

Introduction

Olive oil is a premium vegetable oil highly appreciated and consumed [1]. It constitutes the main lipid source in the Mediterranean diet due to its sensory and healthy properties [2]. The compositional profile of olive oil is strictly connected to the raw material characteristics but the operative conditions adopted to extract the oil fraction can deeply impact on its final chemical composition [3]. Several studies have pointed out the importance of the different virgin olive oil processing stages on the extraction yield as well as the minor composition found in the final product. The technological operations that most affect the composition and quality of oil during the manufacturing process are crushing and malaxation [4].

Although the public demand for adequate and sustainable quantities of good-quality food throughout the food chain stimulated the study and application of promising new and emerging food technologies [5], the entire virgin olive oil (VOO) process has changed very little over the last twenty years [1]. With the aim to contribute to advance in VOO technology, at DAFE of University of Pisa, an innovative VOO extraction technology (Patent n° IT1405173-B) involving the addition of a cryogen (solid CO₂) to the olives [6] were developed, in order to increase the extraction yield and to obtain an oil characterized by higher concentration of phenolic compounds and a stronger link with the raw material and its production area [7].

As a function of the temperature adopted, at atmospheric pressure carbon dioxide (CO₂) can be present only in a solid (CO_{2,s} = carbonic snow; T < -78,5°C) and/or in a gaseous state (T > -78,5°C) [8]. The pre-milling addition of solid carbon dioxide CO_{2,s} (carbonic snow)

to the olives, causes a partial solidification of endocellular water as a consequence of the direct contact between the cryogen (carbonic snow) and the olives. Because of the volume occupied by the same amount of water in the solid state is greater than that in the liquid phase, a corresponding increase of endocellular volume occurs and the consequent laceration of cellular membranes (cellular crash) promotes the immediate diffusion in the liquid phase of many cellular compounds like phenols and oil. In this way, it could be possible to obtain extra virgin olive oils characterized by a greater amount of metabolic compounds deriving from the olives even because the relevant amount of gas (gaseous CO_{2,g}), which evolves during the direct contact between olives and cryogen, decreases the amount of atmospheric oxygen diffused in the liquid phase and than inhibits the possible oxidation of the phenolic substances.

To verify if there are compositional differences between the cryomacerated oils and the traditional ones which make it possible to ensure the traceability and safety of these new products, it's important

*Corresponding author: Francesca Venturi, DAFE – University of Pisa, Italy, Tel: +39 0502216632; Fax: +39 0502216636; E-mail: francesca.venturi@unipi.it

Received April 16, 2014; Accepted May 28, 2014; Published May 30, 2014

Citation: Zoani C, Zappa G, Venturi F, Sanmartin C, Andrich G, et al (2014) Preliminary Results on the Influence of Carbonic Snow Addition during the Olive Processing: Oil Extraction Yield and Elemental Profile. J Nutr Food Sci 4: 277. doi: 10.4172/2155-9600.1000277

Copyright: © 2014 Zoani C, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

to develop analytical methods useful for protecting producer and consumers' interests [9,10]. Suitable traceability systems are needed to document the history of a product along the entire supply chain (from primary production to the final use), to protect high quality productions from frauds and sophistications and contain health safety risks [11-13].

Trace quantities of elements are naturally present in olive oil. Natural composition of olive fruit, natural contamination from soil, use of fertilizers, industrial applications or highways near the plantations are the main sources of toxic or potentially toxic elements in olive oils. Contamination can also occur during the production process and contact with storage materials.

In this context, the objective of the paper is twofold:

a) The first goal was to verify the influence of the addition of cryogen (CO_{2,s}), directly to olives during pre-milling phase, on the yield of the oil production, in order to obtain a preliminary evaluation of the suitability of the new proposed methodology for VOO production;

b) The second goal was to develop specific analytical methods for evaluating the elemental profile of olive oil and to collect some preliminary data about the possibility to use these methods in order to put in evidence the possible influence of different process conditions (extraction with or without addition of carbonic snow) on the olive oil elemental profile. In particular, olive oil samples were submitted - after appropriate pre-treatments - to chemical analyses focused to establish the elemental composition (macro-, micro- and trace elements) and to define characteristic elemental patterns. The following analytical techniques were applied and compared: Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), and Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS). These techniques allow the determination (also simultaneously) of nutritional elements (e.g.: Fe, Zn, Cu), environmental contaminants and contaminants from transformation processes, storage and packaging (e.g.: Pb, Cd, Ni), as well as of trace elements to be used for the definition of characteristic fingerprints linked to the territorial product origin.

