

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

3 **Authors**

4 Aurélien Cuchet<sup>a,b,\*</sup>; Anthony Anchisi<sup>b</sup>; Philippe Telouk<sup>c</sup>; Yijiao Yao<sup>c</sup>; Frédéric Schiets<sup>b</sup>;  
5 François Fourel<sup>d</sup>; Yohann Clément<sup>b</sup>; Pierre Lantéri<sup>b</sup>; Elise Carénini<sup>a</sup>; Patrick Jame<sup>b</sup>; Hervé  
6 Casabianca<sup>b</sup>

7 **Affiliations**

8 <sup>a</sup>Albert Vieille SAS, 629 Route de Grasse, 06220 Vallauris, France

9 <sup>b</sup>Univ Lyon, CNRS, Université Claude Bernard Lyon 1, Institut des Sciences Analytiques, UMR  
10 5280, 5 rue de la Doua, F-69100 Villeurbanne, France

11 <sup>c</sup>Université de Lyon, ENSL, UCBL, CNRS, LGL-TPE, F-69364 Lyon, France

12 <sup>d</sup>Univ Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA, F-69622  
13 Villeurbanne, France

14

15 **\*Corresponding author**

16 Aurélien Cuchet, [acuchet@albertvieille.com](mailto:acuchet@albertvieille.com); [aurelien.cuchet@isa-lyon.fr](mailto:aurelien.cuchet@isa-lyon.fr)

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 ( $^{13}\text{C}$ ,  $^2\text{H}$ ,  $^{18}\text{O}$ ,  $^{15}\text{N}$ , and  $^{34}\text{S}$ ) 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  $^{13}\text{C}$  and  $^2\text{H}$  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  $^{34}\text{S}$  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 ( $\delta$ ) 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,  $\delta^2H_{VSMOW}$   
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  $\text{H}_2$  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  $^{13}\text{C}$  or  $^2\text{H}$  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  $\mu\text{L}$  of diluted liquid sample (10  $\mu\text{L}$  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  $\mu\text{m}$  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<sup>-1</sup>, increased to 210°C at a rate of 5°C min<sup>-1</sup>,  
182 increased to 280°C at a rate of 20 °C min<sup>-1</sup>, and held for 5 min. Compounds were separated at  
183 a flow rate of 1 mL.min<sup>-1</sup>.

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 <sup>2</sup>H measurement as H<sub>2</sub> 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 H<sub>2</sub>. 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  $^{32}\text{S}$  ( $\text{O}_2$ ) and  $^{34}\text{S}$  ( $^{33}\text{S}^1\text{H}$  and  $\text{O}_2$ ). Due to the low abundance of  $^{33}\text{S}$  (0.76%), ratio precision with  
213  $^{33}\text{S}$  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  $-35.41\text{‰}$  to  $-36.79\text{‰}$ , 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  $-33.36\text{‰}$  and  $-34.17\text{‰}$ , and onion essential oils have  
258  $\delta^{13}\text{C}$  values between  $-31.67\text{‰}$  and  $-33.34\text{‰}$ . Shallot essential oils have a wider range of  $\delta^{13}\text{C}$   
259 values, spreading between  $-31.73\text{‰}$  and  $-35.19\text{‰}$ . 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  $-34\text{‰}$  to  $-24\text{‰}$  (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  $26.5\text{‰}$  to  $-25.4\text{‰}$ , 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  $\text{SO}_4^{2-}$  sulfate ion form. Depending on the origin of the fertilizers used, the global  
305 isotopic ratio of  $^{34}\text{S}$  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  $^{13}\text{C}$ ,  $^2\text{H}$ , and  $^{34}\text{S}$  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  $\text{SO}_4^{2-}$ , and the  
360 photosynthesis pathways of  $\text{CO}_2$ . 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  $\text{SO}_3^{2-}$  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.

