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1 **Multi-element (¹³C, ²H and ³⁴S) bulk and compound-specific stable isotope**
2 **analysis for authentication of *Allium* species essential oils.**

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17

18 **Abstract**

19 **Pure and natural essential oils of *Allium* species** are primarily used in the food industry, but
20 due to their low yield, production quantities can be difficult to achieve and thus can be highly
21 expensive. Due to these problems, adulterations are a reality in this field. The purpose of this
22 research is to develop a multi element stable isotope analysis methodology for authentication
23 of the essential oils of leek, onion, shallot, and chive. Naturalness of these essential oils was
24 achieved by assessment of compound specific $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$: an addition of 5% of synthetic
25 compound can be detected. Chemometric models were undertaken to assess purity by
26 distinction of the *Allium* samples according to their species origin using bulk stable and
27 compound specific $\delta^{13}\text{C}$, $\delta^2\text{H}$, $\delta^{34}\text{S}$, and compositions of their major metabolites dipropyl
28 disulfide, dipropyl trisulfide, methyl propyl disulfide, and methyl propyl trisulfide.

29 **Keywords**

30 ***Allium* essential oil; Authentication; Multicollector-inductively coupled plasma mass**
31 **spectrometry; Isotope ratio mass spectrometry**

32

33 **1. Introduction**

34 The *Allium* genus from the Alliaceae family includes many vegetal species principally cultivated
35 as culinary ingredients, due to their strong aromas. *Allium* genus is widely distributed in North
36 Africa, Europe, Asia and America (El-Wakil et al., 2015). Some of these species, such as bulbs
37 of onion, garlic, and leek, are used to obtain essential oils by steam distillation and are
38 incorporated as flavourings in the food industry. The essential oils extracted from these
39 vegetal species have similar medicinal properties: diuretic properties (Satyal et al., 2017),
40 antimicrobial activities (Casella et al., 2013), and antioxidant activities (Ye et al., 2013) which
41 have been reported in previous studies. For instance, chinese chive and onion essentials oils
42 (EOs) have a high antibacterial activity whereas shallot and leek EOs were the strongest
43 antioxidants (Mnayer et al., 2014).

44 The composition of these oil extracts is relatively similar, as they all contain a high level of
45 sulphur compounds. Dipropyl disulfide (DPDS) and dipropyl trisulfide (DPTS) are the main
46 constituents of essential oils of the leek *Allium porrum*, the shallot *Allium ascalonicum*, the
47 onion *Allium cepa*, and the chive *Allium schoenoprasum*. The relative compositions of DPDS
48 are approximately 45% in leek, 30% in onion, 20% in chive and 15% in shallot, and the content
49 of DPTS is close to 15% in leek, onion, and chive, and near 10% in shallot (Mnayer et al., 2014).
50 Other major components of these essential oils are methyl propyl disulfide (MPDS) and methyl
51 propyl trisulfide (MPTS). Essential oils of garlic *Allium savitum*, wild garlic *Allium ursinum* and
52 garlic chive *Allium tuberosum* are principally composed of diallyle disulfides (DADS), diallyle
53 trisulfides (DATS), methyl allyl disulfides (MADS) and methyl allyl trisulfides (MATS) (Dziri et
54 al., 2014).

55 Similarly, with other natural products that present low extraction yields leading to expensive
56 production costs, essential oils from *Allium* species are subject to fraud (e.g., the price of leek
57 essential oil is near 1000 €/kg). To obtain a quantity of 100 g of leek essential oils, it is
58 necessary to treat approximately 1 T of leek raw material. For the same quantity of essential
59 oil, raw materials needed for chive, shallot, onion, and garlic are 2.5 T, 1.5 T, 0.8 T and 0.1 T
60 respectively. It is then necessary to ensure the naturalness of these essential oils by
61 highlighting any adulteration with synthetic compounds. Furthermore, their similar chemical
62 composition makes the mixture detection of these essential oils more difficult.

63 Authentication of naturalness of *Allium* essential oils based on chiral properties cannot be
64 achieved because these molecules of interest do not present any stereoselectivity.
65 Identification of synthetic impurities is also hardly achievable as residual impurities such as
66 halogens precursors can be eliminated with a purification process. Additionally, the
67 complexity of the matrix, with many different metabolites (Mnayer et al., 2014) and the
68 tendency of sulphur-containing molecules to rearrange during gas chromatography (GC)
69 injections, lead to the formation of derived sulphur components (Cantrell et al., 2020). Among
70 the various analytical techniques involved for the assessment of the authenticity of natural
71 products, stable isotope analyses have been widely used to trace or to ensure the origins.

72 Multi-isotopic (^{13}C , ^2H , ^{18}O , ^{15}N , and ^{34}S) approaches sometimes associated with inorganic
73 multi-elemental analyses have been used to track the geographical origin of allium products:
74 Korean onions (Park et al., 2019), Korean garlic (Choi et al., 2020) or Italian red garlic (Pianezze
75 et al., 2019). In these studies, the geographical origins are correlated to specific cultivation
76 practices allowing the production of high quality of these food condiments. Regarding
77 essential oils, bulk stable isotope analysis was recently used to authenticate naturalness of

78 wintergreen essential oils (Cuchet et al., 2019). Bulk stable isotope analyses are usually
79 performed using an elemental analyser linked to an isotope ratio mass spectrometer in
80 continuous flow mode for determination of $\delta^2\text{H}$ via pyrolysis (EA-P-IRMS) and $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$
81 via combustion (EA-C-IRMS). Over a period of a few years, several systems have been
82 developed and manufactured using a combustion approach to simultaneously analyse C, N
83 and S either based on chromatographic separation of the gases with a flash Thermo system
84 (Kracht, 2011) or with a purge-and-trap separation device for the Elementar system (Fourel et
85 al., 2014).

86 However, these methods only produce bulk analyses, and the study of the targeted main
87 secondary metabolites could supply new criteria to improve authentication on more complex
88 essential oils matrices. Gas chromatography – combustion / pyrolysis – isotope ratio mass
89 spectrometry (GC-C/P-IRMS) is an alternative technique allowing to assess compound-specific
90 ^{13}C and ^2H compound-specific isotope ratios. Authentication of naturalness and purity of
91 several essential oils have been achieved on this GC-IRMS system: oregano and thyme (Nhu-
92 Trang et al., 2006a), lemongrass (Nhu-Trang et al., 2006b), or citrus (Schipilliti et al., 2012)
93 essential oils for example.

94 However, due to the high oxidation degree of sulphur, a combustion interface with gas
95 chromatography is difficult to set up for ^{34}S assessment, and therefore, it has not yet been
96 achieved via GC-IRMS. An alternative is to use gas chromatography (GC) connected on line to
97 a multi-collector-inductively coupled plasma mass spectrometry coupling system (GC/MC-ICP-
98 MS) to assess the compound-specific isotopic ratio of this element. This technique has been
99 developed during the past ten years (Amrani et al., 2009; Clough et al., 2006; Hanousek et al.,
100 2016; Said-Ahmad and Amrani, 2013) essentially applied to petrochemical products. Several

101 recent papers also describe the use of GC/MC-ICP-MS for the analysis of organic sulphur
102 compounds (Kümmel et al., 2020; Said-Ahmad et al., 2017) but not for food chemistry yet.

103 In this study, we propose a novel methodology to authenticate the naturalness and the purity
104 of the extracted essential oils of *Allium* species based on $\delta^{13}\text{C}$, $\delta^2\text{H}$, and $\delta^{34}\text{S}$ performed with
105 both bulk stable isotope analysis via EA-C/P-IRMS as well as compound-specific stable isotope
106 analysis via GC-C/P-IRMS and GC/MC-ICP-MS. The aim of this work is to develop a method
107 that supplies naturalness authentication of these essential oils and highlights the isotope
108 differences between the different *Allium* species, using chemometric tools. Simultaneous
109 determination of bulk and compound specific values for $\delta^{13}\text{C}$, $\delta^2\text{H}$ and $\delta^{34}\text{S}$ can lead to the
110 discrimination of the origins of authentic essential oils and synthetic molecules. This method
111 is tested on essential oils of leek, onion, shallot and chive that share similar metabolites.