The accurate determination of trace elements in olive oil is an analytical challenge due to their low concentration and the difficulties arising from the high organic content of the oil matrix. In particular, the high organic content imply that sample pre-treatment could represent a critical step in the olive oil analysis [14].

Materials and Methods

The study was conducted on samples of olive oil produced from monovarietal and polyvarietal (*mix*) olives collected in two different Italian regions (from Tuscany and Basilicata) during the same season (Table 1). In each experimental runs, olive oil samples obtained from the same raw material were submitted or not to the treatment with carbon dioxide in the solid state ("carbonic snow") and were analyzed,

Sample ID		Geographical origin	Cultivar
"Traditional"	"Cryo"		
A	1	Tuscany (GR)	frantoio
B	2	Tuscany (SI)	<i>mix</i> (frantoio, leccino, corregiolo)
C	3	Tuscany (SI)	<i>mix</i> (frantoio, leccino, corregiolo)
D	4	Tuscany (SI)	<i>mix</i> (frantoio, leccino, corregiolo)
E	5	Basilicata	coratina
F	6	Basilicata	coratina
G	7	Basilicata	coratina

Table 1: Experimental runs: origin and cultivar of the raw matter.

so to compare the oil yield as well as the elemental profiles obtained as a function of different process conditions.

Determination of oil extraction yield

The extraction runs were carried out utilising a micro oil mill (Oliomio Baby[®], Toscana Enologica Mori), which is able to mill 20-30 kg of olives, and it was suitably modified to allow the addition of carbonic snow directly to olive fruits and/or to their paste.

The main process steps followed by this micro oil mill, can be so schematically presented: olives properly cleaned and washed, are poured into the receiving hopper, where a screw feeds the crusher equipped with a hollow knife impeller. The produced paste falls into the lower mixer, where an helicoidally shaped stirrer promotes its malaxation, the temperature reached by the paste is maintained in the wished range by a thermal regulation system (temperature sensor put inside the olive paste, connected with an heat exchanger). The wished flow of the olive paste is then sent to a biphasic decanter by a pump equipped with a speed change gear. The decanter (4200 rpm) promotes the separation of oil from the solid parts of olive mixed with vegetable water plus the fraction possibly added to allow an efficient separation of these two phases.

The separation efficiency of this decanter can be enhanced by a suitable regulation of its nozzles, which determine the point of oil picking and then also the purity degree of the product.

To allow a suitable comparison between the results obtained in the presence or in the absence of solid CO₂ during the oil extraction process, the olive fruits utilised in each experimental runs (~ 60 kg) were preliminary and suitably mixed to ensure homogeneous feeds.

In all the experimental run the ratio "mass of cryogen"/"mass of fruits" was maintained constant, close to 0,2.

With the aim to avoid that the differences in the composition of olives (i.e. cultivar and/or ripening stage as well as water content) utilised in different experimental runs can influence determination of the oil extraction yield, this parameter has been expressed as "extractability" according to the following equation:

$$\text{Extractability index (E)} = \frac{(\text{kg of extracted oil})}{(\text{kg of milled olives}) \times \% \text{ of oil inside the olives}} \quad (1)$$

Determination of oil elemental profile

All reagents used were of analytical grade. High-purity water (resistivity > 18MΩ cm) was used in all dilutions. The elemental standard solutions were prepared by diluting stock solutions (TraceCERT[®] Fluka Standards) of 1000 mg/l.

ICP-AES and ICP-MS analysis:

Dissolution of the test material: Olive oil dissolution was performed by acid attack in a Microwave High Pressure Digestion System MILESTONE MLS 1200 MEGA. Different reagent mixtures were tested in order to define the better conditions for obtaining a total dissolution with a complete destruction of the organic component of the matrix. In particular the use of different reagent mixtures was investigated: 0,5 g of olive oil was treated with concentrated H₂O₂ (30%v/v) and concentrated HNO₃ (69.9%v/v) in different proportions (H₂O₂ ranged from 1 ml up to 2 ml while HNO₃ ranged from 2 ml up to 6 ml). Complete dissolution of oil was obtained by employing 6 ml HNO₃+2 ml H₂O₂ and applying the following digestion cycle: 1 min for 250 W, 1 min at 0 W, 5 min at 400 W, 5 min at 650 W and 5 min vent

(with free pressure rise). The obtained solutions were filled up to the 25 ml final volume with high-purity water in PP flasks and submitted to ICP-AES and ICP-MS analysis. Process blank solutions were prepared and submitted to elemental analysis under identical conditions.