#### 488 **Acknowledgments**

489 This work was conducted in partial fulfilment of the PhD requirements for Aurélien Cuchet  
490 who has received a grant from the Association Nationale de la Recherche et de la Technologie  
491 (ANRT, Paris, France). The research was supported by a contract between Albert Vieille SAS  
492 and ISA (Institut des Sciences Analytiques, Villeurbanne, France). The authors are grateful to  
493 Ferrant PHE (Rodelinghem, France) for providing authentic samples of leek, onion, chive, and  
494 shallot essential oils, and to Thermo Fisher Scientific for providing the Trace 1300 GC and GCI  
495 300 interface. This study was partially performed using the INSU/CNRS MC-ICP-MS national  
496 facility at ENS-LYON. This study was also partially realized on the Isoprime 100 IRMS hosted  
497 by the platform “Ecologie Isotopique” of the ‘Laboratoire d’Ecologie des Hydrosystèmes  
498 Naturels et Anthropisés’ (LEHNA, UMR 5023, Villeurbanne, France), a member of the RÉGEF  
499 network.

#### 500 **Declaration of Competing Interests**

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

502

503 **References**

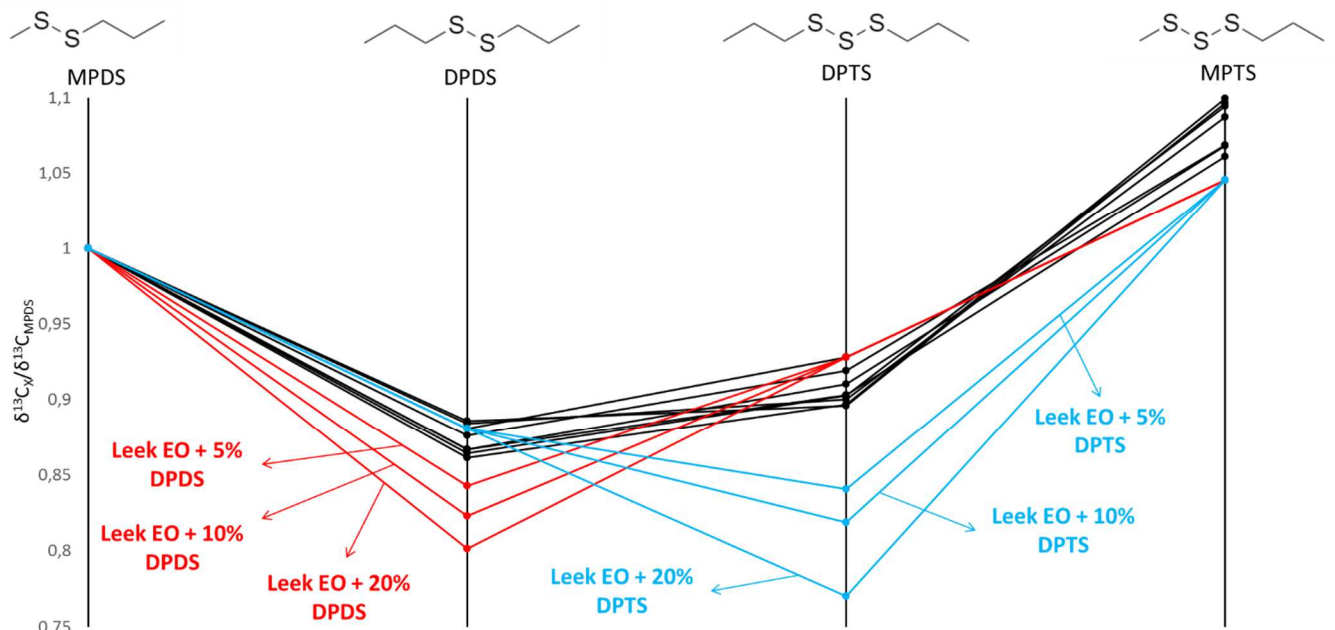
- 504 Abbasi, M., Mohammadizadeh, M.R., Saeedi, N. (2016). The synthesis of symmetrical disulfides by  
505 reacting organic halides with Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O in DMSO. *New Journal of Chemistry*, *40*, 89–92.  
506 <https://doi.org/10.1039/C5NJ01885D>
- 507 Amrani, A., Sessions, A.L., Adkins, J.F. (2009). Compound-Specific  $\delta^{34}\text{S}$  Analysis of Volatile Organics by  
508 Coupled GC/Multicollector-ICPMS. *Analytical Chemistry*, *81*, 9027–9034.  
509 <https://doi.org/10.1021/ac9016538>
- 510 Bhaumik, I., Misra, A. (2017). Rapid Transformation of Alkyl Halides into Symmetrical Disulfides Using  
511 Sodium Sulfide and Carbon Disulfide. *SynOpen*, *01*, 0117–0120. [https://doi.org/10.1055/s-](https://doi.org/10.1055/s-0036-1588573)  
512 [0036-1588573](https://doi.org/10.1055/s-0036-1588573)
- 513 Brand, W.A., Coplen, T.B., Vogl, J., Rosner, M., Prohaska, T. (2014). Assessment of international  
514 reference materials for isotope-ratio analysis (IUPAC Technical Report). *Pure and Applied*  
515 *Chemistry*, *86*, 425–467. <https://doi.org/10.1515/pac-2013-1023>
