

1 **Interregional traceability of Tunisian olive oils to the provenance soil by multielemental**
2 **fingerprinting and chemometrics**

3 Fadwa Damak^{a,c,e*}, Maki Asano^{a,f}, Koji Baba^b, Aomi Suda^b, Daisuke Araoka^c, Ahmed Wali^d,
4 Hiroko Isoda^{e,f}, Mitsutoshi Nakajima^f, Mohamed Ksibi^d, Kenji Tamura^{a,f}

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6 *^aEnvironmental Soil Chemistry Laboratory, University of Tsukuba, 1-1-1 Tennodai, Tsukuba,*
7 *Ibaraki 305-8577, Japan*

8 *^bInstitute of Agro-Environmental Sciences, NARO, 3-1-3 Kannondai Tsukuba, Ibaraki 305-*
9 *0856, Japan*

10 *^cGeological Survey of Japan (GSJ), National Institute of Advanced Industrial Science and*
11 *Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8567, Japan*

12 *^dEnvironmental Engineering and Ecotechnology Laboratory(LGEET), National School of*
13 *Engineers of Sfax (ENIS), University of Sfax, Route de Soukra Km 4 Po. Box 1173, 3038 Sfax,*
14 *Tunisia*

15 *^eTsukuba Life Science Innovation (T-LSI) Program, School of Integrative and Global Majors,*
16 *University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan*

17 *^fFaculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan*

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19 ***Corresponding author:** Fadwa Damak, E-mail: f.damak@yahoo.fr

20 **Running title**

21 Tunisian olive oils traceability by geochemical fingerprints

22 **Abstract**

23 This study aims at proving the usefulness of multielements as provenance markers of olive oils by
24 evaluating their source, their link with the soils and their discriminating power. Eleven elements in
25 twenty one olive oils and their paired soils from four Tunisian producing regions were
26 characterized. The analytical results were then combined with various chemometric methods.
27 Principal component analysis demonstrated the predominant geochemical source of the oils
28 elements based on their associations according to Goldshmidt rule. Even though a clear correlation
29 couldn't be proven, a correspondence was identified between the discriminating elements for both
30 soil and olive oil represented by Fe, Rb, Mg and Pb. Linear discriminant analysis achieved
31 classification and prediction rates of 92.1% and 87.3%, respectively. Our study shows the validity
32 of multielements as good markers of the provenance for olive oils and that elemental
33 fingerprinting approach can be applied to build a Tunisian olive oils database.

34 **Keywords:** Olive oil, Soil, ICP-MS, Geographical traceability, Geochemical fingerprints

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36 **Chemical compounds studied in this article:**

37 Sodium (PubChem CID: 5360545); Magnesium (PubChem CID: 888); Iron (PubChem CID:
38 23925); Zinc (PubChem CID: 32051); Strontium (PubChem CID: 104798); Manganese
39 (PubChem CID: 23930), Barium (PubChem CID: 104810); Lead (PubChem CID: 5352425);
40 Vanadium (PubChem CID: 5460753); Rubidium (PubChem CID: 105153).

41 **1. Introduction**

42 Olive oil production in Tunisia has increased due the development of modern orchards,
43 intensification of the traditional ones and expansion into new crop producing areas, making it
44 the second largest producer after the European Union (IOC, 2017). Besides this, Tunisian
45 olive orchards are characterized by rich genotypic heritage approximating seventy olive tree
46 varieties each having unique attributes (Laroussi-Mezghani et al., 2015). Yet, this key food
47 product suffers from a low international recognition since about 99% of Tunisia's olive oil
48 has traditionally been exported in bulk with relatively cheap price (Kashiwagi, 2012). With a
49 growing international competitiveness, Tunisian authorities have aimed at increasing the
50 bottled olive oil exports by the establishment in 2006 of the Fund for the Promotion of
51 Packaged Olive Oil (FOPROHOC) and developing new products based on the organic
52 production certification in order to enhance the recognition of Tunisian olive oil and to reach
53 new markets such as Japan (Kashiwagi, 2012). Despite these efforts, exports of packaged
54 olive oils are still far from the targets set of 20% (IOC, 2017). To solve this problem and to
55 push Tunisia's olive oil producers to export less oil in bulk and more bottled and branded
56 products, development of differentiated olive oil products such as geographical indications
57 (GI) labels is highly recommended to introduce a product with a higher added value and fit
58 with consumers' preferences in a highly competitive market. Therefore, Tunisia is aiming at
59 the high-end market, seeking to enhance its competitiveness. In 2008, a study conducted by
60 the Agency for the Promotion of Agricultural Investments (APIA) has identified 21 areas
61 eligible for obtaining the registered designation of origin qualification. This selection is based
62 on the differentiation of the areas according to variety, soil, climate and the know-how, but it
63 does not take into account the physico-chemical characteristics of the oils (Laroussi-Mezghani
64 et al., 2015).

65 During the previous decade, intensive research efforts have been carried out to develop
66 methodological approaches to characterize and prove the uniqueness of Tunisian olive oils
67 according to their geographical and/or varietal origin. Most of these efforts with a view to
68 selecting the suitable geographical marker, focused on its molecular and biochemical
69 composition and sensory profile. These two later parameters are highly dependent on the
70 storage conditions of olive oils (Gomez-Alonso, Mancebo-Campos, Desamparados-Salvador
71 & Fregapane, 2007; El Haouhay et al., 2018); hence their use as geographical markers appears
72 unsafe. In addition to that, molecular markers are more appropriate to check for the varietal
73 origin rather than the geographical origin (Adamo et al., 2012). An alternative and reliable
74 marker of the provenance is therefore needed.

75 Numerous studies have shown that element patterns, in combination with multivariate
76 statistics, can be successfully used to identify the geographical origin of foodstuff in general
77 (Ariyama, Nishida, Noda, Kadokura & Yasui, 2006; Greenough, Fryer & Mallory-Greenough,
78 2010) and olive oil in particular in specific geographical contexts (Jimenez, Velarte, Gomez &
79 Castillo, 2004; Benincasa, Lewis, Perri & Sindona, 2007; Camin et al., 2010a; Camin et al.,
80 2010b; Cabrera-Vique, Bouzas & Oliveras-Lopez, 2012; Beltrán, Sanchez-Astudillo, Aparicio
81 & Garcia-Gonzalez, 2015). These studies have laid the foundation for testing the suitability of
82 multielements as potential provenance marker within the Tunisian geographical and
83 geological context.

84 The success of multielements as geographical markers is essentially due to the fact that food
85 elemental profile reflects its complex interaction with the environment including climate, rock
86 mineralogy, elements mobility and bioavailability in soils and physiological aspects typical of
87 the specie from which it is derived (Giaccio & Vicentini, 2008). And even though the
88 composition of olive oil could be affected by cultivation practices, extraction process and/or
89 environmental pollution, it has been suggested that the selection of suitable elements that

90 reflect the geogenic origin rather than anthropogenic origin such as alkaline metals especially
91 Rubidium (Rb) and Cesium (Cs), being easily mobilized in the soil (Kelly, Heaton &
92 Hoogewerff, 2005), or Lithium (Li) and Rb that don't suffer the influence of technological
93 processing (Giaccio & Vicentini, 2008) can solve this issue. It is therefore necessary to check
94 for the possible origin of the elements contained in the Tunisian olive oil.

95 Apart from being proved successful in other countries, elemental fingerprinting can give
96 valuable information regarding the quality of olive oils expressed as the Maximum Residue
97 Limit which is a quality attribute defined by the international olive council fixing the
98 maximum allowable concentration of some toxic heavy metals (IOC, 2016).

99 Even though multielements (major, minor, trace and rare earth elements) were extensively
100 used to relate olive oils to their provenance, information on how the natural factors of the
101 provenance, including the geochemical factors, correlate with the elemental profile of olive
102 oil is very scarce (Beltrán et al. 2015) unlike other tracers, for instance the stable isotope
103 ratios of light elements, whose variability in olive oils according to origin was correlated with
104 climatic and geographical parameters (Chiocchini, Portarena, Ciolfi, Brugnoli & Lauteri,
105 2016).