ICP-AES analysis: Solutions obtained by olive oil dissolution were analyzed as it were (TQ) using a Varian VISTA MPX (Axial Configuration; simultaneous 1.12 Mpixel CCD detector).

First of all tests to optimize gas flows basing on signal-to-background ratio (S/B) were conducted. Then samples were analyzed with a method allowing to perform the study of spectral interferences and the preliminary qualitative analysis (simultaneous detection of the presence of 72 elements). Finally quantitative analysis was carried out, under the conditions reported in Table 2.

ICP-MS analysis: Solutions obtained by olive oil dissolution were 1:10 diluted with high-purity water and then analyzed using a Bruker Aurora M90 (90 degree ion mirror ion optics; Collision Reaction Interface). The ICP-MS was tuned with a with a 5 µg/l Be, Mg, Co, In, Ba, Ce, Ti, Pb and Th solution for sensitivity and resolution optimization and mass calibration. The level of oxide ions were checked by the CeO⁺/Ce⁺ ratio (< 2%), while double charged ions were monitored by the signal ¹³⁷Ba⁺⁺/¹³⁷Ba⁺ (< 3%).

The isotopes to be investigated were selected basing on potential interferences and relative abundances. In order to check/correct the presence of interferences, for some elements the method was optimized by analyzing more isotopes (e.g.: Rb85 and Rb87; Sr88 and Sr87; Nd142, Nd146 and Nd144; Se77 and Se78; Sm147, Sm148 and Sm152; Gd157 and Gd154; Er166 and Er168; Yb170 and Yb172), even applying correction equations with respect to isotopes of other elements potentially interfering (e.g.: (e.g.: Nd142 corrected by analyzing Ce140, Nd144 by Sm147, Sm152 by Gd157, Rb87 by Sr88)). For lead, considering the great variability of the relative abundances of its isotopes in nature, a mathematical combination of the results obtained for the three stable isotopes Pb206, Pb207 and Pb208 was employed. The dwell time was optimized for each element in order to obtain the best sensitivity.

For some elements, analyses were performed operating both in *Normal* and in *High Sensitivity Mode*, so to focus the ion beam enhancing sensitivity.

Instrumental conditions		
RF Frequency	40 MHz (free running, air-cooled)	
RF Power	1.2 kW	
Gas (plasma, auxiliary, nebulizer)	Argon	
Plasma flow	15.0 l/min	
Auxiliary flow	1.5 l/min	
Nebulizer flow	1.10 l/min	
Replicates	5	
Replicate read time	5 s	
Analytical wavelengths (nm)		
Al - 396.152 nm	K - 766.491 nm	Rb - 780.026 nm
As - 188.980 nm	Li - 670.783 nm	S - 181.972 nm
B - 249.772 nm	Mg - 279.553 nm	Sb - 206.834 nm
Ba - 455.403 nm	Mn - 257.610 nm	Si - 251.611 nm
Ca - 396.847 nm	Na - 589.592 nm	Sr - 407.771 nm
Cr - 267.716 nm	Ni - 231.604 nm	Ti - 334.941 nm
Cu - 327.395 nm	P - 213.618 nm	V - 311.837 nm
Fe - 238.204 nm	Pb - 220.353 nm	Zn - 206.200 nm

Table 2: Operating parameters for ICP-AES quantitative analysis.

Instrumental conditions					
Gas flow parameters (Argon)	Plasma flow	17.00 l/min			
	Auxiliary flow	1.80 l/min			
	Nebulizer flow	0.94 l/min			
	Sheat flow	0.25 l/min			
Plasma power	RF power	1.40 kW			
Sample introduction	Sampling depth	7.50 mm			
	Pump rate	2 rpm			
Ion optics	First extraction lens	- 1.00 V			
	Second extraction lens	- 176.00 V			
	Third extraction lens	- 210.00 V			
	Corner lens	- 213.00 V			
	Mirror lens left	42.00 V			
	Mirror lens right	24.00 V			
	Mirror lens bottom	26.00 V			
Quadrupole scan	Fringe bias	- 2.40 V			
	Scan mode	Peak hopping			
	Dwell time	30 ÷ 60 ms			
	Points per peak	1			
	Scans/Replicate	10			
	Replicates/Sample	5			
Analyzed isotopes					
As75	Cu65	La139	Ni60	Sc45	Sr88
Cd114	Er166	Mg32	Pb(206,207,208)	Se78	Th232
Ce138	Er168	Mn55	Pr141	Sm147	Y89
Ce140	Eu153	Nd142	Rb85	Sm148	Yb170
Co59	Gd154	Nd144	Rb87	Sm152	Yb172
Cr53	Gd157	Nd146	Sb121	Sr87	V51

Table 3: Operating parameters for ICP-MS analysis *Normal Sensitivity Mode* (without CRI).