- 516 Cantrell, M.S., Seale, J.T., Arispe, S.A., McDouglas, O.M. (2020). Determination of Organosulfides from  
517 Onion Oil. *Foods*, *9*, 884. <https://doi.org/10.3390/foods9070884>
- 518 Casella, S., Leonardi, M., Melai, B., Fratini, F., Pistelli, L. (2013). The Role of Diallyl Sulfides and Dipropyl  
519 Sulfides in the *In Vitro* Antimicrobial Activity of the Essential Oil of Garlic, *Allium sativum* L.,  
520 and Leek, *Allium porrum* L. *Phytotherapy Research*, *27*, 380–383.  
521 <https://doi.org/10.1002/ptr.4725>
- 522 Choi, S.-H., Bong, Y.-S., Park, J.H., Lee, K.-S. (2020). Geographical origin identification of garlic cultivated  
523 in Korea using isotopic and multi-elemental analyses. *Food Control*, *111*, 107064.  
524 <https://doi.org/10.1016/j.foodcont.2019.107064>
- 525 Clough, R., Evans, P., Catterick, T., Evans, E.H. (2006).  $\delta^{34}\text{S}$  Measurements of Sulfur by Multicollector  
526 Inductively Coupled Plasma Mass Spectrometry. *Analytical Chemistry*, *78*, 6126–6132.  
527 <https://doi.org/10.1021/ac060875h>
- 528 Cuchet, A., Jame, P., Anchisi, A., Schiets, F., Oberlin, C., Lefèvre, J.-C., Carénini, E., Casabianca, H. (2019).  
529 Authentication of the naturalness of wintergreen (*Gaultheria* genus) essential oils by gas  
530 chromatography, isotope ratio mass spectrometry and radiocarbon assessment. *Industrial*  
531 *Crops and Products*, *142*, 111873. <https://doi.org/10.1016/j.indcrop.2019.111873>
- 532 Diomande, D., Antheaume, I., Leroux, M., Lalande, J., Balayssac, S., Remaud, G.S., Tea, I. (2015). Multi-  
533 element, multi-compound isotope profiling as a means to distinguish the geographical and  
534 varietal origin of fermented cocoa (*Theobroma cacao* L.) beans. *Food Chemistry*, *188*, 576–582.  
535 <https://doi.org/10.1016/j.foodchem.2015.05.040>
- 536 Drivelos, S.A., Georgiou, C.A. (2012). Multi-element and multi-isotope-ratio analysis to determine the  
537 geographical origin of foods in the European Union. *TrAC Trends in Analytical Chemistry*, *40*,  
538 38–51. <https://doi.org/10.1016/j.trac.2012.08.003>
- 539 Dziri, S., Casabianca, H., Hanchi, B., Hosni, K. (2014). Composition of garlic essential oil (*Allium sativum*  
540 L.) as influenced by drying method. *Journal of Essential Oil Research*, *26*, 91–96.  
541 <https://doi.org/10.1080/10412905.2013.868329>