112 **2. Material and methods**

113 **2.1. *Sample description***

114 **2.1.1. *Essential oils***

115 Six Egyptian leek (*Allium porrum* L.) essential oils (EOs), nine German Shallot (*Allium*
116 *ascalonicum* L.) EOs, and eleven Egyptian onion (*Allium cepa* L.) EOs were provided by Albert
117 Vieille SAS (Vallauris, Alpes Maritime, France). Two leek EOs, two shallot EOs, ten chives
118 (*Allium ascalonicum* L.) EOs, and two onion EOs originating from France were supplied by
119 Ferrand PHE (Rodelinghem, Pas de Calais, France). More information can be found in
120 **Supplementary Table S1.**

121 **2.1.2. *Organosulfides standards***

122 Commercial synthetic molecules of organosulfides (propyl sulfide, dipropyl disulfide, dipropyl
123 trisulfide, methyl propyl disulfide, and methyl propyl trisulfide) were purchased from TCI
124 (Tokyo, Japan) and Sigma Aldrich (Saint Louis, MO, USA). Chemosynthetic origin was
125 confirmed by radiocarbon activity assessment. The undertaken method is fully described in a
126 previous study (Cuchet et al., 2019).

127 **2.2. Stable Isotope Ratio Analysis**

128 Isotopic results are expressed in the delta unit (δ) according to the following formula (Brand
129 et al., 2014):

$$130 \quad \delta^i E = \frac{({}^i R_{Sample} - {}^i R_{Reference})}{{}^i R_{Reference}}$$

131 where i is the mass number of the heavier isotope of element E , R_{Sample} is the respective
132 isotope ratio of a sample, $R_{Reference}$ is the relevant internationally recognized reference
133 material. The delta values are multiplied by 1000 and are expressed in units "per mil" (‰). SI
134 unit for isotopic ratio is the Urey (Ur), but for practical reasons, the unit used here is the per
135 mil: 1 per mil = 1 mUr.

136 High-purity gases of carbon dioxide 99.998% (4.8 Messer), hydrogen 99.999% (5.0 Messer).
137 and N45 high-purity SO_2 (Linde Gas France), previously calibrated versus international
138 references (VPDB, VSMOW, and VCDT), were used as the working monitoring gas with each
139 sample. Helium flow at 99.999% was used as a carrier gas.

140 International reference materials IAEA CH7 (polyethylene foil: $\delta^{13}C_{VPDB}$ (‰) -32.15, δ^2H_{VSMOW}
141 (‰) -100), USGS40 (L-glutamic acid: $\delta^{13}C_{VPDB}$ (‰) -26.39), NBS127 (barium sulfate, $BaSO_4$:
142 $\delta^{34}S_{VCDT} = +20.3\%$), (Halas and Szaran, 2001) and S1 (silver sulfide, Ag_2S : $\delta^{34}S_{VCDT} = -0.3\%$),

143 (Robinson, 1995), supplied by IAEA (International Agency of Atomic Energy, Vienna, Austria)
144 were used to calibrate all of our studied samples, which allowed us to anchor our $\delta^{13}\text{C}$ results
145 to the Vienna Pee Dee Belemnite (VPDB) scale, our $\delta^2\text{H}$ results to the Standard Mean Ocean
146 Water (VSMOW) scale and our $\delta^{34}\text{S}$ results to the Vienna-Canyon Diablo Troilite (V-CDT) scale.
147 Each sample was analysed versus a monitoring gas for which two pulses were admitted with
148 each analyte. All sample values were analysed in duplicate and verified by bracketing the
149 analytical sequence with international reference materials every six analyses. The $\delta^{13}\text{C}$ values
150 are given within a maximum standard deviation of $\pm 0.28\text{‰}$, $\delta^2\text{H}$ within SD = $\pm 5\text{‰}$ and $\delta^{34}\text{S}$
151 within SD = $\pm 0.34\text{‰}$.

152 **2.2.1. EA-C/P-IRMS**

153 The $\delta^2\text{H}$ measurement was conducted using a TC-EA elemental analyser linked to a Delta V
154 Plus via a Conflo IV interface (all Thermo Fisher Scientific, Bremen, Germany). Samples
155 weighed in silver capsules (3.3 X 5 mm, Säntis Analytical AG, Teufen, Switzerland) were
156 dropped into the pyrolysis unit held at 1400°C. This high temperature reactor was constructed
157 of an inner glassy carbon tube half filled with glassy carbon granules (IVA Analysentechnik,
158 Meerbusch, Germany) integrated into an external ceramic tube. Hydrogen is converted into
159 H_2 and separated on a gas chromatography column prior to the introduction in the IRMS via
160 the same Conflo IV interface. The interface allows the introduction of monitoring gas pulses.

161 Carbon and sulphur isotope compositions were measured using a Vario PYRO cube elemental
162 analyser (Elementar GmbH, Langenselbold, Germany) equipped with a “purge and trap” gas
163 separation module in combustion mode. The elemental analyser was interfaced in
164 continuous-flow mode with an Isoprime 100 isotope ratio mass spectrometer (Elementar UK
165 Ltd, Cheadle, UK). The applied technique is fully described in a previous study (Fourel et al.,

166 2015). For each sample, 3 aliquots of 200 mg of essential oils were loaded in tin foil capsules.
167 Data are reported as $\delta^{34}\text{S}$ in ‰ vs. V-CDT.

168 **2.2.2. GC-C/P-IRMS**

169 GC-IRMS analyses were performed using a 6890N GC gas chromatograph system (Agilent)
170 connected to an Isoprime GV isotope ratio mass spectrometer (Elementar UK Ltd, Cheadle,
171 UK) via the GC5MK1 GV combustion or pyrolysis interface. The system can be configured for
172 either ^{13}C or ^2H analyses. All samples were analysed in triplicate and verified by bracketing the
173 analytical sequence with organosulfides standards every six analyses. Organosulfides
174 standards were calibrated to the international references V-PDB and VSMOW using the EA-
175 IRMS technique.

176 An amount of 1 μL of diluted liquid sample (10 μL in 1 mL of ethanol) was injected using a 7683
177 Agilent Technologies auto-sampler into the 6890N gas chromatograph equipped with an
178 Agilent HP-5 column (60 m*0.320 mm*0.25 μm I.D film thickness) used in chromatographic
179 separation. The injection port was held at 250°C, fitted with a split liner containing glass wool,
180 and operated in split mode (10:1). The oven temperature programme started at 50°C during
181 2 min, increased to 120°C at a rate of 10 °C min⁻¹, increased to 210°C at a rate of 5°C min⁻¹,
182 increased to 280°C at a rate of 20 °C min⁻¹, and held for 5 min. Compounds were separated at
183 a flow rate of 1 mL.min⁻¹.

184 A heart split valve connected to the outlet of the GC column enabled the components to be
185 obtained either in the combustion/pyrolysis tube or in the FID detector. This process allows
186 elimination of the solvent peak as well as unwanted compounds.

187 The combustion of carbon in carbon dioxide was undertaken using a tube containing copper
188 oxide maintained at 850°C in a furnace. Silver wool was placed in the second portion of the
189 tube to trap halogens and sulphur. A Nafion water trap, placed between the combustion
190 furnace and the IRMS, was used to remove water produced during the combustion.

191 Pyrolysis of the sample for ²H measurement as H₂ was conducted at higher temperature
192 (1050°C) using a tube containing chromium powder. Chromium was used as a pyrolysis
193 catalyser to convert organic components into H₂. Furthermore, chromium is not affected by
194 any carryover effects and directly traps other elements such as halogens and sulphur (Kelly et
195 al., 2001).

196 The targeted molecules present a high percentage of sulphur, which can generate disruptions
197 to metabolite combustion and/or pyrolysis within the interface. To prevent such
198 perturbations, it is necessary to retain the sulphur by addition of silver wool in the combustion
199 tubes. The pyrolysis temperature is too high for silver wool use, but fortunately, the chromium
200 pyrolysis catalyser has a sulphur-trapping property.