Instrumental conditions				
Gas flow parameters (Argon)	Plasma flow	17.00 l/min		
	Auxiliary flow	1.70 l/min		
	Nebulizer flow	1.00 l/min		
	Sheat flow	0.16 l/min		
Plasma power	RF power	1.40 kW		
Sample introduction	Sampling depth	6.50 mm		
	Pump rate	15 rpm		
Ion optics	First extraction lens	- 456.00 V		
	Second extraction lens	- 826.00 V		
	Third extraction lens	- 600.00 V		
	Corner lens	- 544.00 V		
	Mirror lens left	69.00 V		
	Mirror lens right	48.00 V		
	Mirror lens bottom	51.00 V		
Quadrupole scan	Fringe bias	- 3.00 V		
	Scan mode	Peak hopping		
	Dwell time	40 ÷ 60 ms		
	Points per peak	1		
	Scans/Replicate	10		
	Replicates/Sample	5		
Analyzed isotopes				
Ce138	Eu153	Nd142	Sc45	Th232
Ce140	Gd154	Nd144	Sm147	Y89
Er166	Gd157	Nd146	Sm148	Yb170
Er168	La139	Pr141	Sm152	Yb172

Table 4: Operating parameters for ICP-MS analysis *High Sensitivity Mode*.

Tables 3 and 4 show the analyzed isotopes and the instrumental conditions for the ICP-MS analysis, in *Normal Sensitivity Mode* and in *High Sensitivity Mode* respectively. In addition, for As and Se a specific method in *Normal Sensitivity Mode* was developed in order to reduce interferences by using the Collision Reaction Interface (Skimmer gas:

H₂; Skimmer flow = 60 ml/min); the potentials of ion optics, Ar flows and dwell time (100 ms) were appropriately optimized.

As internal standard, Y89 (except when Y was an analyte) and In115 were employed.

External calibration was performed by employing 5 standard solution and a blank solution; working ranges were:

- 0.05 ÷ 5 µg/l – for As, Cd, Ce, Er, Eu, Gd, La, Mn, Nd, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sr, Th, V, Y, Yb
- 0.1 ÷ 10 µg/l – for Co, Cr, Cu, Mg, Ni

Results and Discussion

Determination of oil extraction yield indexes: preliminary results

In Table 5 are reported the different indexes of extractability calculated for each experimental run according to eq. 1, with (EC) or without (ET) the addition of cryogen during pre-milling phase.

In order to evaluate the effect induced by the addition of cryogen on the oil extraction yield, it was determined the Extractability Index Variation (EIV) as the percentage of the variation of oil extractability using CO_{2,s} compared with the same parameter obtained by a traditional extraction process:

$$EIV = (EC-ET)/ET \cdot 100 \quad (2)$$

The experimental values (Table 5) showed as the direct addition of cryogen to the olives during pre-milling phase could induce a general increase in the oil extraction yield, ranging from 2 to 12,4%.

Even if the number of experimental runs carried out until now could appear quite reduced, the data obtained have given a positive indication about the suitability of the new proposed method for the oil extraction so that it will be used during the next crop season. In order to individuate the best working conditions which have to be adopted to obtain the best oil extraction yield as a consequence of the addition of cryogen, it will be necessary to apply the new extraction process to a greater number of experimental runs adopting several combinations of the working parameters (i.e. amount of cryogen/amount of olives, fruit ripening stage, etc.).

Moreover, in order to define the best working conditions, it will be taken into account the values of the concentrations of high quality components (such as phenols and aromatic compounds) diffused from the solid parts of the olives into the lipid phase during the different experimental runs.

Because of the cellular crash induced by the cryogen addition, it would be possible to move up the olive harvest time in order to greatly reduce the damages due to the third fly attack of *Bactrocera oleae* that

can induce a significant loss in oil production (up to 60%) as well as a decrease in oil quality. In fact the extent of the changes was much greater when the olives were attacked and harvested at the advanced stage of maturity [15].