106 In order to prove the usefulness of the multielement fingerprint as a tool for origin
107 authentication, the relationship with the provenance environment should be proved. Firstly,
108 the source of the elements in olive oil should be evaluated whether conditioned upon
109 geochemical or anthropogenic factors. Secondly, the strength of the relationship between the
110 geochemistry of orchard soil and elemental composition of olive oil needs to be quantified.

111 The present work constitutes a pilot study within a research project aiming at identifying the
112 origin of Tunisian olive oils using chemical markers. The objectives of the present study were
113 to:

- 114 (i) characterize the elemental profile of Tunisian olive oils from four geographical origins,
115 (ii) check the quality of the oils based on the maximum residue limit criteria,
116 (iii) verify the source of the elements in the Tunisian oils based on their associations,
117 (iv) evaluate the ability of multielements analysis in classifying and predicting the
118 geographical origin of Tunisian olive oils,
119 v) study the link between the chemical compositions of olive oils and their provenance soils.

120 To the best of our knowledge, no previous study on Tunisian olive oil have ever used the
121 geochemical fingerprints (i.e. major and trace elements) to trace back its geographical origin.
122 The only available multi-element data concerned olive oils obtained from trees irrigated with
123 treated wastewater in one limited geographical area (Benincasa et al., 2012).

124 **2. Materials and methods**

125 *2.1. Sampling areas*

126 The olive oils used in this study originated from four producing regions namely Sfax (2 sites)
127 and Monastir (5 sites) in the center-east and Gafsa (5 sites) and Medenine (2 sites) in the
128 south of Tunisia. These regions are considered important olive oil producing areas whose
129 considerable amount of their production is destined for exportation. The climate in these
130 regions varies from arid to semi-arid Mediterranean. During the time period comprised
131 between 1991 and 2015, the average annual temperature and rainfall for the four regions was
132 as follows: Gafsa: 20.4°C, 194.3 mm/year; Medenine: 21.2°C, 215.8 mm/year; Sfax: 20°C,
133 260 mm/year and Monastir: 20.7°C, 346.5 mm/year (World Bank Group, 2018).

134 From a geological point of view, the outcrops in the sampling areas are indicated in Fig. 1.
135 Gafsa samples were located on recent and actual (Holocene) sandy alluvial sediments and
136 sand dune sediments. For Sfax samples, the first site was located on old alluviums that are

137 mostly aeolian sand-loam with limestone concretions and gypsum crusts (Middle-Late
138 Pleistocene); the other sampling site was located on Mio-Pliocene continental sediments made
139 of conglomerates and sandy clays. The sampling sites of Monastir samples were located on
140 the same old alluviums and Mio-Pliocene sediments of Sfax sites. Medenine samples
141 originated from a site lying above the same old alluviums and Villafranchian - lower
142 Pleistocene conglomerates and carbonate crusts (Cohen, Schamel & Boyd-Kaygi, 1980;
143 Sghari, 2014).

144 2.2. Olive and soil sampling

145 Only varietal olive fruits were hand-picked from three representative trees for each sample.
146 These were autochthonous Tunisian varieties like Chemleli Sfax, Chemleli Sahli and
147 Chemchali of Gafsa and introduced foreign varieties like Spanish Arbosana, Italian
148 Frangivento and Greek Koroneiki (Table S1). The introduced varieties were mostly from
149 Gafsa where they are extensively cultivated in new intensive systems compared to other
150 regions. The sampling followed other criteria so as to sample paired olive fruit and soil
151 samples from the same orchard to allow evaluating the relationship between provenance soil
152 and olive oil compositions. Top and subsoil samples were collected from two layers at the
153 depths of 0-30 and 30-60 cm using hand auger. Soil samples were taken from under each
154 sampled tree and then mixed by weight to form a representative sample of each sampling site
155 at each depth. Fig. 1 shows the locations of the total fourteen sampling sites recorded by a
156 handheld GPS receiver (eTrex Legend HCx, GARMIN, UK). All samples were collected in
157 polyethylene plastic bags and rapidly transported to the laboratory for preparation to the
158 chemical analyses. Sampling was conducted from November to December 2015.

159 2.3. Samples preparation and analyses

160 2.3.1. Olive oil samples

161 2.3.1.1. Oil extraction

162 Olive oil was extracted in Sfax Olive Institute from 1.5 kg of whole washed olive fruits by
163 mechanical means alone. A laboratory scale 2 phases oil mill was used following the standard
164 methods employed in industrial olive oil mills. This method consists of crushing, malaxation
165 for 30 minutes at ambient temperature, centrifugation without addition of water and finally
166 natural decantation. This process yields an oily phase and a very wet pomace phase. Olive oil
167 samples were stored in amber glass bottles at 4 degrees Celsius until major and trace elements
168 determinations at the Advanced Analysis Center, National Agriculture and Food Research
169 Organization (NARO) in Japan.

170 *2.3.1.2.Digestion*

171 Mineralization of olive oil samples was carried out according to the method of Llorent-
172 Martinez, Fernandez-de Cordova, Ortega-Barrales and Ruiz-Medina (2014) with minor
173 modifications. The method consists of weighing 0.5 g of sample vigorously shaken directly
174 into the digestion vessel and adding 7 ml of 61% electronic-grade (EL) nitric acid HNO₃
175 (Cica-Merck Kanto Chemicals, Japan) and 1 ml of 30% atomic absorption spectrometry-
176 grade hydrogen peroxide H₂O₂ (Wako Pure Chemical Industries, Japan) for ultratrace analysis.
177 The vessels were placed in a microwave digestion system (ETHOS 1600 Advanced
178 Microwave Labstation, Milestone Inc., Italy). The program of the microwave consisted of a
179 ramp of 15 minutes to reach 200°C and 1000 W that were maintained for additional 15
180 minutes. After cooling down to room temperature, samples were transferred into DigiTUBES
181 that have an ultra-low leachable metal content, of class A tolerance at the 25 ml graduation
182 (DigiTUBES, SCP Science, Canada) and diluted to volume with ultrapure water (Milli-Q
183 Integral 3, Nihon Millipore, Japan). Samples were filtered using a 0.20 µm pore size syringe
184 filter (Captiva econofilter, Agilent Technology, USA). Vessels were cleaned using the same
185 microwave operating program after each digestion batch and rinsed with Milli-Q water. The
186 use of glassware was avoided to prevent metal release.

187 *2.3.1.3. Quality control*

188 Confidence in measurement results is important for food analysis especially when it comes to
189 a geographic marker that will be used to check for authenticity. Evaluating reliability of trace
190 elements measurement results in olive oil is hindered by the absence of a matrix matching
191 certified reference material. The accuracy of the method was therefore evaluated using a
192 multielement oil standard S23-100Y of 100 ppm concentration (SPEXCertiPrep, USA). The
193 limit of detection (LOD) and limit of quantification (LOQ) of each element were calculated as
194 three and ten times the standard deviation of the concentrations determined in 9 independent
195 method blank samples, respectively. The method blank sample was prepared using Conostan
196 75 cSt blank oil (SCP Science, Canada) to substitute olive oil sample in the extraction step.
197 Each olive oil sample was digested and measured three times to ensure precision. Each
198 digestion batch contained one method blank to monitor contamination. Influence of
199 instrumental drift was corrected by using 10 µg L⁻¹ internal standard solution of Sc, Y, In, Tb,
200 Ho and Bi prepared from 10 mg L⁻¹ CLISS-1 standard (SPEX CertiPrep, USA).