201 **2.2.3. GC-MC-ICP-MS**

202 A Trace 1300 gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) was coupled
203 to a Neptune Plus MC-ICP-MS (Thermo Fisher Scientific) using a GCI 300 interface (Thermo
204 Fisher Scientific). GC setting parameters were as follows: 1 µL of each diluted sample (20 µL in
205 0.5 mL of ethanol) or standard (10 µL in 1 mL of ethanol) was injected in split mode (120:1)
206 with the injector temperature maintained at 300 °C. The helium carrier gas was regulated at
207 a constant flow rate of 2 mL/min. Separation of volatile compounds was realised on a
208 TraceGOLD TG-5MS capillary column (30 m × 0.25 mm × 0.25 µm; Thermo Fisher Scientific).

209 Initial oven temperature was held at 150 °C for 4 min, then ramped at 20 °C/min to 300 °C and
210 held for 2 min. The transfer line temperature was maintained at 280 °C.

211 The Neptune Plus MC-ICP-MS was used in high-resolution mode to avoid any interference for
212 ^{32}S (O_2) and ^{34}S ($^{33}\text{S}^1\text{H}$ and O_2). Due to the low abundance of ^{33}S (0.76%), ratio precision with
213 ^{33}S was poor and finally not recorded. After acquisition (parameters are described in
214 **Supplementary Table S2**), data were analysed using IsoDat Software Version 3.0.94.12
215 (Thermo Fisher Scientific) for background subtraction, peak integration, and ratio calculation.
216 MC-ICP-MS optimisation was performed prior to connection with the GCI 300 interface using
217 an Aridus II desolvating system (Teledyne CETAC Technologies, Omaha, NE, USA) with a 1 mg/L
218 sulphur solution. Argon (1 L/min) was added via T piece inside the Trace 1310 GC to enhance
219 the low helium flow rate for separation (2 mL/min). The capillary exit from the GC column
220 entered the GCI 300 interface for approximately 13 cm with this piece. Argon surrounded the
221 capillary tube for this length and was mixed with helium inside the GCI 300 interface before
222 entering the torch injector. **Table S2** describes the applied parameters.

223 All samples were analysed in triplicate and were verified by bracketing the analytical sequence
224 with organosulfide standards every six analyses. Organosulfide standards were calibrated to
225 the international standard V-CDT (Vienna Canyon Diablo Troilite) using the EA-IRMS
226 technique.

227 In order to obtain an accurate isotope ratio measurement (Yang et al., 2018), the MC-ICP-MS
228 mass bias needs to be corrected, according to the Russell law (Russell et al., 1978):

$$229 \quad R (^{34}\text{S}/^{32}\text{S})_{True} = R (^{34}\text{S}/^{32}\text{S})_{Measured} * \left(\frac{34}{32}\right)^f \Leftrightarrow f = \frac{\ln \left[\frac{R (^{34}\text{S}/^{32}\text{S})_{True}}{R (^{34}\text{S}/^{32}\text{S})_{Measured}} \right]}{\ln \left[\frac{34}{32} \right]}$$

230 Where $R (^{34}\text{S}/^{32}\text{S})_{\text{Measured}}$ is the integrated $^{34}\text{S}/^{32}\text{S}$ ion-current ratio of samples; $R (^{34}\text{S}/^{32}\text{S})_{\text{True}}$ is
231 the true $R (^{34}\text{S}/^{32}\text{S})$ value after correction; and f is the correction factor.

232 **2.3. Statistical Analysis**

233 Univariate and multivariate statistical analyses were performed using Xlstat© (Addinsoft,
234 Paris). The Pearson correlation test was carried out to determine pairwise correlations
235 between isotope ratio values. Unidimensional test of equality was realized to determine
236 significant differences between species, within a confidence level p-value < 0.05.

237 Principal component analysis (PCA) (Jolliffe, 2002) was preliminary used in this study to
238 describe and to assess the quality and homogeneity of the 42 samples of interest. Machine
239 learning algorithm linear discriminant analysis (LDA) (Haghighat et al., 2016), and partial least
240 square method – discriminant analysis (PLS-DA) (Tenenhaus, 1998) were then used to classify
241 the different Allium species on the basis of their isotopic signature and chemical composition.
242 Due to a small number of data points, a 7-fold stratified cross validation was repeated 20 times
243 to avoid overfitting the small number of data-points. Features were ranked according to the
244 variable importance projection (VIP) from PLS-DA. The model's quality was evaluated based
245 on the residuals ($R^2\text{X}$, $R^2\text{Y}$) and the model's predictive ability parameter ($Q2_{\text{cum}}$) (Levet et al.,
246 2013). More information can be found in **Supplementary data**. The overall quality of models
247 was also assessed using confusion matrix.

248 **3. Results and discussion**

249 **3.1. Variations of the isotopic composition**

250 The Bulk Stable Isotope Analysis (BSIA) and Compound Specific Isotope Analysis (CSIA) $\delta^{13}\text{C}$,
251 $\delta^2\text{H}$, and $\delta^{34}\text{S}$ values of leek, shallot, chive, and onion essential oils are reported in **Table 1** and
252 **Supplementary Table S3**.

253 **3.1.1. BSIA and CSIA $\delta^{13}\text{C}$ analyses**

254 Bulk $\delta^{13}\text{C}$ stable isotope ratio analyses show differences amongst the studied species (p-value
255 $p < 0.05$). Leek essential oils present $\delta^{13}\text{C}$ values in the range of -35.41‰ to -36.79‰ , which
256 are slightly depleted compared with the values measured for other species. Chive essential
257 oils have $\delta^{13}\text{C}$ values measured between -33.36‰ and -34.17‰ , and onion essential oils have
258 $\delta^{13}\text{C}$ values between -31.67‰ and -33.34‰ . Shallot essential oils have a wider range of $\delta^{13}\text{C}$
259 values, spreading between -31.73‰ and -35.19‰ . These $\delta^{13}\text{C}$ isotopic values correspond to
260 essential oils from C3 plant, which is in line with the C3 plant origin of *Allium* species (Choi et
261 al., 2020; Park et al., 2019; Pianezze et al., 2019). $\delta^{13}\text{C}$ typical values of C3 plants generally
262 range from -34‰ to -24‰ (Krueger and Reesman, 1982) but it has been reported in some
263 cases more negative values for $\delta^{13}\text{C}$ in essential oils extracted from C3 plants (Cuchet et al.,
264 2019). To our knowledge, stable isotopic analysis on *Allium* essential oils have never been
265 referenced in the literature before. However, isotopic analyses were realized on *Allium* raw
266 materials. Park et al. (2019) presented an average $\delta^{13}\text{C}$ value of Korean onions ranging from -
267 26.5‰ to -25.4‰ , which differs from the analyses undertaken on onion essential oils. This
268 could be due to the fact that raw material isotope signature could be influenced by cellulose,
269 proteins, carbohydrates, and other constituents which are not present in essential oils.
270 Moreover, this can also be attributed to isotopic fractionation during distillation processes.
271 These *Allium* essential oils are peculiar amongst essential oils as they are produced from
272 degradation when exposed to air, and from heating processes. This generates organosulphur
273 compounds from precursors, such as allicine (Ramirez et al., 2017), which **could** result to more
274 negative $\delta^{13}\text{C}$ values.

275 The global isotopic values for $\delta^{13}\text{C}$ are similar to the measured CSIA values, especially for DPDS
276 and DPTS. However, the $\delta^{13}\text{C}_{\text{MPDS}}$ and $\delta^{13}\text{C}_{\text{MPTS}}$ values are systematically depleted compared

277 with $\delta^{13}\text{C}_{\text{DPDS}}$ and $\delta^{13}\text{C}_{\text{DPTS}}$ in all samples analysed. The $\delta^{13}\text{C}$ CSIA values are different amongst
278 several vegetal species of this study, especially for leek essential oils, for which it can be
279 noticed that the $\delta^{13}\text{C}_{\text{MPTS}}$ values (-40.41‰ to -43.59‰) are significantly different from those
280 of the other essential oils: -33.98‰ to -36.91 ‰ in shallot essential oils, -34.31‰ to -35.15‰
281 in chive essential oils, and -35.42‰ to -37.81‰ in onion essential oils. The $\delta^{13}\text{C}_{\text{DPDS}}$ (-33.50‰
282 to -35.22‰) and $\delta^{13}\text{C}_{\text{DPTS}}$ (-34.88‰ to -35.97‰) values for leek essential oils are also more
283 depleted.