Determination of oil elemental profile: preliminary results

The analytical methods for evaluating the olive oil elemental profile by ICP-MS and ICP-MS were set up. ICP-AES and ICP-MS techniques are particularly advantageous for the application in the definition of the elemental profiles of olive oil, since they allow simultaneous multielemental analysis and are characterized by wide linear ranges and (especially ICP-MS) very low Detection Limits. Main analytical issues are related to the hard organic content of the oil matrix, which requires appropriate sample pre-treatments. Microwave-assisted digestion permitted to obtain a complete dissolution of olive oil samples, but resulted in the obtainment of highly acidic solutions not directly detectable by ICP-MS, with a consequent reduction of sensitivity (due to the preliminary 1:10 dilution of the mineralized olive oil). To address this issue, it is possible to apply matrix modification procedures (e.g. solvent extraction – also ultrasound assisted -, addition of organic solvents, or emulsification) or to employ systems for direct oil sample introduction in torch (e.g.: Flow Injection Analysis systems). In the next experimental campaign, it will be then possible to apply these methodologies for the analysis of a higher number of samples, so to study in depth the influence of different process conditions on the olive oil elemental profile. Additional experimental tests have been planned also in order to evaluate the possibility to employ a Laser Ablation (LA) system or an ElectroThermal Vaporization (ETV) system for direct sample introduction in ICP-AES and ICP-MS.

Table 6 shows the elemental content in olive oil samples. Preliminary results show a rather homogeneous elemental content among all the analyzed samples of olive oil. Generally, samples of olive oil obtained by the “traditional” process showed a higher content of Ca, Cr, Mg, Si and Zn, compared to those obtained by the addition of “carbonic snow”. However also in this case it will be necessary to increase the number of experimental runs and vary the operating conditions adopted during the extraction with “carbonic snow” (i.e. ratio “cryogen”/“olives”; ripening stage; etc.).

As showed in Figure 1, experimental concentrations fell within the ranges reported in literature [16] for all elements. In respect to these ranges, analyzed samples showed a high content of B, S and Si, while Fe, Na, Pb, Ba, Cr and Cu contents are quite low.

Although in this first stage of our work the main purpose was to develop the analytical methods for elemental analysis of olive oil by ICP-AES and ICP-MS, the results obtained on this first set of samples have been submitted to multivariate statistical analysis in order to better interpret the data and perform a preliminary evaluation about the possibility to differentiate the samples in respect to the process.

Principal Component Analysis (PCA) was performed with XLStat 2014 Software package (Addinsoft) used as a Microsoft Excel plug-in. Considering that below DL values are unreadable when using XLSTAT, PCA was applied to the elemental concentration of each sample by using the approximation to the DL value (set of 39 observations for 14 variables). The first 5 principal components extracted account together for 80.64% of the variability in the original data. The elements that mainly describe the first two variables (accounting together for 55.84% of the variability of the original data) are: Ba, Er, Gd, Se, Ce, Y, Nd, Mn and Pb for F1 (40.02%); Zn, Sr, Cr, Na, Li, K and V for F2 (15.82%). A further data processing was performed by considering

Run	EC	ET	(EC-ET) · 100 ET
1/A	85.8	82.5	4.0%
2/B	85.5	77.2	10.8%
3/C	91.2	82.7	10.3%
4/D	84.1	74.8	12.4%
5/E	80.8	79.3	1.9%
6/F	73.6	68.9	6.8%
7/G	84.4	77.4	9.0%

Table 5 : Oil extraction yield. EC = extractability with cryogen; ET = extractability without cryogen.