- 542 El-Wakil, E.A., El-Sayed, M.M., Abdel-Lateef, E.E.-S. (2015). GC-MS Investigation of Essential oil and  
543 antioxidant activity of Egyptian White Onion (*Allium cepa* L.). *International Journal of Pharma*  
544 *Sciences and Research*, 6, 537–543.
- 545 Filippi, J.-J., Fernandez, X., Duñach, E. (2007). Volatile organosulfur compounds (VOSCs) in flavor  
546 chemistry. *Sciences des Aliments*, 27, 23–46.
- 547 Fourel, F., Lécuyer, C., Balter, V. (2015). New Frontiers for Sulfur Isotopic Analyses. *Procedia Earth and*  
548 *Planetary Science*, 13, 232–239. <https://doi.org/10.1016/j.proeps.2015.07.055>
- 549 Fourel, F., Martineau, F., Seris, M., Lécuyer, C. (2014). Simultaneous N, C, S stable isotope analyses  
550 using a new purge and trap elemental analyzer and an isotope ratio mass spectrometer. *Rapid*  
551 *Communications in Mass Spectrometry*, 28, 2587–2594. <https://doi.org/10.1002/rcm.7048>
- 552 Haghghat, M., Abdel-Mottaleb, M., Alhalabi, W. (2016). Discriminant Correlation Analysis: Real-Time  
553 Feature Level Fusion for Multimodal Biometric Recognition. *IEEE Transactions on Information*  
554 *Forensics and Security*, 11, 1984–1996. <https://doi.org/10.1109/TIFS.2016.2569061>
- 555 Halas, S., Szaran, J. (2001). Improved thermal decomposition of sulfates to SO<sub>2</sub> and mass spectrometric  
556 determination of  $\delta^{34}\text{S}$  of IAEA SO-5, IAEA SO-6 and NBS-127 sulfate standards. *Rapid*  
557 *Communications in Mass Spectrometry*, 15, 1618–1620. <https://doi.org/10.1002/rcm.416>
- 558 Hanousek, O., Brunner, M., Pröfrock, D., Irrgeher, J., Prohaska, T. (2016). The performance of single  
559 and multi-collector ICP-MS instruments for fast and reliable  $^{34}\text{S}/^{32}\text{S}$  isotope ratio  
560 measurements. *Analytical Methods*, 8, 7661–7672. <https://doi.org/10.1039/C6AY02177H>
- 561 Hoefs, J. (2018). *Stable Isotope Geochemistry* (8<sup>th</sup> edition), Springer Textbooks in Earth Sciences,  
562 Geography and Environment. Springer International Publishing. [https://doi.org/10.1007/978-](https://doi.org/10.1007/978-3-319-78527-1)  
563 [3-319-78527-1](https://doi.org/10.1007/978-3-319-78527-1)
- 564 Jolliffe, I.T. (2002). *Principal Component Analysis* (2<sup>nd</sup> Edition). Springer Series in Statistics.  
565 <https://doi.org/10.1007/b98835>
- 566 Jones, M.G., Hughes, J., Tregova, A., Milne, J., Tomsett, A.B., Collin, H.A. (2004). Biosynthesis of the  
567 flavour precursors of onion and garlic. *Journal of Experimental Botany*, 55, 1903–1918.  
568 <https://doi.org/10.1093/jxb/erh138>
- 569 Kelly, S.D., Heaton, K.D., Brereton, P. (2001). Deuterium/hydrogen isotope ratio measurement of water  
570 and organic samples by continuous-flow isotope ratio mass spectrometry using chromium as  
571 the reducing agent in an elemental analyser. *Rapid Communications in Mass Spectrometry*, 15,  
572 1283–1286. <https://doi.org/10.1002/rcm.303>
- 573 Kracht, O. (2011). *Simultaneous N, C and S Isotope Ratio Determination on a DELTA V Isotope Ratio MS*  
574 *using a Flash Elemental Analyzer*
- 575 Krueger, H.W., Reesman, R.H. (1982). Carbon isotope analyses in food technology. *Mass Spectrometry*  
576 *Reviews*, 1, 205–236. <https://doi.org/10.1002/mas.1280010302>
- 577 Kümmel, S., Horst, A., Gelman, F., Strauss, H., Richnow, H.H., Gehre, M. (2020). Simultaneous  
578 Compound-Specific Analysis of  $\delta^{33}\text{S}$  and  $\delta^{34}\text{S}$  in Organic Compounds by GC-MC-ICPMS Using  
579 Medium- and Low-Mass-Resolution Modes. *Analytical Chemistry*, 92, 14685-14692.  
580 <https://doi.org/10.1021/acs.analchem.0c03253>