284 **3.1.2. BSIA and CSIA $\delta^2\text{H}$ analyses**

285 Bulk $\delta^2\text{H}$ analyses from all of the studied species are relatively similar (leek, shallot and chive
286 essential oil $\delta^2\text{H}$ values are spread between -218‰ and -227‰, p-value > 0.05), including
287 samples from various geographical origins. However, deuterium isotope fractionation is
288 noticeably more important in the case of onion essential oils ($\delta^2\text{H}$ between -172‰ and -
289 251‰). Onion samples with higher $\delta^2\text{H}$ isotopic ratios were resulting from a different
290 extraction process (OE9, OE10, and OE11, see **Supplementary table S1**), where the whole
291 onion bulb was crushed. This grinding process accelerates the organosulfur precursors
292 oxidation (Tocmo et al., 2014; Zhang et al., 2021), leading to a possible isotopic enrichment.
293 The CSIA $\delta^2\text{H}$ natural isotopic values are significantly lower than the $\delta^2\text{H}$ synthetic values.
294 Amongst the essential oils, the CSIA $\delta^2\text{H}$ values are relatively similar, except for onion essential
295 oil batches in which we noticed a larger isotopic fractionation.

296 **3.1.3. BSIA and CSIA $\delta^{34}\text{S}$ analyses**

297 The $\delta^{34}\text{S}$ isotopic fingerprint of sulphur-containing volatile metabolites which is the
298 components of *Allium* essential oils is dependent on sulphur issued from rainwater, soil, and
299 external supply. The sulphur $^{34}\text{S}/^{32}\text{S}$ isotope ratio in plants might be influenced by several

300 factors, including geological characteristics of a location (Sulfides abundance) (Choi et al.,
301 2020), climatic conditions (Nehlich, 2015), sea spray effect in coastal regions (Norman et al.,
302 2006), and soils bacterial processes (Pianezze et al., 2019). $\delta^{34}\text{S}$ values are also affected by
303 anthropogenic effects such as the sulphur supplied by fertilizers (Mizota and Sasaki, 1996)
304 under the SO_4^{2-} sulfate ion form. Depending on the origin of the fertilizers used, the global
305 isotopic ratio of ^{34}S can theoretically differ, offering the potential to observe different
306 agricultural practices.

307 Egyptian leek essential oils have $\delta^{34}\text{S}$ values ranging from 9.0‰ to 10.4‰, as compared with
308 $\delta^{34}\text{S}$ values from French essential oils, which show significantly more depleted $\delta^{34}\text{S}$ values
309 (from 1.0‰ to 2.6‰). Egyptian samples are located in the Nile Delta region, and are probably
310 affected by sulphates from the Nile Delta, which could explain these higher values. These
311 results are also observed amongst onion samples of Egyptian and French origins.

312 The CSIA $\delta^{34}\text{S}$ values of DPDS, DPTS, and MPTS were determined via GC/MC-ICP-MS. In order
313 to validate this recent technique for our type of samples, comparison measurements between
314 CSIA and BSIA analyses were first done. Similarities were observed between CSIA $\delta^{34}\text{S}$ and BSIA
315 $\delta^{34}\text{S}$, as the isotopic values were on the same scale, between -2.3 and 15.2‰. Furthermore,
316 similar isotopic trends were observed between species and geographical origin. For example,
317 the $\delta^{34}\text{S}_{\text{Bulk}}$ values of French leek essential oils were depleted compared to their Egyptian
318 equivalent, as well as the compound-specific $\delta^{34}\text{S}$ values for DPDS, DPTS, and MPTS. This
319 demonstrates the accuracy of values measured with the ICP methodology. However, a
320 systematic discrepancy was also observed between IRMS and MC-ICP-MS values. The MC-ICP-
321 MS is a precise isotope ratio measurement method, which usually achieve precise ratio
322 determination using 20 to 40 cycles (4 to 8 seconds per cycle). However, in our configuration,

323 with the fast-transient signal obtained from the GC system, the MC-ICP-MS technology was at
324 the limit of the Faraday cup detector response time, and to correct for that drawback, we used
325 a Tau correction for the amplifiers. It is well known that MC-ICP-MS suffers mass bias problems
326 from the torch, the interface, the lens stack, or the mass spectrometer, and it should be
327 corrected to obtain the true ratio (Yang et al., 2018). On Sr, Hf, or Nd isotope measurements,
328 it is possible to use a stable ratio for mass bias correction. When this is not possible (Pb or Cu
329 for example), a correction with another element (like Zn or Tl) can be used. However, for sulfur
330 measurements, there is no stable ratio available, and the addition of another element for mass
331 bias correction is not possible because the mass dispersion authorized by the spectrometer is
332 not sufficient. The only way to do the measurement is the sample bracketing technique
333 (Amrani et al., 2009; Clough et al., 2006, Said-Ahmad and Amrani, 2013). Unfortunately, the
334 absolute value for $^{32}\text{S}/^{34}\text{S}$ cannot be obtained due to the impossibility to correct for mass bias,
335 which explains the systematic discrepancy observed between IRMS and MC-ICP-MS results.
336 The relative differences between the samples obtained with GC/MC-ICP-MS still supply
337 significant $\delta^{34}\text{S}$ to characterise our samples.

338 Measured $\delta^{34}\text{S}$ isotopic values differed amongst species and geographical origins. Egyptian
339 leek essential oils presented higher $\delta^{34}\text{S}_{\text{DPDS}}$ (11.7-15.2‰) and $\delta^{34}\text{S}_{\text{DPTS}}$ (10.4-12.8‰) values
340 compared with French leek values ($\delta^{34}\text{S}_{\text{DPDS}}$, 6.9-7.7‰; $\delta^{34}\text{S}_{\text{DPTS}}$, 2.4-4.9‰). However, the
341 ranges were similar for $\delta^{34}\text{S}_{\text{MPTS}}$ values, between 2.5 and 11.2‰. German shallot samples
342 presented higher $\delta^{34}\text{S}_{\text{DPTS}}$ values (4.6-7.8‰) than French samples (2.7- 4.6‰). However,
343 shallot essential oil values for $\delta^{34}\text{S}_{\text{DPDS}}$ (6.4- 8.8‰) and $\delta^{34}\text{S}_{\text{MPTS}}$ (0.1-5.4‰) were within the
344 same range for both geographical origins. Sulphur isotope fractionation was noticeably
345 important in the case of onion essential oils. Egyptian and French onion samples presented
346 $\delta^{34}\text{S}_{\text{DPDS}}$ values between 5.1 and 10.7‰, $\delta^{34}\text{S}_{\text{DPTS}}$ values between 3.7 and 10.2‰, and $\delta^{34}\text{S}_{\text{MPTS}}$

347 values between -2.30 and 6.1‰. Moreover, chive essential oils presented $\delta^{34}\text{S}_{\text{DPDS}}$ values
348 ranging between 3.92 and 8.8‰; $\delta^{34}\text{S}_{\text{DPTS}}$ values between 1.6 and 3.5‰; and $\delta^{34}\text{S}_{\text{MPTS}}$ values
349 between -0.2 and 3.5‰.

350 **3.2. Naturalness authentication**

351 ^{13}C , ^2H , and ^{34}S stable isotope signature can differ between natural and synthetic molecules.
352 Synthetic-origin molecules are not subject to the same isotopic fractionation phenomenon
353 than natural metabolites, as they are not produced from similar pathways. Whereas *Allium*
354 natural organosulfides compounds are produced from biosynthesis pathways, synthetic
355 components are produced from petrochemical processes. Alkyl and allyl sulphides, from
356 *Allium* essential oils, are generated during heating process from allicin and other thiosulfinates
357 degradation (Ramirez et al., 2017). These secondary metabolites represent the enzymatic
358 hydrolysis product of alkyl cysteine sulfoxides (ACSO) (Jones et al., 2004) which are produced
359 from several biosynthesis steps influenced notably by the assimilation of SO_4^{2-} , and the
360 photosynthesis pathways of CO_2 . Syntheses of organic disulfides molecules has been usually
361 prepared by nucleophile substitution reactions of alkyl halides with sulphur containing
362 products (Abbasi et al., 2016; Bhaumik and Misra, 2017; Soleiman-Beigi and Mohammadi,
363 2012). All of these pathways generate symmetric alkyl disulfides with elevated yields. Other
364 common synthetic pathways use a sulfonate (Filippi et al., 2007).