201 *2.3.1.4. Calibration*

202 External calibration curves were built on eight different mass concentrations. Standard
203 solutions were prepared by diluting in 5.0% w/w nitric acid matrix a multielement solution
204 (XSTC-622B) containing Na, Mg, V, Fe, Mn, Zn, As, Rb, Sr, Ba, Pb of 10 mg L⁻¹
205 concentration (SPEXCertiPrep, USA). The concentration range for all the elements was 0-50
206 µg L⁻¹.

207 *2.3.1.5. ICP-MS measurements*

208 The quantification of the elements was carried out with an ICP-MS instrument (Elan DRC-e,
209 Perkin-Elmer SCIEX, Canada). Oil samples were introduced by means of a borosilicate glass

210 nebulizer. The ICP torch was a standard torch (Fassel type torch) with ceramic injector. ICP-
211 MS is known to suffer from unwanted polyatomic isobaric interferences. Therefore, the
212 elements had been monitored in standard and CH₄ dynamic reaction cell (DRC) modes to
213 check for and reduce polyatomic interferences and the appropriate isotopes were used.
214 Instrument performance was checked by a midrange continuing calibration verification (5 µg
215 L⁻¹) every ten samples.

216 The operating conditions and parameters of ICP-MS were as follows: Rf power, 1100 W;
217 plasma Ar flow rate, 15 L min⁻¹; auxiliary Ar flow rate, 1.3 L min⁻¹; nebulizer (carrier gas)
218 flow rate, 0.77 L min⁻¹; sampler and skimmer cones, nickel; lens voltage, 7.5 V; analog stage
219 voltage, -1700 V; pulse stage voltage, 950 V; discriminator threshold, 70 V; quadrupole rod
220 offset, -1.5 V; detector, dual; speed of peristaltic pump, 20 rpm; sweeps/reading, 20;
221 replicates, 3; dwell time, 50 ms; scan mode, peak hopping; in DRC mode CH₄ reaction gas
222 flow, 0.6 L min⁻¹; rejection parameter *a*, 0 and Rejection parameter *q*, 0.65. Monitored
223 isotopes: ²³Na, ²⁴Mg, ⁵¹V, ⁵⁶Fe, ⁵⁵Mn, ⁶⁶Zn, ⁷⁵As, ⁸⁵Rb, ⁸⁸Sr, ¹³⁸Ba, ²⁰⁸Pb.

224 2.3.2. *Soil samples*

225 Soil samples were dried in a thermostatic oven at 40°C for 24 hours to remove moisture as a
226 pre-treatment step prior to subsequent analyses. Major and trace elements in soil samples
227 were measured at Geological Survey of Japan (GSJ) of National Institute of Advanced
228 Industrial Science and Technology (AIST) in Japan following a method developed by the GSJ.
229 It consists of preparing high dilution-ratio fused glass beads for X-Ray Fluorescence (XRF)
230 determination of major element oxides (Ejima, Kon, Kawano & Araoka, 2018) and using the
231 same beads for trace elements measurement by femtosecond ultraviolet Laser Ablation ICP-
232 MS (LA-ICP-MS) (Kon & Hirata, 2015).

233 2.3.2.1. *XRF analysis*

234 The glass beads were prepared by mixing 0.5 g of powdered soil sample with 5.0 g of lithium
235 tetraborate flux. The mixture was heated to 1200°C during 10 minutes in a 95% Pt- 5% Au
236 crucible using a semi-automatic fusion device (HAG-M-HF, Herzog, Germany). Four major
237 elements in soil samples (Fe, Mn, Mg and Na) were quantified by XRF instrument (ZSX
238 Primus III+, Rigaku Corp., Japan) with an Rh tube. The calibration lines of each element were
239 prepared by using 14 GSJ geochemical reference samples (JA-1, JA-2, JA-3, JA-1a, JB-3, JB-
240 1b, JG-2, JG-3, JG-1a, JGb-1, JGb-2, JR-1, JR-2 and JR-3) with their chemical compositions
241 varying from mafic to felsic (Imai et al., 1995). The accuracy was verified each day with two
242 reference samples (JB-1b and JG-3). The analytical uncertainties of each element were better
243 than 1.5% as estimated from the long-term reproducibility of measurements of JB-1b.

244 2.3.2.2. LA-ICP-MS analysis

245 To obtain reliable abundance data for seven trace elements in the soil samples, we used a
246 quadrupole ICP-MS system (Agilent 7500cx, Agilent Tehnologies Japan Ltd., Japan) coupled
247 with a 260 nm (UV) Titanium-Sapphire femtosecond laser-ablation system (IFRIT, Cyber
248 Laser Inc., Japan). The Laser ablation was operated using a crater size of 20µm, a pulse
249 energy of 10 J cm⁻², an emission repetition rate of 1000 Hz and a pulse duration of 150 s. A
250 galvanometric optical scanner was employed to minimize elemental fractionation and for
251 effective ablation of the glass beads with a rastering speed of 10,000 µm s⁻¹ in 400 µm x 400
252 µm rastered area. The plasma parameters of the ICP-MS instrument were optimized to
253 minimize the production of ²³²Th¹⁶O⁺ (²³²Th¹⁶O/²³²Th) and the doubly charged ion
254 interferences (¹⁰³Rh/²⁰⁶Pb) and to maximize the intensity of ⁷Li, ²⁹Si, ⁸⁹Y, ¹³⁹La, ²⁰⁸Pb by
255 using the NIST SRM 610 glass standard. To quantify the concentration of each element, GSJ
256 geochemical reference samples (JP-1, JB-1b, JB-2, JB-3, JA-1, JA-2, JA-3, JR-1, JR-2, JR-3,
257 JGb-1, JGb-2, JG-1a, JG-2, JG-3) were used to build the calibration curves. Calibration lines
258 were established by plotting Li normalized signal intensities against the reference values of

259 the standards. The operating conditions and parameters of ICP-MS were as follows: Rf power,
260 1600 W; cool gas flow rate, 15 L min⁻¹; auxiliary gas flow rate, 1 L min⁻¹; carrier gas flow
261 rate, 0.8 L min⁻¹; scanning mode, peak jump; analysis mode, time-resolved analysis;
262 integration time, 240 s/ sample; dwell time, 10 ms for Li, 20 ms for V, Cr, Rb, Sr, Ba and Pb
263 and 40 ms for other elements; sweep time, 1,6 s; detector mode, analog for Li and P/A mode
264 for other elements. Isotopes measured: ⁶Li, ⁵¹V, ⁶⁶Zn, ⁷⁵As, ⁸⁵Rb, ⁸⁸Sr, ¹³⁷Ba, ²⁰⁸Pb. The
265 repeatabilities were better than 5% for V, Rb, Sr, Pb and Ba; and 9% for Zn and As (Kon &
266 Hirata, 2015).

267 *2.4. Data analysis*

268 The approach used in this study to reveal related features and samples distribution in the olive
269 oil and soil datasets according to geographical provenance included uni- and multivariate
270 statistical algorithms. The analysis of variance (ANOVA) is a parametric test used to
271 determine the statistical effect of geographical origin on the multi-elemental profile of the
272 different groups based on the variability of their means. After checking for normality of
273 distribution and homogeneity of variance assumptions in the oil dataset, the non-parametric
274 Kruskal-Wallis test was applied to detect location shift in the distribution. The unsupervised
275 dimension-reducing Principal Component Analysis (PCA) procedure creates a new feature
276 space defined by uncorrelated principal components that are linear combinations of the
277 original variables, to reduce redundancy. PCA with Varimax rotation was applied to assess
278 the multi-element associations in the oil dataset and reveal the underlying processes
279 responsible for such associations. The Linear discriminant analysis (LDA), a supervised class
280 modeling approach, was applied to evaluate the multielements performance to classify the
281 olive oils and soils according to their origin providing a way for validating the predictive and
282 generalization ability of the model. LDA maximizes the ratio of between class variance to
283 within class variance based on a defined classification. The sensitivity of the established

284 LDA models was evaluated using the leave one out cross-validation (LOOCV) technique
285 considering the relatively small number of cases. Spearman's rank correlation coefficient was
286 calculated to evaluate the relationship between soil and olive oil chemical composition.
287 Kruskal-Wallis, PCA, LDA and Spearman's correlation were performed using IBM SPSS
288 Statistics software for Windows, version 24.0 (IBM Corp., Armonk, N.Y., U.S.A.).