Sample		Al	B	Ba	Ca	Fe	K	Li	Na	P	S	Si	Ti	Zn
		mg/kg												
1	Trad	0.9	24	0.02	7.9	0.50	0.6	0.25	2.9	1.4	20	16	0.12	1.4
A	Cryo	0.9	26	0.01	5.5	0.28	< 0.4	0.26	2.9	1.3	25	10	0.21	1.4
2	Trad	2.8	26	0.13	21	1.3	1.1	0.25	2.8	1.6	24	25	0.21	1.4
B	Cryo	1.0	24	0.01	7.1	0.40	0.5	0.24	2.7	1.5	24	15	0.27	1.2
3	Trad	1.1	26	0.01	7.3	0.32	< 0.4	0.27	3.0	2.6	15	8	0.10	1.5
C	Cryo	0.9	25	< 0.01	6.2	0.26	0.5	0.25	2.8	2.0	30	7	0.11	1.4
4	Trad	0.8	26	0.01	11	0.28	0.5	0.25	2.8	1.1	29	84	0.12	1.4
D	Cryo	< 0.8	25	< 0.01	3.8	0.62	0.4	0.26	2.8	1.4	25	14	0.08	1.3
5	Trad	1.2	25	0.02	9.5	0.42	0.5	0.26	2.8	2.7	30	16	0.11	1.4
G	Cryo	2.3	23	0.01	10	0.43	0.4	0.26	3.0	2.0	28	20	0.11	1.4
6	Trad	2.3	24	0.04	13	1.0	1.1	0.26	2.9	1.6	23	18	0.14	1.7
F	Cryo	1.0	23	0.02	12	0.39	0.8	0.26	2.9	1.8	25	21	0.11	1.5
7	Trad	1.2	24	0.02	7.7	0.49	0.5	0.24	2.8	1.2	28	13	0.14	1.4
G	Cryo	1.3	24	0.03	12	0.58	1.2	0.26	2.9	1.5	< 10	19	0.13	1.5

Table 6a – Elemental content in olive oil (mg/kg).

Sample		As	Cd	Ce	Co	Cr	Cu	Er	Eu	Gd	La	Mg	Mn	Nd	Ni	Pb	Pr	Rb	Sb	Sc	Se	Sm	Sr	Th	V	Y	Yb	
		µg/kg																										
1	Trad	24	< 5	< 2.5	18	283	< 100	< 2.5	< 2.5	< 2.5	< 2.5	306	< 25	< 2.5	< 250	10	< 2.5	< 25	< 15	448	< 25	< 2.5	< 25	36	214	< 2.5	4	
A	Cryo	35	28	< 2.5	< 5	119	106	< 2.5	< 2.5	< 2.5	< 2.5	858	< 25	< 2.5	< 250	126	< 2.5	78	< 15	428	< 25	< 2.5	32	36	192	< 2.5	4	
2	Trad	< 15	< 5	6	< 5	133	< 100	4	< 2.5	4	12	1040	41	9	< 250	3255	< 2.5	< 25	< 15	580	38	< 2.5	27	288	222	3.0	12	
B	Cryo	40	< 5	< 2.5	< 5	99	120	< 2.5	< 2.5	< 2.5	< 2.5	523	< 25	5	< 250	70	< 2.5	< 25	< 15	538	< 25	< 2.5	< 25	53	243	< 2.5	10	
3	Trad	29	10	< 2.5	< 5	61	173	< 2.5	< 2.5	< 2.5	< 2.5	789	< 25	< 2.5	< 250	64	< 2.5	65	< 15	261	< 25	< 2.5	34	108	83	< 2.5	5	
C	Cryo	< 15	< 5	< 2.5	< 5	54	437	< 2.5	< 2.5	< 2.5	< 2.5	164	< 25	< 2.5	< 250	139	< 2.5	< 25	< 15	395	< 25	< 2.5	< 25	54	161	< 2.5	4	
4	Trad	43	< 5	< 2.5	< 5	230	201	< 2.5	< 2.5	< 2.5	< 2.5	1467	< 25	< 2.5	< 250	93	< 2.5	< 25	< 15	205	< 25	< 2.5	32	41	118	< 2.5	4	
D	Cryo	44	< 5	< 2.5	< 5	190	288	< 2.5	< 2.5	< 2.5	< 2.5	611	< 25	< 2.5	< 250	1028	< 2.5	< 25	< 15	448	< 25	< 2.5	< 25	49	123	< 2.5	4	
5	Trad	< 15	< 5	< 2.5	< 5	< 50	125	< 2.5	< 2.5	< 2.5	< 2.5	244	< 25	< 2.5	< 250	8	< 2.5	< 25	< 15	588	< 25	< 2.5	< 25	39	< 50	< 2.5	4	
G	Cryo	25	< 5	< 2.5	< 5	401	< 100	< 2.5	< 2.5	< 2.5	< 2.5	218	< 25	< 2.5	< 250	54	< 2.5	< 25	< 15	258	< 25	< 2.5	47	45	56	< 2.5	4	
6	Trad	63	< 5	< 2.5	6	539	< 100	< 2.5	< 2.5	< 2.5	5.7	581	< 25	3	< 250	109	< 2.5	< 25	< 15	431	< 25	< 2.5	50	189	116	< 2.5	6	
F	Cryo	30	< 5	< 2.5	< 5	725	< 100	< 2.5	< 2.5	< 2.5	< 2.5	303	< 25	< 2.5	< 250	163	< 2.5	< 25	< 15	320	< 25	< 2.5	< 25	41	81	< 2.5	4	
7	Trad	30	< 5	< 2.5	< 5	149	< 100	< 2.5	< 2.5	< 2.5	< 2.5	334	31	< 2.5	< 250	39	< 2.5	< 25	< 15	386	< 25	< 2.5	< 25	37	173	< 2.5	4	
G	Cryo	51	< 5	< 2.5	< 5	680	320	< 2.5	< 2.5	< 2.5	14	661	28	< 2.5	< 250	42	< 2.5	< 25	< 15	436	< 25	< 2.5	42	48	118	< 2.5	4	