365 **3.2.1. Analysis of synthetic organosulfides**

366 In order to compare natural and synthetic compounds, synthetic $\delta^{13}\text{C}_{\text{DPDS}}$ were measured,
367 generating values between -28.52‰ and -26.26‰, significantly different from natural values
368 between -31.66‰ and -36.79‰. The same observations were noted for DPTS and MPTS.
369 Nevertheless, the $\delta^{13}\text{C}$ value of one MPDS synthetic compound (-37.17‰) is comparable to

370 the $\delta^{13}\text{C}$ value from MPDS in essential oils, whereas, considering hydrogen data, its $\delta^2\text{H}$ value
371 is enriched, which outlines the power of multi-isotope approaches. Most synthetic commercial
372 standards have shown enriched $\delta^{13}\text{C}$ values (-26.18‰ to -28.68‰) compared with the natural
373 essential oils reported in the study. Those results are in line with the literature. Synthetic
374 compounds are petrochemicals by-products, which have generally a $\delta^{13}\text{C}$ value ranging
375 between -25 and -30‰ (Krueger and Reesman, 1982). Based on this significant $\delta^{13}\text{C}$ isotopic
376 gap between natural and synthetic origins for those compounds, adulteration of essential oils
377 with addition of synthetic molecules to natural products could be clearly established with $\delta^{13}\text{C}$
378 bulk analyses. Therefore, we have established an efficient indicator of authentication for
379 *Allium* essential oils.

380 As it was the case for $\delta^{13}\text{C}$ values, synthetic standard molecules show more positive $\delta^2\text{H}$ values
381 (ranging from -105‰ to -170‰), and thus adulteration of natural *Allium* extract with synthetic
382 compounds is expected to increase the $\delta^2\text{H}$ bulk value and could also be detected. A majority
383 of synthetic samples present $\delta^{34}\text{S}$ values close to 0‰ (ranging from 2.0‰ to -3.1‰), except
384 for one MPDS sample that has a more positive value (7.3‰). Differentiation was realised
385 between synthetic DPDS and DPTS molecules and natural essential oils of *Allium* species.
386 Synthetic DPDS values of $\delta^{34}\text{S}$ (-0.8‰ and -3.0‰) and $\delta^{13}\text{C}$ (-28.52‰ and -26.26‰) and the
387 synthetic DPTS $\delta^{13}\text{C}$ value (-26.18‰) differed significantly from natural isotopic values.
388 However, synthetic (2.0‰) and natural $\delta^{34}\text{S}_{\text{DPTS}}$ values were within the same range.
389 Comparisons between $\delta^{34}\text{S}$ values from natural and synthetic compounds are also promising.

390 **3.2.2. Correlation study**

391 A univariate Pearson's correlation is realized on the data set to determine correlation
392 coefficients and pairwise correlations, described in **Supplementary Table S4**. It appears that

393 several data are linearly correlated with each other: a correlation of $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values
394 determined on *Allium* metabolites DPDS, DPTS, MPDS, and MPTS, was established, especially
395 $\delta^{13}\text{C}_{\text{DPDS}}$ and $\delta^{13}\text{C}_{\text{DPTS}}$ ($r=0.94$), $\delta^{13}\text{C}_{\text{DPDS}}$ and $\delta^{13}\text{C}_{\text{MPTS}}$ ($r=0.84$), and $\delta^{34}\text{S}_{\text{DPDS}}$ and $\delta^{34}\text{S}_{\text{DPTS}}$ ($r=0.84$).
396 These $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ isotopic filiations between organosulphur metabolites were expected as
397 these metabolites are sulphur-enriched from the same sulphite SO_3^{2-} source. Furthermore,
398 the biosynthesis pathways at the origin of their formation during plant photosynthesis are
399 composed of similar precursors. All metabolites DPDS, DPTS, MPDS, and MPTS were affected
400 by the same isotopic fractionation effects. However, no correlation was observed with $\delta^2\text{H}$
401 analysis, which is probably because $\delta^2\text{H}$ values are mainly affected by climatic effects /
402 geographical origins. This $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ linear correlation is useful in authentication as the
403 potential addition of the same metabolite from a different source (synthetic or natural) will
404 not show the same correlation. For instance, this means that an addition of DPDS or DPTS
405 from another source can be highlighted. This is an important result for the use of the CSIA
406 method in an authentication approach.

407 **3.2.3. Adulteration of samples by synthetic samples**

408 To evaluate the detection limits for the addition of synthetic molecules in essential oil, a
409 known sample of leek essential oil was adulterated with 5%, 10%, and 20% of DPDS and DPTS
410 from synthetic origins. Results are presented in **Fig. 1**. It is observed that due to a different
411 isotopic ratio between DPDS/DPTS from synthetic and natural origins, the adulterated
412 samples no longer fall on the previously obtained correlation line.

413 The CSIA approach allows the comparison between metabolites contained in the various
414 essential oil that were investigated. The result of those isotopic measurements is that the
415 combination of $\delta^{13}\text{C}$, $\delta^2\text{H}$, and $\delta^{34}\text{S}$ values supply useful information for deciphering the

416 authentication of *Allium* natural products. Moreover, the combination of $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values
417 can be used to discriminate synthetic molecules and natural essential oils and thereby indicate
418 potential adulterations.

419 **3.3. Multivariate analyses – Chemometric study**

420 In order to understand the capability of our variables to predict the species origin of the *Allium*
421 essential oil samples, a multivariate analysis was carried out on the entire sample data set (42
422 samples), with all the 17 following parameters as variables: BSIA ($\delta^{13}\text{C}_{\text{Bulk}}$, $\delta^2\text{H}_{\text{Bulk}}$, $\delta^{34}\text{S}_{\text{Bulk}}$) and
423 CSIA ($\delta^{13}\text{C}_{\text{DPDS}}$, $\delta^{13}\text{C}_{\text{DPTS}}$, $\delta^{13}\text{C}_{\text{MPDS}}$, $\delta^{13}\text{C}_{\text{MPTS}}$, $\delta^2\text{H}_{\text{DPDS}}$, $\delta^2\text{H}_{\text{DPTS}}$, $\delta^2\text{H}_{\text{MPDS}}$, $\delta^{34}\text{S}_{\text{DPDS}}$, $\delta^{34}\text{S}_{\text{DPTS}}$, $\delta^{34}\text{S}_{\text{MPTS}}$)
424 stable isotope data, as well as relative concentration of major organosulfides (DPDS, DPTS,
425 MPDS, and MPTS). This statistical analysis consisted in a preliminary PCA to describe the
426 dataset, followed by a LDA and a PLS DA analyses to classify the samples and exhibit clusters.
427 The main objective of this statistical multivariate analysis is to authenticate the species origin
428 of the samples. Although many studies describe the possibility to assess the geographical
429 origin of samples using isotopic data and chemometric (Drivelos and Georgiou, 2012;
430 Diomande et al., 2015), it has not been realised here as the dataset does not have enough
431 representative samples of each origin.

432 **3.3.1. Principal Component Analysis**

433 We depict scores and loadings plots of the first two principal components (F1 and F2) of the
434 PCA in **Fig. 2**, which constituted 63.5% of the total variance. PCA supported our earlier
435 observations. Species differentiation was observed, especially for leek essential oils. Onion
436 essential oils were highly scattered, because of their large isotopic fractionation. Individual
437 clusters were identified for shallot and chive essential oils. Shallot and chive essential oils were

438 closely grouped however, even if a slight separation was exhibited on F2. These two essential
439 oils have closely related isotopic range values and share similar compositions.

440 Essential oils from leek were also grouped according to the geographical origin of the raw
441 material, being the two samples from France allocated in more negative values of F2. The
442 variables involved in this separation are hydrogen isotopic ratios ($\delta^2\text{H}_{\text{Bulk}}$, $\delta^2\text{H}_{\text{DPDS}}$, $\delta^2\text{H}_{\text{DPTS}}$, and
443 $\delta^2\text{H}_{\text{MPDS}}$), which are known to be highly dependent on geographical origins (Pianezze et al.,
444 2019; Hoefs, 2018). Shallot essential oils were also geographically separated in component F2,
445 making the use of $\delta^2\text{H}$ ratios more robust for geographic assignments.