289 **3. Results and discussion**

290 *3.1. Quality control*

291 As it can be seen from table 1, accuracy results were in the range 84–102% for almost all the
292 elements measured in the oil standard except for Mg that gave 66%. All of the reported
293 elements had concentrations 100% greater than LOD except for Zn and Pb whose
294 concentrations were 95% and 32% greater than LOD, respectively. As for LOQ, the results
295 were less satisfactory and only Na, Rb and Sr were 100% measured with quantifiable amounts
296 in olive oil samples. The rest of the elements presented concentrations comprised between 0%
297 (Pb) and 76% (As) higher than the LOQ. The linearity was satisfactory with $R^2 \geq 0.999$ for all
298 the elements. For the statistical treatments, we used the original concentrations of all of the 11
299 elements (Table 1) even those with values below the LOD and/or LOQ.

300 *3.2. Elemental profile of olive oils*

301 Table 2 shows descriptive statistics of detected elements in olive oil samples from the four
302 geographical origins, all cultivars taken together. As expected, Na and Mg were the most
303 abundant elements followed by Fe, Zn, Sr, As, Mn, Ba, Pb, V and Rb. As most of Tunisia's
304 bedrocks are of sedimentary nature, Na, Mg and Fe are found in high concentrations in soils
305 of different types (Table 4). Therefore, finding them as major elements in olive oils as well
306 testifies to their absorption by olive trees from soil in higher proportions compared to
307 micronutrients and trace elements.

308 The elemental profiling of olive oils can give valuable information about their quality
309 expressed as total content of specific elements. A quality criteria known as Maximum Residue
310 Limits (MRL) was established by the International olive council (IOC, 2016) fixing maximum
311 levels of metal ions; such as As and Pb to $100 \mu\text{g kg}^{-1}$ and Fe to $3000 \mu\text{g kg}^{-1}$; known to
312 increase the rate of oil oxidation. All of the olive oil samples had a concentration of Fe, As
313 and Pb far below the maximum limits which shows the good quality of Tunisian olive oils.

314 Since data on Tunisian olive oil are practically inexistent, concentrations found in the present
315 work were also compared to concentrations measured in olive oils from several
316 Mediterranean countries (Table S2). The reviewed literature showed a wide variability in the
317 concentrations of many elements even at the level of the same country. Our results agreed the
318 most with those on Tunisian olive oils from Sfax region (Benincasa et al., 2012), Italian Olive
319 oils from southern and central Italy (Benincasa et al., 2007) and Croatian olive oils (Zeiner,
320 Steffan & Juranovic-Cindric, 2005; Juranovic-Cindric, Zeiner & Steffan, 2008; Zeiner et al.,
321 2010) but differed significantly from other results for the majority of elements.

322 Concentrations of Na found in this study were in the range $1.6\text{-}4.3 \text{ mg kg}^{-1}$ similar to Sfax
323 olive oils (4.6 mg kg^{-1}) reported by Benincasa et al. (2012) and to Croatian olive oils (3.4 mg
324 kg^{-1}) reported by Zeiner et al. (2010) but 50 times lower than Portuguese oils (Gouvinhas et
325 al., 2016) and 1000 times higher than Spanish oils ($3.8\text{-}7.8 \text{ mg kg}^{-1}$) (Beltran et al., 2015). The
326 data of Mn in the present study ranged between $12\text{-}18 \mu\text{g kg}^{-1}$ and was 10 times lower than in
327 Sfax oils (Benincasa et al., 2012), within the range of Italian olive oils from five different
328 geographic regions of central and southern Italy (Rossano, Andria, Lamezia, Spoleto and
329 Pescara) (n.d.- $25 \mu\text{g kg}^{-1}$) (Benincasa et al., 2007) and about 100 times higher than Italian
330 olive oils from Sicily and Tuscany ($< 0.20 \mu\text{g kg}^{-1}$) (Camin et al., 2010a). Mn concentrations
331 also varied from 100 times higher to 1.8 times lower than Mn quantified in Spanish olive oils.

332 If this variability had been solely due to the geochemistry of the provenance soil, then this

333 evidence would further prove the usefulness of elemental fingerprinting. However, we
334 presume that such variability could have been partially affected by the use of different
335 analytical methods for elements quantification in olive oils. As previously proved in a
336 comparative study, significant differences were found between element concentrations after
337 different pre-treatment procedures (Bakircioglu et al., 2013) and this variability was suggested
338 in another study (Kara, Fisher & Hill, 2015). This situation highlights the need for a standard
339 official method of multielements quantification in olive oils for the purpose of geographical
340 traceability to make rigorous comparisons possible.

341 The nonparametric Kruskal-Wallis test was applied to evaluate the difference between the four
342 origins since data failed the normality of distribution and homogeneity of variance
343 assumptions assessed through Shapiro-Wilk and Levene's tests respectively. Mn and Zn were
344 significantly different among three pairs of origin ($p < 0.01$) (Table 2). Nine out of eleven
345 elements showed significant difference between the mean ranks of at least one pair of origin
346 ($p < 0.05$) demonstrating that oils from different regions have a characteristic elemental
347 profile. There was no evidence of difference in the concentrations of V and Fe ($p > 0.05$)
348 between any pair of the four origins. Among all origins, the highest concentrations were
349 found in oils from Gafsa and Medenine except for Rb, Ba and Pb that had higher
350 concentrations in Monastir and Sfax oils. As pointed out in a previous study on wine
351 traceability, the soil and dust are probable source of **Ba and Sr in food, so variations of Ba, Rb**
352 **and Pb** found in olive oils from different origins; in this case Monastir and Sfax versus the rest
353 of the origins; can be explained by differences in regional soil geochemistry (Fiket, Mikac &
354 Kniewald, 2011).

355 *3.3. Multivariate analyses*

356 *3.3.1. PCA*

357 Univariate methods alone are insufficient to get a global view with regards to elements
358 discriminating potential taking into consideration the complex interactions between the
359 different elements. Multivariate approaches such as PCA can help understanding the
360 relationship between the predictors. PCA can be useful in depicting elements associations and
361 evaluating whether such associations are conditioned upon natural or anthropogenic processes.
362 In our study, PCA was applied to the correlation Matrix with Varimax rotation and four
363 components were extracted explaining 75% of the original variance in the dataset.
364 Examination of the component loadings (Table 3) shows that Rb, Mg, Sr and Ba loaded high
365 in the first component that explained 27% of the overall variance. Bearing in mind that strong
366 associations of the elements in olive oil reflect similar behaviour or bioavailability from the
367 soil, the first component represents elements grouped according to Goldshmidt's geochemical
368 classification (Greenough et al., 2010). Rb, Sr, Mg and Ba are classified as lithophile elements
369 and occupy the first two groups of the periodic table which displays elements with similar
370 chemical characteristics such as solubility. Taking into account Kruskal-Wallis test results, it
371 is interesting to note that the elements of the first PC were found in higher concentrations in
372 Monastir and Sfax olive oils except for Mg and Sr. Thus it appears that this component groups
373 the elements that also show significant variation between Monastir and Sfax and the rest of
374 the origins. This occurrence can be related to the composition of the soils on which the olive
375 trees were grown. As shown on the geological map, samples originating from Monastir and
376 Sfax were located on the same mixture of conglomerates sand and clay. Shales are known to
377 accumulate rare elements such as Rb and Ba (Krauskopf, 1995). The second component was
378 dominated by Mn, Fe, Zn and Na. Jimenez et al. (2004) mentioned that the relationship
379 between Mn and Alkali elements and soil has been proven and used in geographical
380 traceability issues. On the other hand, Mn and Fe have very similar geochemical behaviour
381 and are common in Fe-Mg silicates and carbonates given the similar ionic radius of Mn, Fe,

382 Mg and Ca that favours substitutions in minerals (Pohl, 2011). Similarly to what was
383 previously mentioned for the first PC, the elements grouped in the second PC were more
384 concentrated in Gafsa olive oils, derived from olives located on sandy soils rich in silicates,
385 and showed a significant difference between Gafsa and the rest of the origins except for Fe.
386 The elements distribution in the third and fourth component is less clear and didn't allow us to
387 determine the predominant cause of the associations. The elements grouping in PCA suggests
388 that olive oils elemental profile is substantially shaped by geochemical processes. This agrees
389 with findings of Ariyama et al. (2006) on element contents in Japanese onions and Greenough
390 et al. (2010) on Canadian Maple syrups and wines where a tendency for elements of the same
391 group in the periodic table or from similar geochemical groups to form clusters was related to
392 similar behaviour of elements in the foodstuff in question. This information supports the
393 utility of using multielements for olive oil traceability.