- 581 Levet, A., Bordes, C., Clément, Y., Mignon, P., Chermette, H., Marote, P., Cren-Olivé, C., Lantéri, P.  
582 (2013). Quantitative structure–activity relationship to predict acute fish toxicity of organic  
583 solvents. *Chemosphere*, *93*, 1094–1103. <https://doi.org/10.1016/j.chemosphere.2013.06.002>
- 584 Mizota, C., Sasaki, A. (1996). Sulfur isotope composition of soils and fertilizers: Differences between  
585 Northern and Southern hemispheres. *Geoderma*, *71*, 77–93. [https://doi.org/10.1016/0016-7061\(95\)00091-7](https://doi.org/10.1016/0016-7061(95)00091-7)
- 587 Mnayer, D., Fabiano-Tixier, A.-S., Petitcolas, E., Hamieh, T., Nehme, N., Ferrant, C., Fernandez, X.,  
588 Chemat, F. (2014). Chemical Composition, Antibacterial and Antioxidant Activities of Six  
589 Essentials Oils from the Alliaceae Family. *Molecules*, *19*, 20034–20053.  
590 <https://doi.org/10.3390/molecules191220034>
- 591 Nehlich, O. (2015). The application of sulphur isotope analyses in archaeological research: A review.  
592 *Earth-Science Reviews*, *142*, 1–17. <https://doi.org/10.1016/j.earscirev.2014.12.002>
- 593 Nhu-Trang, T.-T., Casabianca, H., Grenier-Loustalot, M.-F. (2006a). Deuterium/hydrogen ratio analysis  
594 of thymol, carvacrol,  $\gamma$ -terpinene and p-cymene in thyme, savory and oregano essential oils by  
595 gas chromatography–pyrolysis–isotope ratio mass spectrometry. *Journal of Chromatography*  
596 *A*, *1132*, 219–227. <https://doi.org/10.1016/j.chroma.2006.07.088>
- 597 Nhu-Trang, T.-T., Casabianca, H., Grenier-Loustalot, M.-F. (2006b). Authenticity control of essential oils  
598 containing citronellal and citral by chiral and stable-isotope gas-chromatographic analysis.  
599 *Analytical and Bioanalytical Chemistry*, *386*, 2141–2152. <https://doi.org/10.1007/s00216-006-0842-2>
- 601 Norman A.-L, Anlauf, K., Hayden, K., Thompson, B., Brook, J.R., Li, S.-M., Bottenheim, J. (2006). Aerosol  
602 sulphate and its oxidation on the Pacific NW coast: S and O isotopes in PM<sub>2.5</sub>. *Atmospheric*  
603 *Environment*, *40*, 2676–2689. <https://doi.org/10.1016/j.atmosenv.2005.09.085>
- 604 Park, J.H., Choi, S.-H., Bong, Y.-S. (2019). Geographical origin authentication of onions using stable  
605 isotope ratio and compositions of C, H, O, N, and S. *Food Control*, *101*, 121–125.  
606 <https://doi.org/10.1016/j.foodcont.2019.02.023>
- 607 Pianezze, S., Perini, M., Bontempo, L., Ziller, L., D’Archivio, A.A. (2019). Geographical discrimination of  
608 garlic (*Allium Sativum* L.) based on Stable isotope ratio analysis coupled with statistical  
609 methods: The Italian case study. *Food and Chemical Toxicology*, *134*, 110862.  
610 <https://doi.org/10.1016/j.fct.2019.110862>
- 611 Ramirez, D.A., Locatelli, D.A., González, R.E., Cavagnaro, P.F., Camargo, A.B. (2017). Analytical methods  
612 for bioactive sulfur compounds in *Allium*: An integrated review and future directions. *Journal*  
613 *of Food Composition and Analysis*, *61*, 4–19. <https://doi.org/10.1016/j.jfca.2016.09.012>
- 614 Robinson, B.W. (1995). Sulphur isotope standards (Proceedings of a Consultants’ Meeting held in  
615 Vienna, 1–3. Dec. 1993. No. IAEA-TECDOC-825).
- 616 Russell, W.A., Papanastassiou, D.A., Tombrello, T.A. (1978). Ca isotope fractionation on the Earth and  
617 other solar system materials. *Geochimica et Cosmochimica Acta*, *42*, 1075–1090.  
618 [https://doi.org/10.1016/0016-7037\(78\)90105-9](https://doi.org/10.1016/0016-7037(78)90105-9)
- 619 Said-Ahmad, W., Amrani, A. (2013). A sensitive method for the sulfur isotope analysis of dimethyl  
620 sulfide and dimethylsulfoniopropionate in seawater. *Rapid Communications in Mass*  
621 *Spectrometry*, *27*, 2789–2796. <https://doi.org/10.1002/rcm.6751>