446 **3.3.2. Discriminant models (LDA and PLS-DA)**

447 Classification of the oil extracts according to allium species was first addressed by means of
448 linear discriminant analysis (LDA) using all the stable isotopes and the chemical composition.
449 This statistical model is one of the most popular multivariate analytical tool to highlight data
450 clusters in authentication. Unlike PCA, LDA considers differences between data classes. The
451 analytical target was **set** to increase the separation between species. LDA scores and loadings
452 plots can be observed in **Fig. 3**; grouped by species. The confusion matrix for estimated
453 samples is displayed on **Table 2**. Only BSIA isotope values were first included in the LDA study,
454 but only 85.7% of samples were well classified, with several shallot and onion essential oils
455 samples wrongly attributed. This study showed that the use of CSIA isotope values and relative
456 concentration of principal metabolites lead to 100% of classification of species origin. With
457 regard to species, score plots of the linear discriminants F1 (80.0%) and F2 (14.5%) constituted
458 94.5% of the total variance. Several parameters highly contribute to the clustering. F1
459 correlated positively notably with $\delta^{34}\text{S}_{\text{Bulk}}$ (0.602), $\delta^{34}\text{S}_{\text{DPDS}}$ (0.622), $\delta^{34}\text{S}_{\text{DPTS}}$ (0.534), $\delta^{34}\text{S}_{\text{MPTS}}$
460 (0.542), $\%_{\text{DPDS}}$ (0.909), and $\%_{\text{MPDS}}$ (0.721), and negatively with $\delta^{13}\text{C}_{\text{Bulk}}$ (-0.819), $\delta^{13}\text{C}_{\text{DPDS}}$ (-

461 0.701), $\delta^{13}\text{C}_{\text{DPTS}}$ (-0.726), $\delta^{13}\text{C}_{\text{MPDS}}$ (-0.552), $\delta^{13}\text{C}_{\text{MPTS}}$ (-0.937), and $\%_{\text{MPTS}}$ (-0.653); whereas F2
462 correlated negatively with $\delta^{34}\text{S}_{\text{DPTS}}$ (-0.531), and positively with $\%_{\text{MPTS}}$ (0.568).

463 In order to conclude the study and establish a predictive statistical model, a partial least
464 square – discriminant analysis (PLS-DA) was also conducted. PLS-DA scores and loadings plots
465 are represented in **Fig. 4**. The component F1 is mostly correlated positively with $\delta^{13}\text{C}$ isotopic
466 values ($\delta^{13}\text{C}_{\text{Bulk}}$ (0.814), $\delta^{13}\text{C}_{\text{DPDS}}$ (0.839), $\delta^{13}\text{C}_{\text{DPTS}}$ (0.886), and $\delta^{13}\text{C}_{\text{MPDS}}$ (0.934)), and negatively
467 with $\delta^{34}\text{S}$ isotopic values and organosulfides composition ($\delta^{34}\text{S}_{\text{Bulk}}$ (-0.738), $\delta^{34}\text{S}_{\text{DPDS}}$ (-0.828),
468 $\delta^{34}\text{S}_{\text{DPTS}}$ (-0.691), $\delta^{34}\text{S}_{\text{MPTS}}$ (-0.710), $\%_{\text{DPDS}}$ (-0.811), and $\%_{\text{MPDS}}$ (-0.797)). The component F2 is
469 mostly correlated negatively with $\delta^2\text{H}$ isotopic values ($\delta^2\text{H}_{\text{Bulk}}$ (-0.520), $\delta^2\text{H}_{\text{DPDS}}$ (-0.710), $\delta^2\text{H}_{\text{DPTS}}$
470 (-0.535), and $\delta^2\text{H}_{\text{MDPS}}$ (-0.484)). Essential oils from leek presented more negative values on F1
471 than other *Allium* species, whereas shallot, onion, and chive essential oils were separated in
472 component F2. According to the **Table 2**, varieties were significantly clustered with 100% of
473 sample classification. The performances of this model in this study are comparable to the LDA.
474 Both models successively allow to distinguish the different classes of *Allium* species.

475 **Conclusion**

476 Authentication of essential oils of *Allium* varieties was performed using bulk stable and
477 compound specific isotope analysis of carbon, hydrogen, and sulphur elements determined in
478 EA-IRMS, and GC-IRMS, as well as GC/MC-ICP-MS. This last technique was used for the first
479 time to measure organic compounds in essential oils. The isotopic filiation demonstrated in
480 $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ produces better precision in authenticating naturalness of these types of
481 essential oils. We demonstrated that an addition of 5% of synthetic-origin DPDS or DPTS can
482 be detected. The results also showed that this methodology is applicable to assess **EOs** purity.
483 Combination of BSIA, CSIA, and metabolite composition leads to varieties discrimination: LDA

484 and PLS-DA models performances achieved 100% of sample classification. Furthermore, the
485 methodology could be employed to improve the authentication of other sulphur component-
486 based essential oils, such as garlic *Allium sativum* and other *Allium* essential oils composed of
487 allyl components, such as *A. ursinum* and *A. tuberosum* essential oils.

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499 network.

500 **Declaration of Competing Interests**

501 The authors declare that there are no conflicts of interest.

502

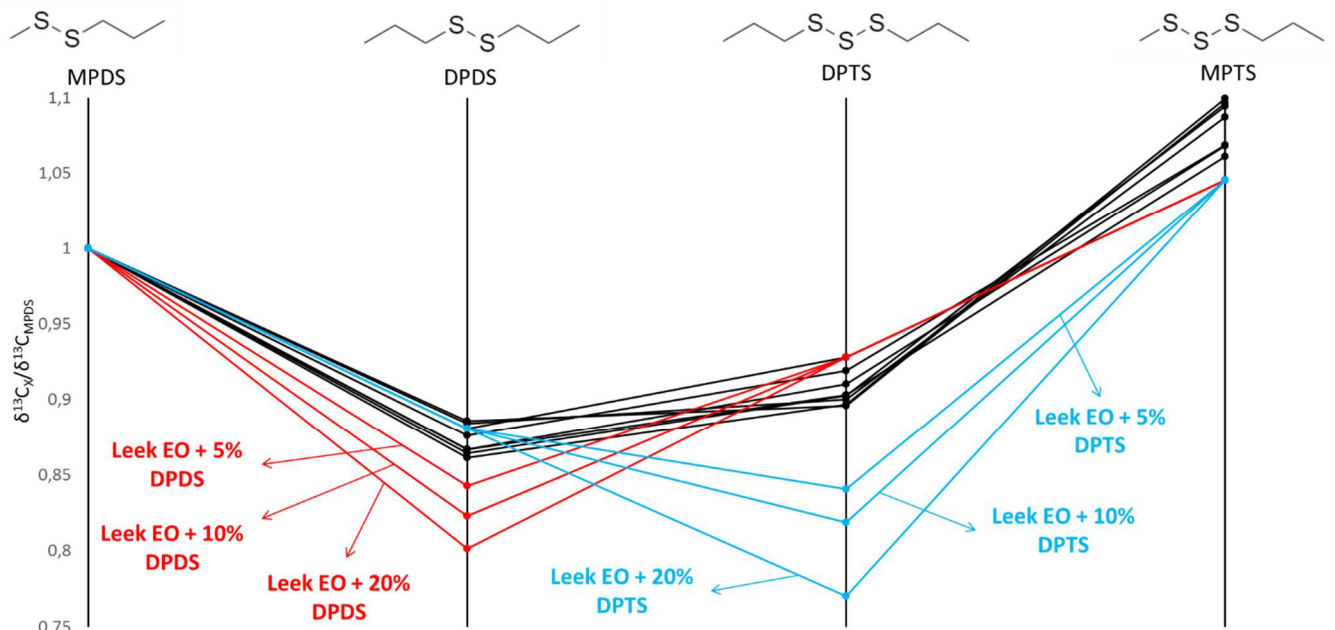
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652 **Fig. 1.** Parallel plots of $\delta^{13}\text{C}_{\text{MPDS}}$, $\delta^{13}\text{C}_{\text{DPDS}}$, $\delta^{13}\text{C}_{\text{DPTS}}$, and $\delta^{13}\text{C}_{\text{MPTS}}$ values compared with $\delta^{13}\text{C}_{\text{MPDS}}$