394 3.3.2. Classification and prediction

395 PCA provides evidence that could only indicate the elements behaviour. In our case, PCA is
396 inappropriate to check the data points pattern based on a bidimensional plot since the variance
397 explained by the first two components was deemed too small to successfully substitute the
398 original dimensions without loss of information. In order to gain information on the
399 classification potential of multielements, LDA was applied to 63 cases (21 samples three
400 times replicated to allow the groups of small size to have a reasonable ratio of cases to
401 variables) organized in four groups corresponding to the four origins and 11 independent
402 variables. The LDA calculated three canonical linear discriminant functions where the first
403 two accounted for 98% of the total variance. A total of 92.1% of the original samples were
404 correctly classified to their origin. The bidimensional plot (Fig. 2a) shows that olive oils from
405 Gafsa, Medenine and Monastir can be differentiated on the basis of the first function. Sfax
406 and Monastir samples cannot be clearly separated on the basis of the first function but are

407 well differentiated along the second function. The analysis of the standardized coefficients
408 indicated that Ba (-1.1), Rb (0.70), Mg (0.53) and Zn (0.45) had the largest absolute values,
409 i.e. the greatest discriminating ability, in the first function while Fe (0.84), Pb (0.74), As
410 (0.52) and V (0.47) showed the largest absolute values in the second function. Indeed, the first
411 set of variables (Ba, Mg and Zn especially) had significant differences between Gafsa and the
412 rest of the oil samples while Pb, featured in the second function, had a significant difference
413 only between Sfax and Monastir olive oils. To verify the predictive and generalization power
414 of the model, LOOCV was applied and 87.3% of the samples were correctly predicted. The
415 lower prediction rate was probably due to the small number of samples used to train the
416 model but the sensitivities achieved are still considered satisfactory. The fact that olive oils
417 from the same origin plotted together; which indicates that they are chemically similar;
418 demonstrates the predominant influence of provenance on olive oils composition. This
419 composition can considerably be attributed to the geochemistry of soils as we showed in the
420 PCA analysis. The homogeneity of soils in Gafsa, mostly sandy, could have played a positive
421 role in achieving high classification rate despite that various cultivars came from that region
422 which again proves the importance of soil composition. However, olive oils from Sfax,
423 Monastir and Medenine were mostly from the same cultivar within the same origin which
424 could also contribute to the classification success achieved if the elements profile would
425 depend on the cultivar as well. At this point it is important to evoke that Beltran et al. (2015)
426 reported that classification of south-western Spanish olive oils to their municipality of origin
427 was not influenced by the cultivars. In another study on olive oils from central and southern
428 Italy, Benincasa et al. (2007) showed that the difference induced by the origin was more
429 significant than by cultivar.

430 3.3.3. Influence of cultivar

431 In order to test this hypothesis, we applied LDA using this time 9 groups corresponding to
432 each single cultivar and origin. This hypothesis can be verified mainly based on Koroneiki
433 olive oils coming from Gafsa and Monastir since other cultivars were sampled from these
434 origins. The LDA calculated 8 canonical linear discriminant functions where the first two
435 accounted for 77% of the total variance. A total of 97% of the original samples were correctly
436 classified to their origin. **The analysis of the standardized coefficients indicated that Na (-1.4),**
437 **Sr (-1.33), Ba (-1.05), Mn (-1.04) and Fe (0.98) had the largest absolute values in the first**
438 **function while Zn (0.87) and Mg (0.83) had the largest absolute values in the second function.**
439 The bidimensional plot (Fig. 2b) shows that olive oils from the same origin tend to plot next
440 to each other even if the cultivar difference was taken into account. This explains the short
441 distance on the plot between Koroneiki from Gafsa (100% correct classification) and other
442 cultivars from Gafsa compared to that between Koroneiki from Gafsa and Koroneiki from
443 Monastir (100% correct classification). This observation is further confirmed by LOOCV
444 results were 100% of Koroneiki from Monastir olive oils samples were ascribed to their
445 correct origin and Koroneiki from Gafsa samples were attributed to olive oils from Gafsa with
446 some misclassifications though (56% Koroneiki Gafsa, 22% Chemlali Gafsa, 11%
447 Frangivento Gafsa and 11% Zalmati Medenine) but 89% of the samples still can be classified
448 as coming from Gafsa. These results agree with the findings of Benincasa et al. (2007) and
449 Beltran et al. (2015). In future work, sufficient data on coexisting cultivars in different regions
450 need to be collected that one may further confirm that trend.

451 *3.4. The link between soil and oil*

452 The key point of using multielements in geographical traceability matter relies on the
453 hypothesis that soil chemistry is the main factor that influences that of olive oil. Accordingly,
454 it is relevant to check the strength of link between olive oil and soil elemental composition.
455 Table 4 shows the concentration of elements in soil samples of the four origins and at the two

456 depths. Welch's test, an equivalent to ANOVA used when group variances are not equal, was
457 employed to test the differences between the two depths and the four origins. The element
458 concentrations didn't show any significant difference between the two depths but all elements
459 had a significant difference between at least two origins. Spearman's rank correlation
460 coefficients were calculated for elements in olive oils and soils taken from the two layers. In
461 the group of the 11 determined elements, only Zn exhibited a statistically significant
462 correlation between its content in olive oil and in soil no matter what the layer depth is but the
463 correlation sign was negative: (c.c. = -0.62, $p = 0.019$ for 0-30 cm) and (c.c. = -0.74, $p =$
464 0.0030 for 30-60 cm). As for the 0-30 cm layer, Pb (c.c. = -0.58, $p = 0.031$) showed
465 statistically significant correlations between its content in olive oil and soil. The comparison
466 of soil and oil concentrations shows that Fe, Mn, Zn and Na and Pb had the highest
467 concentrations in soils of Monastir and lower concentrations in soils of Gafsa. Contradictorily,
468 these elements had higher concentrations in Gafsa olive oils compared to Moanstir. Thus, the
469 significant negative correlation coefficient obtained for Zn and Pb probably means that their
470 content in olive oils depends not only on the element contents in soils but also on other
471 determinants with the closest related factor being climate (Greenough et al., 2010). In fact,
472 Gafsa is located in a middle arid bioclimatic zone characterized by higher temperatures and
473 evapotranspiration rates favouring higher water and elements uptake as opposed to Monastir
474 situated in a lower semi arid climate marked by lower temperatures and higher precipitations
475 (Kefi, Dat Pham, Kashiwagi & Yoshino, 2016). The other elements didn't show any
476 significant correlation between soil and olive oil which could be explained by many reasons:
477 (i) the use of total elements concentrations rather than bioavailable fraction of elements in soil,
478 (ii) elemental fractionation during olive oil extraction process, (iii) physiological aspects of
479 the cultivars that impose a specific elemental uptake pattern and partitioning within the

480 different olive tree tissues and differences in climate conditions which affect water and
481 elements uptake by olive trees might be plausible explanations.
482 Even though most of the elements didn't show a significant correlation, differences in olive
483 oil composition need to be checked whether they can be explained by differences in soil
484 multielemental composition. When stepwise LDA analysis was applied to the soils elemental
485 composition, soil samples were 100% correctly classified and 97% validated to their origin
486 including the two depths and showing less scattering than olive oil samples (Fig. 2c). **The**
487 **analysis of the standardized coefficients of the first two functions out of three calculated**
488 **functions indicated that Fe (6.4), Rb (-5.06), Mg (-4.37), Sr (2.52) and Pb (1.85) had the**
489 **largest absolute values in the first function while Mn (4.61) had the largest absolute value in**
490 **the second function.** Thus, Fe, Mg, Sr, Rb, Mn and Pb were selected by the stepwise LDA as
491 the most discriminating elements. This shows some agreement with olive oil discriminating
492 elements and revealing Fe, Rb, Mg and Pb as common discriminating elements between olive
493 oils and their provenance soils.