Table 6b – Elemental content in olive oil (µg/kg).

Table 6. Elemental profile of olive oil samples.

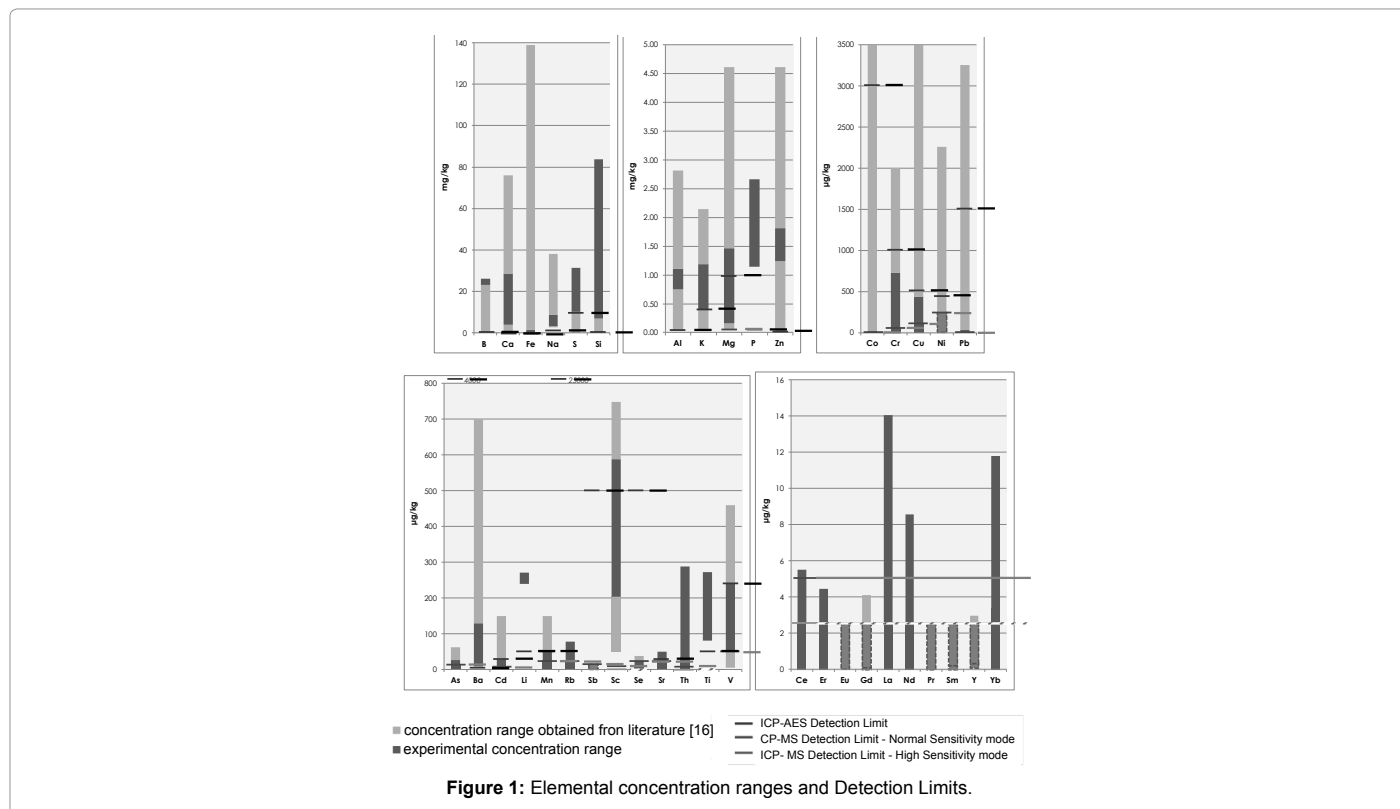


Figure 1: Elemental concentration ranges and Detection Limits.