653 and measured on 8 natural leek essential oils (in black), one essential oil adulterated with 5,

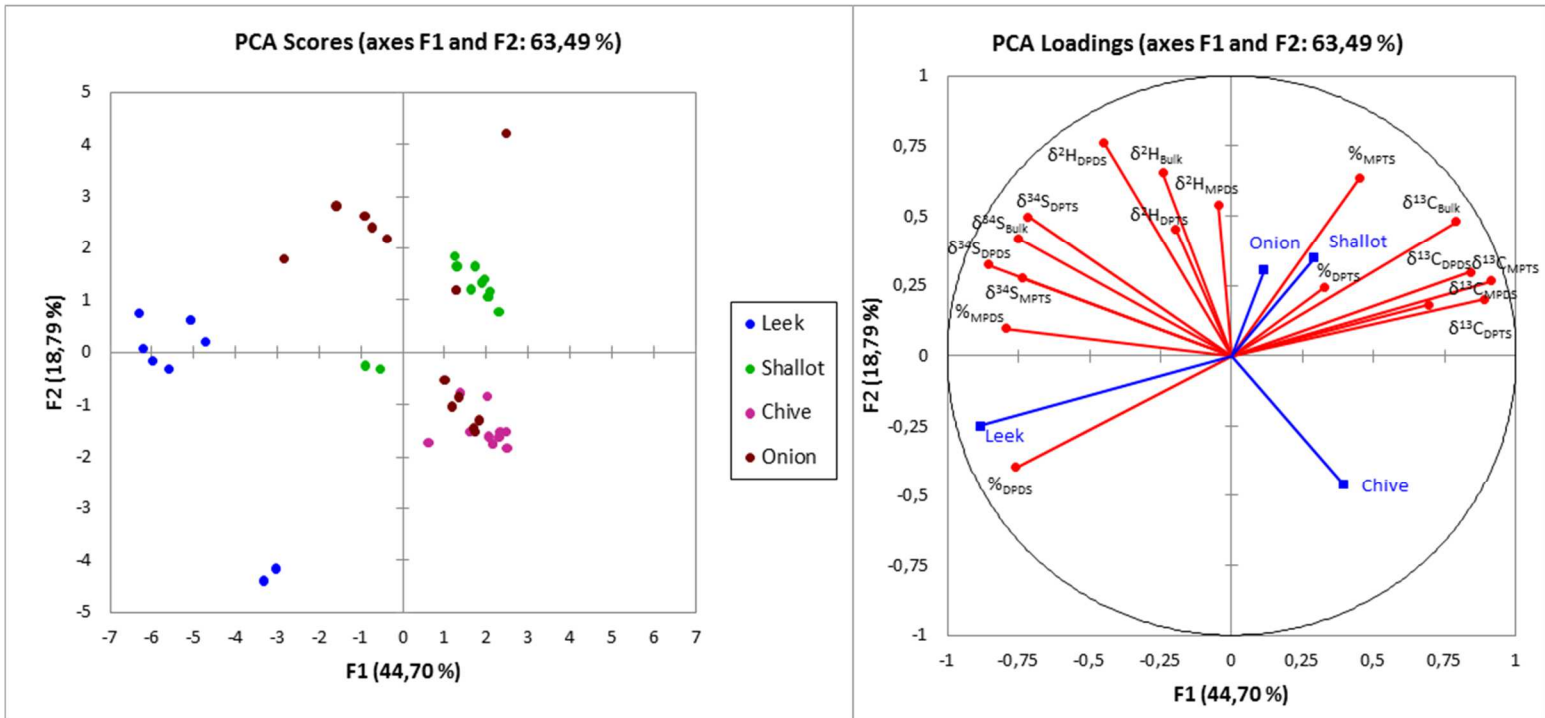
654 10, and 20% of synthetic DPDS (in red), and one essential oil adulterated with 5, 10, and 20%

655 of synthetic DPTS.

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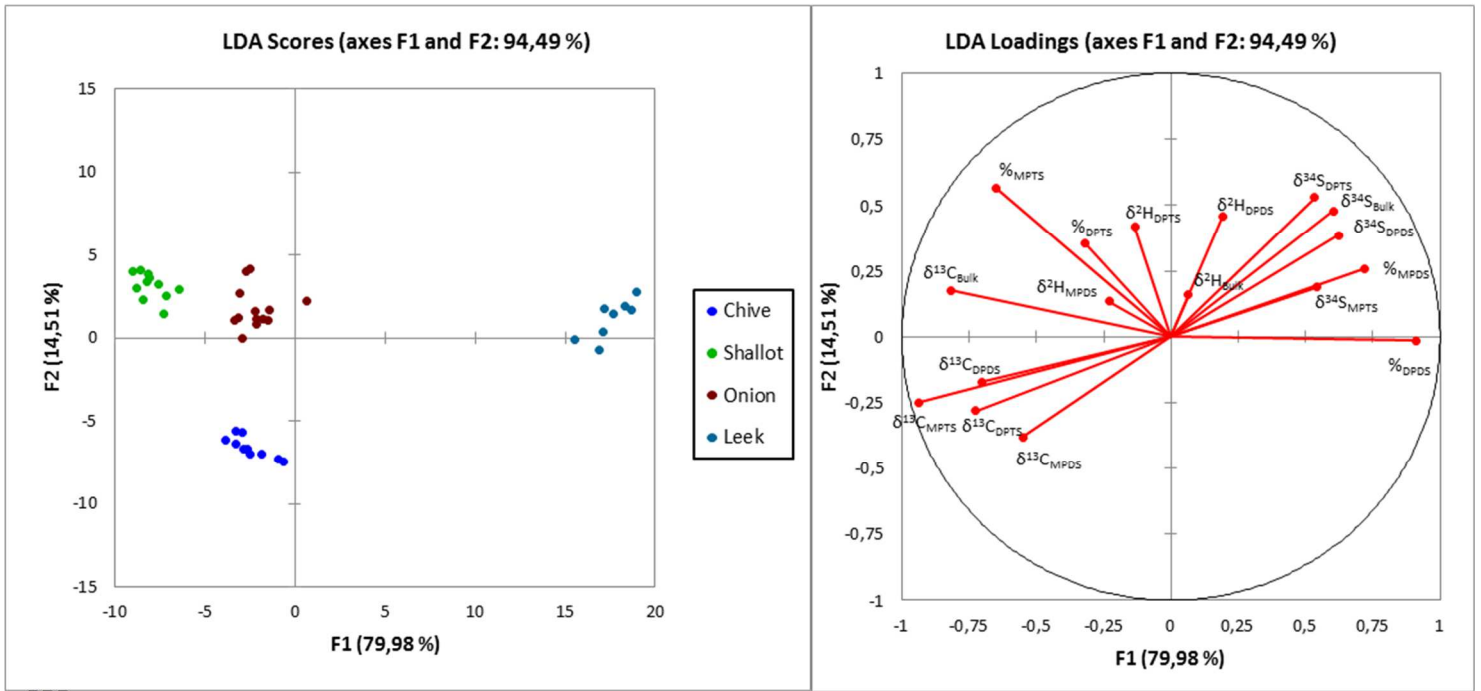


660

661 **Fig. 2.** Scores and loadings plots of principal component F1 and F2 of PCA model developed
 662 from all isotopic values (BSIA and CSIA), and DPDS, DPTS, MPDS, and MPTD chemical
 663 composition, of leek, shallot, chive, and onion essential oils.