- 622 Said-Ahmad, W., Wong, K., Mcnall, M., Shawar, L., Jacksier, T., Turich, C., Stankiewicz, A., Amrani, A.  
623 (2017). Compound-Specific Sulfur Isotope Analysis of Petroleum Gases. *Analytical Chemistry*,  
624 89, 3199–3207. <https://doi.org/10.1021/acs.analchem.6b05131>
- 625 Satyal, P., Craft, J.D., Dosoky, N.S., Setzer, W.N. (2017). The Chemical Compositions of the Volatile Oils  
626 of Garlic (*Allium sativum*) and Wild Garlic (*Allium vineale*). *Foods*, 6.  
627 <https://doi.org/10.3390/foods6080063>
- 628 Schipilliti, L., Dugo, P., Bonaccorsi, I., Mondello, L. (2012). Authenticity control on lemon essential oils  
629 employing Gas Chromatography–Combustion-Isotope Ratio Mass Spectrometry (GC–C-IRMS).  
630 *Food Chemistry*, 131, 1523–1530. <https://doi.org/10.1016/j.foodchem.2011.09.119>
- 631 Soleiman-Beigi, M., Mohammadi, F. (2012). A novel copper-catalyzed, one-pot synthesis of symmetric  
632 organic disulfides from alkyl and aryl halides: potassium 5-methyl-1,3,4-oxadiazole-2-thiolate  
633 as a novel sulfur transfer reagent. *Tetrahedron Letters*, 53, 7028–7030.  
634 <https://doi.org/10.1016/j.tetlet.2012.10.016>
- 635 Tenenhaus, M. (1998). *La régression PLS : théorie et pratique*. Editions TECHNIP.
- 636 Tocmo, R., Lin, Y., Huang, D. (2014). Effect of Processing Conditions on the Organosulfides of Shallot  
637 (*Allium cepa* L. *Aggregatum* Group). *Journal of Agricultural and Food Chemistry*, 62, 5296–5304.  
638 <https://doi.org/10.1021/jf500739n>
- 639 Yang, L., Tong, S., Zhou, L., Hu, Z., Mester, Z., Meija, J. (2018). A critical review on isotopic fractionation  
640 correction methods for accurate isotope amount ratio measurements by MC-ICP-MS. *Journal*  
641 *of Analytical Atomic Spectrometry*, 33, 1849–1861. <https://doi.org/10.1039/C8JA00210J>
- 642 Ye, C.-L., Dai, D.-H., Hu, W.-L. (2013). Antimicrobial and antioxidant activities of the essential oil from  
643 onion (*Allium cepa* L.). *Food Control*, 30, 48–53.  
644 <https://doi.org/10.1016/j.foodcont.2012.07.033>
- 645 Zhang, B., Zheng, Z., Liu, N., Liu, P., Qiu, Z., Qiao, X. (2021). Effect of different combined mechanical  
646 and thermal treatments on the quality characteristics of garlic paste. *Journal of Food Science*  
647 *and Technology*, 58, 1061–1071. <https://doi.org/10.1007/s13197-020-04620-1>
- 648
- 649