494 **4. Conclusion**

495 **The results obtained in this study provide an evidence to the validity of multielements as good**
496 **markers of the provenance for olive oils. The predominant geochemical origin of the elements**
497 **contained in olive oils attests to their link with the provenance soils. Amongst the analyzed**
498 **elements, only Zn exhibited a significant correlation coefficient indicating that the soil total**
499 **concentration is not the best predictor of the oil elemental composition. Even though a**
500 **quantitative estimation of the strength of the relationship between soil and oil total elemental**
501 **concentrations was not possible for most of the elements, a correspondance between the**
502 **discriminating elements was identified represented by Fe, Rb, Mg and Pb as revealed by LDA.**
503 **This shows that the soil's geochemical signature has been transferred to the oil with some**
504 **alterations though.**

505 Another important finding of this study is the good quality of the analyzed oils based on the
506 MRL quality criterion as Fe, As and Pb had concentrations far below the maximum limit. The
507 LDA proved the good inter-regional discrimination of olive oils to their four origins based on
508 multielements by yielding 92.1% and 87.3% classification and prediction rates, respectively.
509 The classifications of olive oils to their origin is furthermore not hindered by the presence of
510 various cultivars as the origin proved to be more significant than the cultivar in the variation
511 of multielements concentrations in olive oils.
512 This work can serve as a reference for further research where olive oils representative of each
513 Tunisian producing origin are characterized for their elemental composition to build
514 comprehensive datasets of each producing region. This way, multielements can be applicable
515 to help provide a scientific evidence of the uniqueness and protect the identity of oils
516 produced inside Tunisia. In future work, correlation of the oil elemental profile with other soil
517 parameters such as the bioavailable fraction and climatic factors need to be investigated to
518 provide an additional proof of the usefulness of multielments as markers of the natural factors
519 of the provenance.

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656 **Figures captions**

657 **Fig. 1.** Geological map showing the location of sampling points in Gafsa, Medenine, Sfax and
658 Monastir.

659 **Fig. 2.** LDA analysis of the element concentrations: scatterplot of the first two canonical
660 functions. (a) in olive oils using 4 groups corresponding to each origin all cultivars taken
661 together as input, (b) in olive oils using 9 groups corresponding to each single origin and
662 cultivar as input: G., Gafsa; Med., Medenine; Sf., Sfax; Mo., Monastir; Kor., Koroneiki;
663 Chemc., Chemchali; Arbos., Arbosana; **Cheml., Chemlali**; Frang., Frangivento and Zal.,
664 Zalmati, (c) in soils using 4 groups corresponding to each origin as input.

Fig. 1

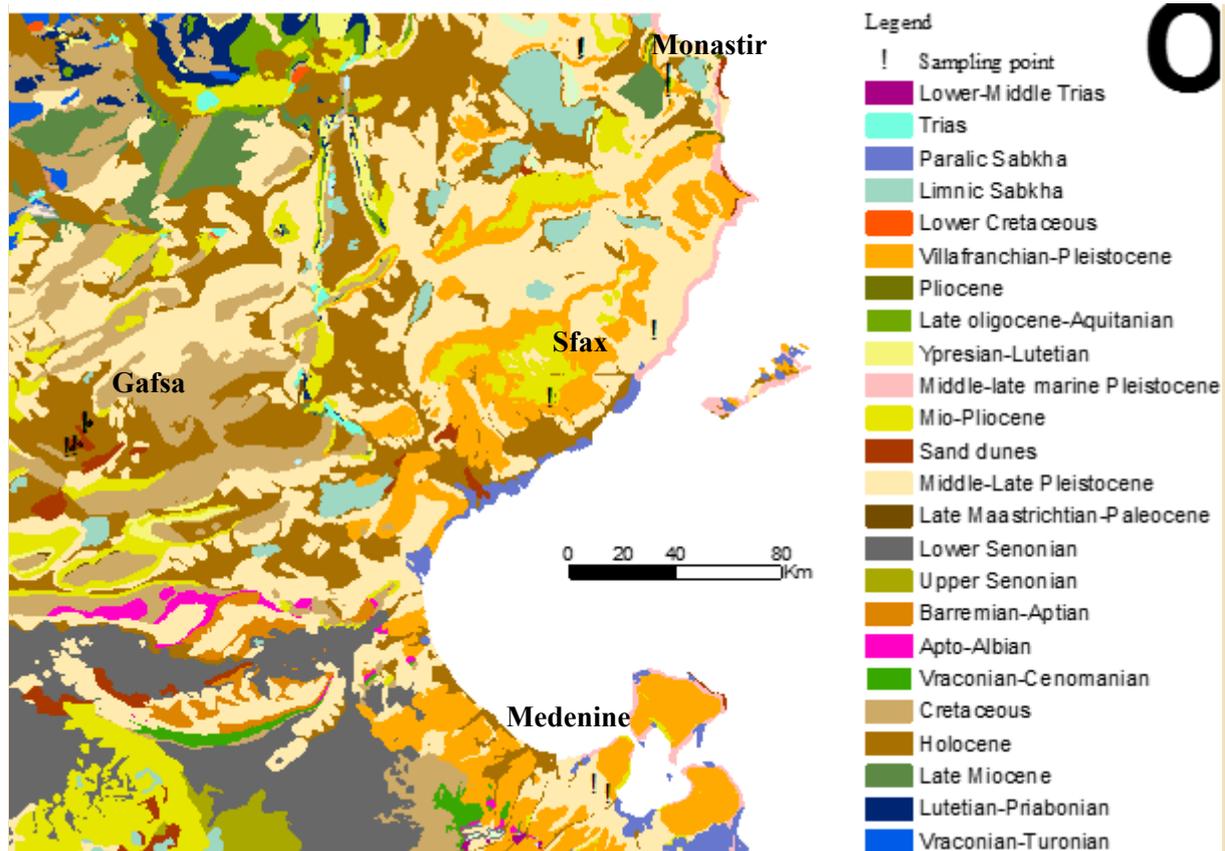
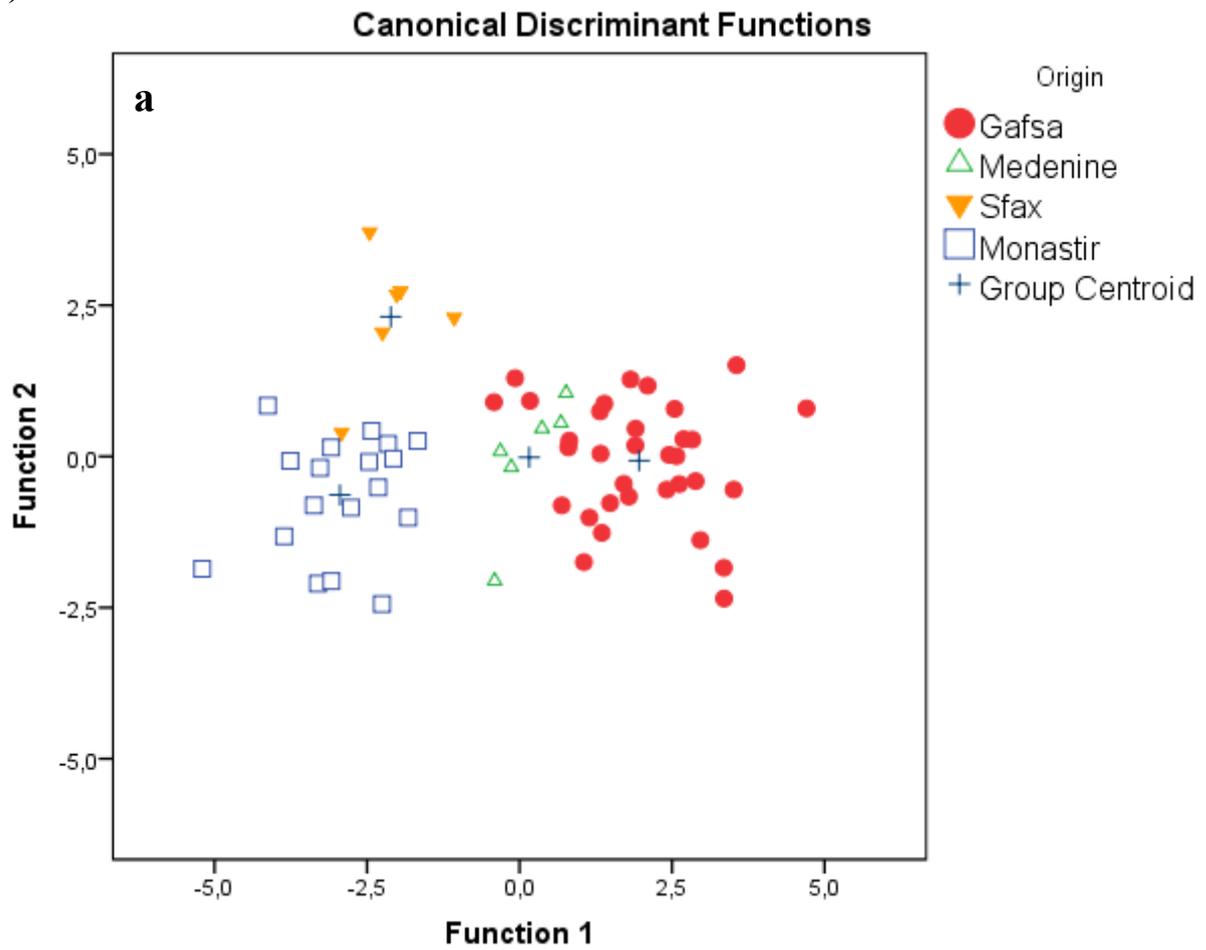