only the elements present at detectable concentrations in at least 50% of the olive oil samples (set of 23 observations for 14 variables). Also in this case the first 5 variables extracted account together for 80.65% of the variability in the original data, but with the F1 (26.87%) mainly described by Ba, Fe, Th, Yb, Pb, Ca, Al and K and the F2 (22.18%) mainly described by Zn, Sr, Na, Cr, Li and V (F1 and F2: 52.04%). The results of the PCA showed a rather homogeneous distribution of samples, in accordance with the rather homogeneous elemental content previously highlighted. This is also due to the reduced number and type of the samples analyzed in this first experimental crop season (suitable to the development of methodologies, in accordance with the aim of our work). In any case, this elaboration allowed to define, in addition to the analytical methods, also the methodologies for data processing and statistical analysis. Further tests conducted on a larger number of olive oil samples, with characteristics as homogeneous as possible in respect to their geographical and botanical origin, differentiated in respect to the production process (addition or not of “carbonic snow” during the extraction, different operating conditions), will allow to better evaluate the effect of the different process conditions on the olive oil elemental profile.

References

1. Clodoveo ML, Hbaieb RH (2013) Beyond the traditional virgin olive oil extraction systems: Searching innovative and sustainable plant engineering solutions. *Food Research International* 54: 1926-1933.
2. Inarejos-García AM, Gómez-Alonso S, Fregapane G, Salvador MD (2013) Evaluation of minor components, sensory characteristics and quality of virgin olive oil by near infrared (NIR) spectroscopy. *Food Research International* 50: 250-258.
3. Clodoveo ML (2013) New advances in the development of innovative virgin olive oil extraction plants: Looking back to see the future. *Food Research International* 54: 726-729.
4. Fregapane G, Salvador MD (2013) Production of superior quality extra virgin olive oil modulating the content and profile of its minor components. *Food Research International* 54: 1907-1914.
5. Helms M (2004) Food sustainability, food security and the environment. *British Food Journal* 106: 380-387.
6. Andrich G, Venturi F, Zinnai A (2010) Innovative technology for the production of olive oil characterized by organoleptic and nutritional high quality. Original Patent of Università di Pisa (Patent n° RM2010A000617).
7. Sanmartin C, Zinnai A, Venturi F, Andrich G (2011) The Utilization of Solid Carbon Dioxide in the Production of Extravirgin Olive Oil, Proceedings of EFFOST 2011, 9-11 November, Berlino (D).
8. Zinnai A, Venturi F, Andrich G (2010) Time evolution of phenol extractions from Sangiovese grapes with and without the addition of solid carbon dioxide. *Agrochimica LV* 54: 1-10.
9. Aung MM, Chang YS (2013) Traceability in a food supply chain: Safety and quality perspectives. *Food Control* 39: 172-184.
10. Bosona T, Gebresembet G (2013) Food traceability as an integral part of logistics management in food and agricultural supply chain. *Food Control* 33: 32-48.
11. Dabbene F, Gay P, Tortia C (2014) Traceability issues in food supply chain management: A review. *Biosystems Engineering* 120: 65-80.
12. Peres B, Barlet N, Loiseau G, Montet D (2007) Review of the current methods of analytical traceability allowing determination of the origin of foodstuffs. *Food Control* 18: 228-235.
13. Luyks DMAM, van Ruth SM (2007) An overview of analytical methods for determining the geographical origin of food products. *Food Chem* 107: 897-911.
14. Zoani C, Zappa G, Zinnai A, Venturi F, Sanmartin C (2013) Analytical methods for olive oil elemental profile. Preliminary results on the influence of carbonic snow addition during the olive processing. Proceedings 10th Euro Fed Lipid Congress and 30th ISF lecture series “Oils, Fats and Lipids: New Strategies for a High Quality Future”, Antalya, Turkey – 27-30 October 2013.
15. Mraicha F, Ksantini M, Zouch O, Ayadi M, Sayadi S, et al. (2010) Effect of olive fruit fly infestation on the quality of olive oil from Chemlali cultivar during ripening. *Food and Chem Toxicol* 48: 3235-3241.
16. Yasar SB, Baran EK, Alkan M (2012) Metal determinations in olive oil. In: *Olive Oil – Constituents, Quality, Health Properties and Bioconversions*. Ed by Boskou Dimitrios 5: 89-108.