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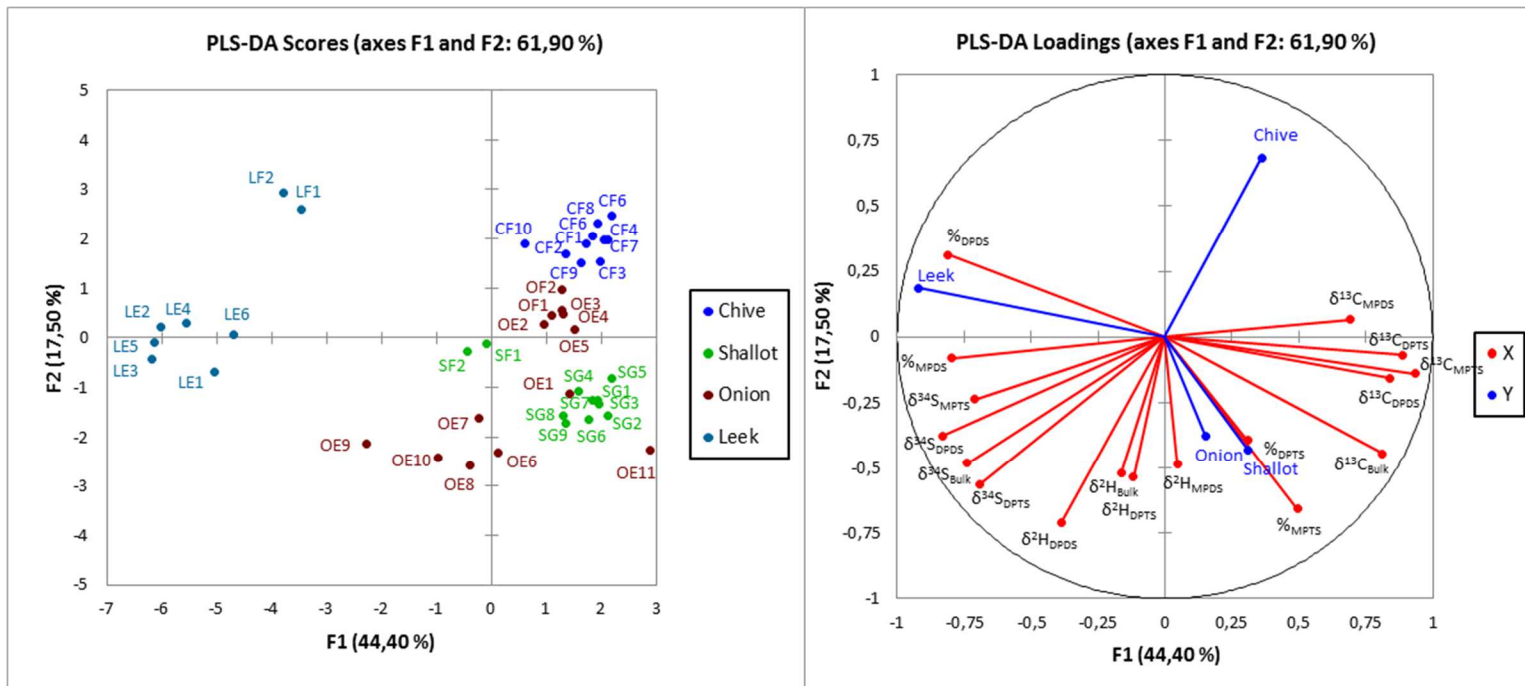
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668 **Fig. 3.** Linear Discriminant Analysis (LDA) scores and loadings plots of all isotopic values (BSIA
 669 and CSIA) and DPDS, DPTS, MPDS, and MPTD chemical composition, determined on *Allium*
 670 essential oils samples grouped by species

671



673

674 **Fig. 4.** Partial Least Square – discriminant analysis (PLS-DA) scores and loadings plots of all
 675 isotopic values (BSIA and CSIA) and DPDS, DPTS, MPDS, and MPTD chemical composition,
 676 determined on *Allium* essential oils samples.

677

678 **Table 1.** Mean, max, min, and standard deviation (SD) of bulk stable and compound-specific
679 $\delta^{13}\text{C}$, $\delta^2\text{H}$, and $\delta^{34}\text{S}$ isotope values, and DPDS, DPTS, MPDS, and MPTD chemical composition,
680 of leek, shallot, chive, and onion essential oils (n=number of samples).

Isotopic Values	Leek essential oils (n=8)				Shallot essential oils (n=11)				Chive Essential oils (n=10)				Onion essential oils (n=13)			
	Mean	Max	Min	SD	Mean	Max	Min	SD	Mean	Max	Min	SD	Mean	Max	Min	SD
$\delta^{13}\text{C}_{\text{bulk}}$ (‰)	-36,02	-34,63	-36,79	0,73	-32,41	-31,73	-35,19	1,35	-33,61	-33,31	-34,17	0,29	-32,63	-31,67	-34,32	0,65
$\delta^2\text{H}_{\text{bulk}}$ (‰)	-218	-211	-227	6	-223	-218	-226	2	-223	-217	-227	3	-209	-172	-251	25
$\delta^{34}\text{S}_{\text{bulk}}$ (‰)	7,5	10,4	1,0	3,7	3,0	4,1	-0,2	1,6	0,7	1,4	0,0	0,5	3,9	7,2	1,2	1,7
$\delta^{13}\text{C}_{\text{DPDS}}$ (‰)	-34,27	-33,50	-35,22	0,64	-32,14	-31,52	-34,11	0,96	-32,08	-31,66	-32,44	0,25	-32,43	-30,00	-34,13	1,00
$\delta^2\text{H}_{\text{DPDS}}$ (‰)	-189	-145	-251	42	-198	-184	-216	9	-227	-216	-252	12	-199	-164	-253	30
$\delta^{34}\text{S}_{\text{DPDS}}$ (‰)	12,1	15,2	6,9	3,2	8,0	8,8	6,4	0,8	6,1	8,8	3,9	1,4	7,9	10,7	5,1	1,9
$\delta^{13}\text{C}_{\text{DPTS}}$ (‰)	-35,59	-34,88	-35,97	0,41	-33,16	-32,36	-35,43	1,12	-32,68	-32,15	-33,14	0,32	-33,27	-31,12	-34,95	1,02
$\delta^2\text{H}_{\text{DPTS}}$ (‰)	-217	-204	-251	15	-196	-154	-228	25	-236	-164	-266	36	-208	-152	-273	37
$\delta^{34}\text{S}_{\text{DPTS}}$ (‰)	9,7	12,9	2,4	3,9	5,8	7,8	2,7	1,4	2,9	3,5	1,6	0,6	6,8	10,2	3,7	1,8
$\delta^{13}\text{C}_{\text{MPDS}}$ (‰)	-39,24	-38,66	-39,81	0,45	-37,55	-36,97	-38,87	0,66	-37,19	-36,10	-38,17	0,63	-38,56	-35,69	-40,13	1,02
$\delta^2\text{H}_{\text{MPDS}}$ (‰)	-229	-211	-253	13	-220	-184	-237	16	-221	-171	-249	20	-201	-162	-231	21
$\delta^{13}\text{C}_{\text{MPTS}}$ (‰)	-42,29	-40,41	-43,59	1,13	-34,78	-33,98	-36,91	1,00	-34,67	-34,31	-35,15	0,24	-36,37	-34,65	-37,81	0,73
$\delta^{34}\text{S}_{\text{MPTS}}$ (‰)	6,5	11,2	2,5	3,0	2,9	5,4	0,1	1,8	1,5	3,5	-0,2	1,3	1,0	6,1	-2,3	2,5
%DPDS	38,4	46,2	34,7	3,5	18,5	20,7	14,0	2,3	22,5	26,6	17,0	3,0	21,5	28,3	14,4	4,1
%DPTS	12,7	14,2	10,4	1,5	16,0	18,5	8,5	3,5	12,7	15,7	8,6	2,1	14,9	17,9	9,5	2,9
%MPDS	5,5	7,9	2,1	2,1	2,4	3,9	1,5	0,7	1,6	2,3	0,7	0,5	1,9	4,6	1,2	0,9
%MPTS	4,9	5,7	4,4	0,6	8,7	9,2	7,9	0,4	5,4	6,3	4,9	0,4	6,9	10,7	5,5	1,3

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682

683 **Table 2.** LDA and PLS-DA confusion matrices using BSIA, CSIA, and chemical composition as
 684 variables (17 variables)

Model	from \ to	Chive	Shallot	Onion	Leek	%correct
LDA	Chive	10	0	0	0	100%
	Shallot	0	11	0	0	100%
	Onion	0	0	13	0	100%
	Leek	0	0	0	8	100%
PLS-DA	Chive	10	0	0	0	100%
	Shallot	0	11	0	0	100%
	Onion	0	0	13	0	100%
	Leek	0	0	0	8	100%

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