650

651

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

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

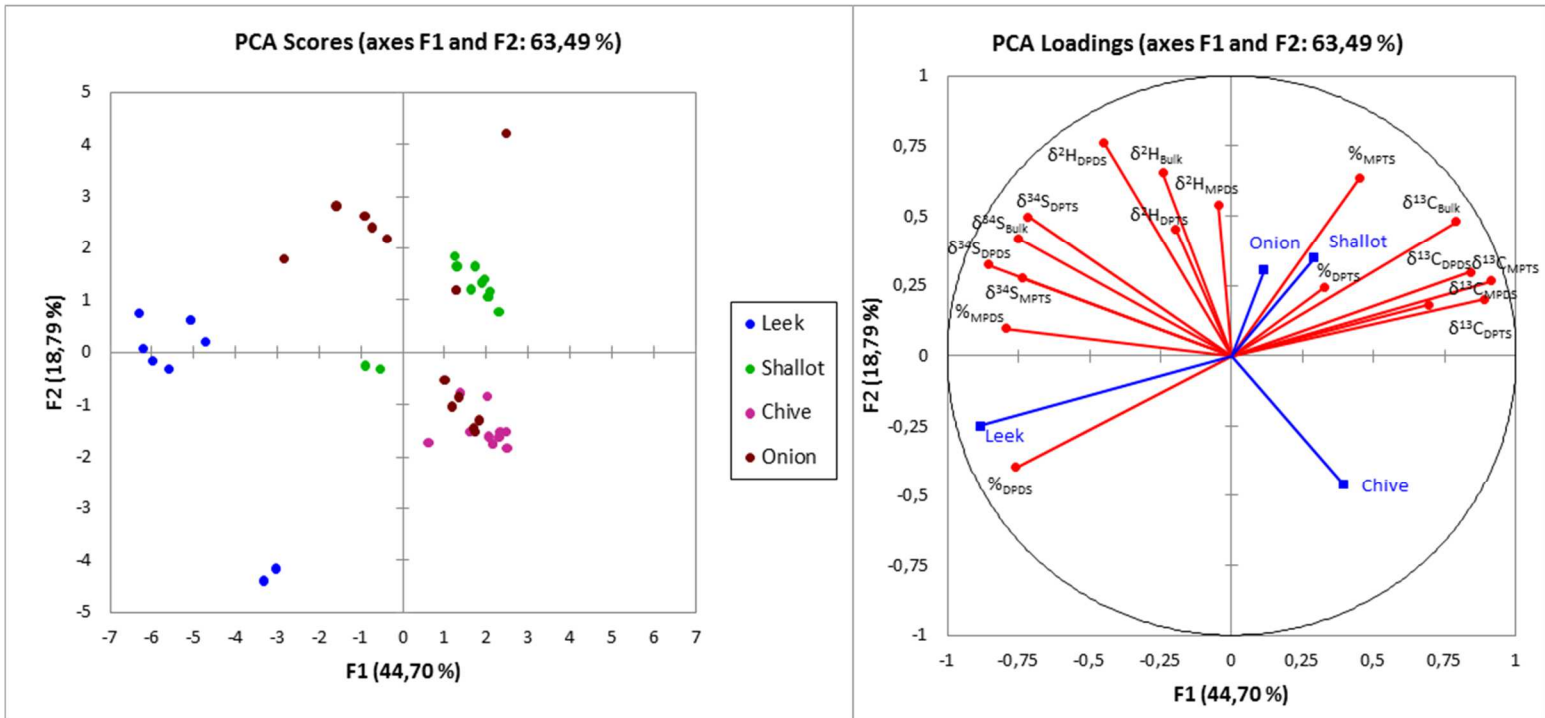
654 10, and 20% of synthetic DPDS (in red), and one essential oil adulterated with 5, 10, and 20%