Fig. 2

(a)



(b)

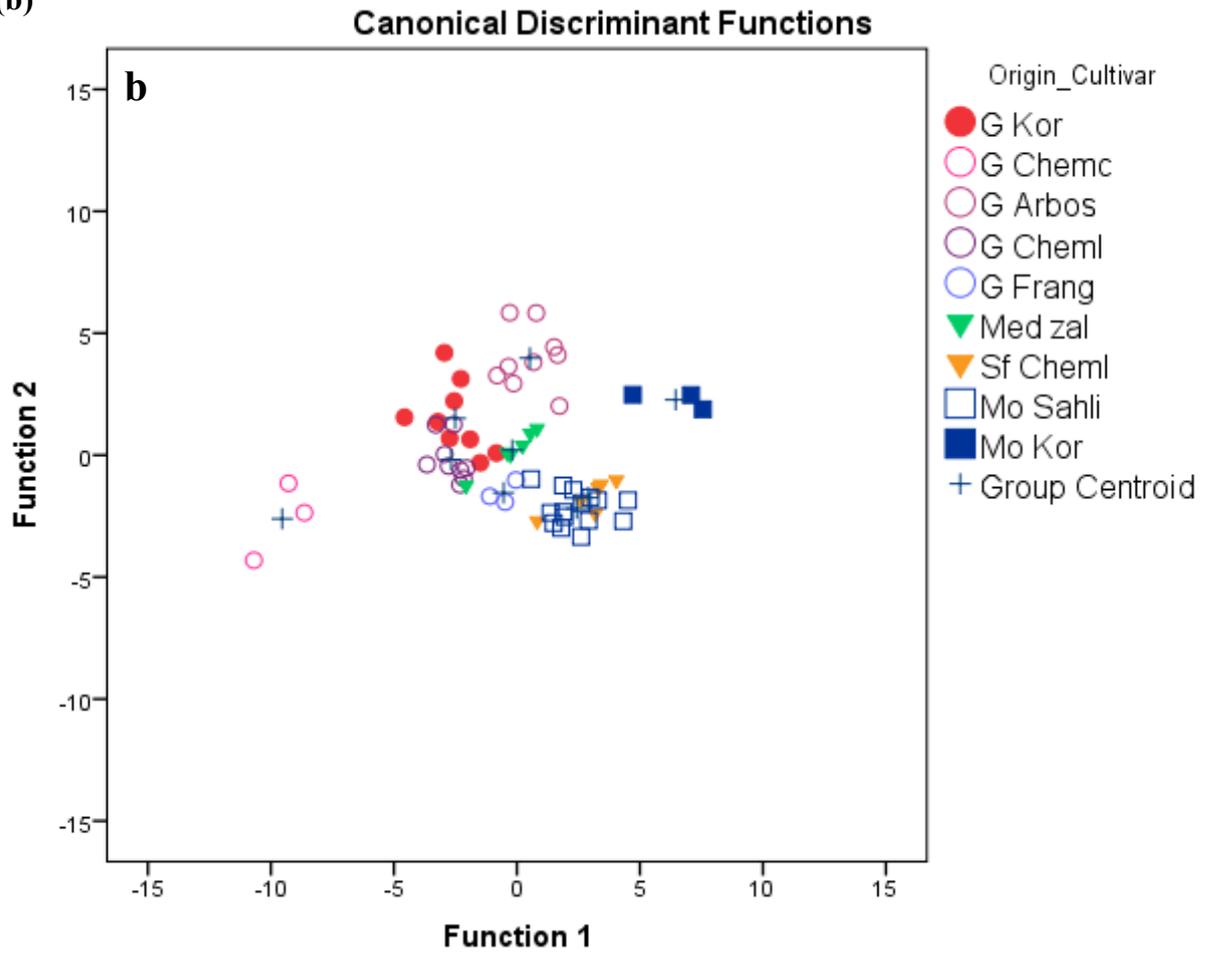


Table 1. Quality control of ICP-MS analysis of olive oil samples (21 samples three times replicated).

| Element | Unit | Isotope | Operation mode | Linearity R ² | Accuracy (%) | LOD | Samples > LOD (%) | LOQ | Samples > LOQ (%) |
|---------|---------------------|---------|----------------|--------------------------|--------------|------|-------------------|------|-------------------|
| Na | mg kg ⁻¹ | 23 | Standard | 0.9998 | 84 | 0.35 | 100 | 0.12 | 100 |
| Mg | mg kg ⁻¹ | 24 | Standard | 0.9991 | 66 | 0.47 | 100 | 0.16 | 6 |
| Fe | mg kg ⁻¹ | 56 | DRC | 0.9999 | 88 | 0.12 | 100 | 0.39 | 52 |
| Zn | mg kg ⁻¹ | 66 | DRC | 0.9997 | 97 | 0.11 | 95 | 0.36 | 19 |
| V | μg kg ⁻¹ | 51 | Standard | 1.0000 | 97 | 1.7 | 100 | 5.6 | 25 |
| Mn | μg kg ⁻¹ | 55 | Standard | 1.0000 | 103 | 6.0 | 100 | 20 | 24 |
| As | μg kg ⁻¹ | 75 | Standard | 0.9990 | - | 0.73 | 100 | 2.4 | 76 |
| Rb | μg kg ⁻¹ | 85 | Standard | 0.9996 | - | 0.30 | 100 | 1.0 | 100 |
| Sr | μg kg ⁻¹ | 88 | Standard | 0.9999 | - | 5.1 | 100 | 17 | 100 |
| Ba | μg kg ⁻¹ | 138 | Standard | 1.0000 | 102 | 4.6 | 100 | 15 | 11 |
| Pb | μg kg ⁻¹ | 208 | Standard | 1.0000 | 97 | 6.9 | 32 | 23 | 0 |

R²: least square regression coefficient; - corresponds to elements not contained in the oil standard.

Table 2. Element contents in Tunisian olive oil samples from the four geographical origins independently of the cultivar.

| Element | Unit | Origin Stat. | Gafsa n=5 | Medenine n=2 | Sfax n=2 | Monastir n=5 | <i>p</i> value |
|---------|---------------------|--------------|-----------|--------------|----------|--------------|----------------|
| Na | mg kg ⁻¹ | Median | 4.3 a | 4.0 a | 1.6 b | 2.3 ab | *** |
| | | SD | 1.2 | 0.62 | 0.33 | 1.8 | |
| Mg | mg kg ⁻¹ | Median | 1.2 a | 1.0 ab | 0.85 b | 0.93 ab | * |
| | | SD | 0.080 | 0.030 | 0.080 | 0.36 | |
| Fe | mg kg ⁻¹ | Median | 0.57 | 0.43 | 0.39 | 0.28 | ns |
| | | SD | 0.49 | 0.18 | 0.15 | 0.17 | |
| Zn | mg kg ⁻¹ | Median | 0.29 a | 0.27 ab | 0.18 bc | 0.17 c | *** |
| | | SD | 0.050 | 0.027 | 0.050 | 0.040 | |
| V | µg kg ⁻¹ | Median | 5.2 | 5.2 | 5.1 | 4.8 | ns |
| | | SD | 0.73 | 0.50 | 1.0 | 0.81 | |
| Mn | µg kg ⁻¹ | Median | 18 a | 13 b | 12 b | 13 b | *** |
| | | SD | 4.3 | 0.74 | 0.020 | 4.0 | |
| As | µg kg ⁻¹ | Median | 25 ab | 29 a | 21 ab | 10 b | *** |
| | | SD | 15 | 22 | 1.9 | 3.3 | |
| Rb | µg kg ⁻¹ | Median | 3.4 ab | 3.5 ab | 2.5 a | 3.5 b | * |
| | | SD | 0.76 | 0.040 | 0 | 3.9 | |
| Sr | µg kg ⁻¹ | Median | 37 a | 35 ab | 33 b | 33 ab | * |
| | | SD | 2.4 | 1.3 | 1.3 | 8 | |
| Ba | µg kg ⁻¹ | Median | 8.7 a | 9.6 ab | 12 b | 11 b | *** |
| | | SD | 0.79 | 0.10 | 0.42 | 6.0 | |
| Pb | µg kg ⁻¹ | Median | 6.0 ab | 6.2 ab | 7.4 a | 5 b | * |
| | | SD | 1.0 | 0.90 | 0.13 | 0.96 | |

SD: standard deviation. Nonparametric Kruskal-Wallis test was applied: ns. not significant at $p > 0.05$; *. $p < 0.05$; **. $p < 0.01$; ***. $p < 0.001$. Dunn's multiple comparison adjusted by Bonferroni correction was used to make pairwise comparisons; different letters a. b. c in the same row indicate significant differences ($p < 0.05$).

Table 3. Loadings of PCA for element concentrations in Tunisian olive oils.

| Component | 1 | 2 | 3 | 4 |
|-----------|--------|--------|--------|--------|
| C.V.% | 27 | 21 | 15 | 11 |
| Rb | 0.899 | 0.059 | -0.041 | -0.212 |
| Mg | 0.789 | 0.378 | 0.144 | -0.116 |
| Sr | 0.782 | 0.196 | 0.041 | -0.089 |
| Ba | 0.716 | -0.150 | -0.298 | 0.244 |
| Mn | 0.371 | 0.835 | 0.073 | 0.001 |
| Fe | 0.074 | 0.806 | -0.326 | -0.342 |
| Zn | -0.183 | 0.629 | 0.162 | 0.280 |
| Na | 0.335 | 0.606 | 0.334 | 0.125 |
| V | 0.195 | -0.009 | 0.841 | -0.144 |
| As | -0.336 | 0.135 | 0.783 | 0.042 |
| Pb | -0.099 | 0.061 | -0.104 | 0.934 |

C.V.; Cumulative variance.

Table 4. Mean \pm standard deviation in ppm of the element contents in soil samples from the four geographical origins at 0-30 and 30-60 cm depths.

| Element | Gafsa | | Medenine | | Sfax | | Monastir | |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|------------------|------------------|
| | 0-30 cm | 30-60 cm | 0-30 cm | 30-60 cm | 0-30 cm | 30-60 cm | 0-30 cm | 30-60 cm |
| Na | 1500 \pm 870 | 880 \pm 220 | 2000 \pm 110 | 1900 \pm 270 | 1100 \pm 280 | 860 \pm 150 | 2000 \pm 860 | 1900 \pm 720 |
| Mg | 3500 \pm 1000 | 3600 \pm 680 | 4600 \pm 17 | 4400 \pm 210 | 2300 \pm 680 | 1700 \pm 260 | 4200 \pm 1400 | 4500 \pm 1400 |
| Fe | 6400 \pm 1700 | 6700 \pm 1300 | 8600 \pm 600 | 8200 \pm 15 | 6000 \pm 1500 | 4100 \pm 300 | 14000 \pm 4400 | 15000 \pm 4700 |
| Zn | 18 \pm 2.8 | 18 \pm 3.0 | 20 \pm 1.0 | 19 \pm 1.6 | 23 \pm 9.9 | 15 \pm 0.48 | 41 \pm 12 | 38 \pm 7.2 |
| V | 29 \pm 7.9 | 25 \pm 3.6 | 34 \pm 4.0 | 32 \pm 7.2 | 23 \pm 6.0 | 17 \pm 1.3 | 45 \pm 11 | 47 \pm 17 |
| Mn | 84 \pm 28 | 84 \pm 18 | 95 \pm 11 | 87 \pm 0 | 79 \pm 13 | 53 \pm 16 | 170 \pm 81 | 170 \pm 76 |
| As | 2.0 \pm 0.53 | 2.2 \pm 0.45 | 3.2 \pm 0.33 | 3.1 \pm 0.13 | 2.3 \pm 0.83 | 1.4 \pm 0.14 | 4.2 \pm 1.6 | 3.9 \pm 1.2 |
| Rb | 23 \pm 2.8 | 22 \pm 3.3 | 34 \pm 2.7 | 33 \pm 0.35 | 21 \pm 5.3 | 15 \pm 1.7 | 40 \pm 13 | 41 \pm 12 |
| Sr | 110 \pm 27 | 130 \pm 30 | 170 \pm 14 | 180 \pm 2.0 | 130 \pm 39 | 100 \pm 12 | 140 \pm 88 | 140 \pm 100 |
| Ba | 190 \pm 9.5 | 190 \pm 16 | 290 \pm 17 | 290 \pm 11 | 210 \pm 46 | 160 \pm 17 | 270 \pm 92 | 270 \pm 84 |
| Pb | n.d. | n.d. | 0.17 \pm 0.25 | 0.18 \pm 0.25 | 0.31 \pm 0.41 | n.d. | 4.4 \pm 3.6 | 4.7 \pm 3.7 |

n.d.; not detected.