655 of synthetic DPTS.

656

657

658

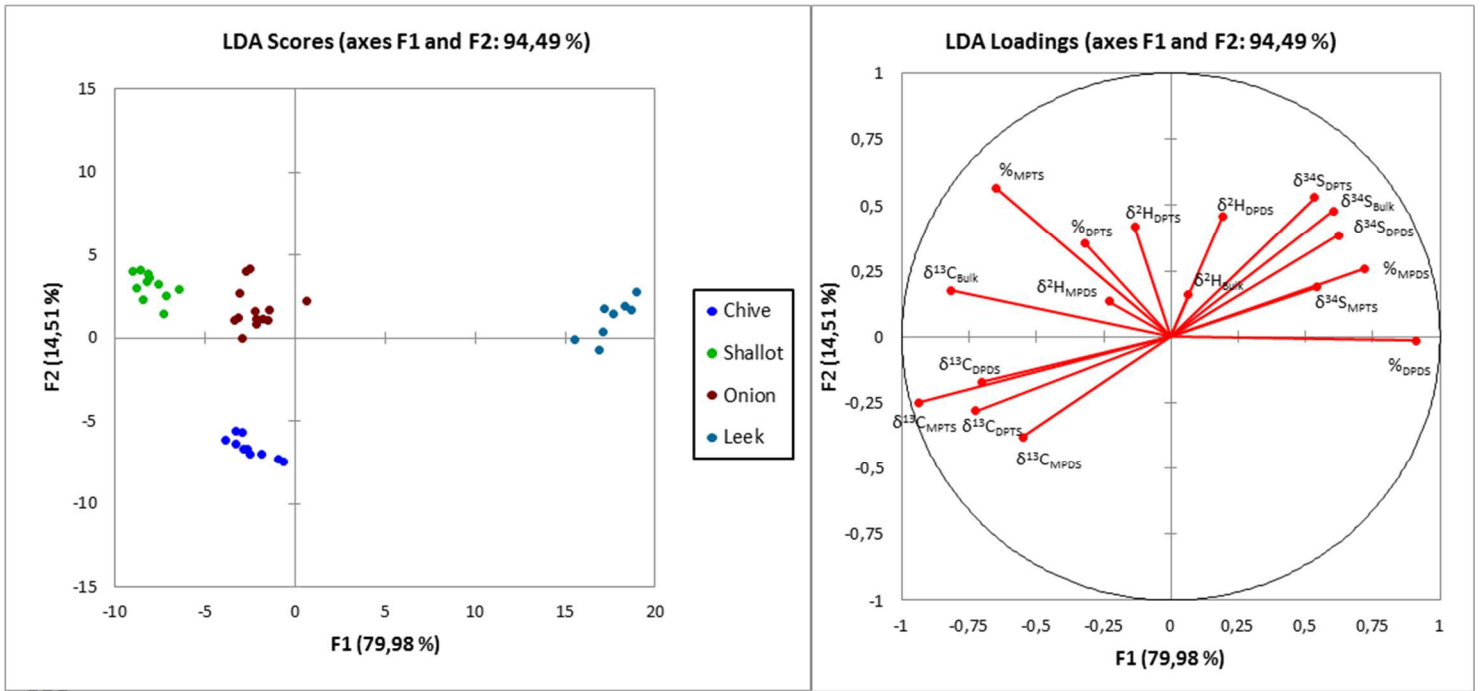


660

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.

664

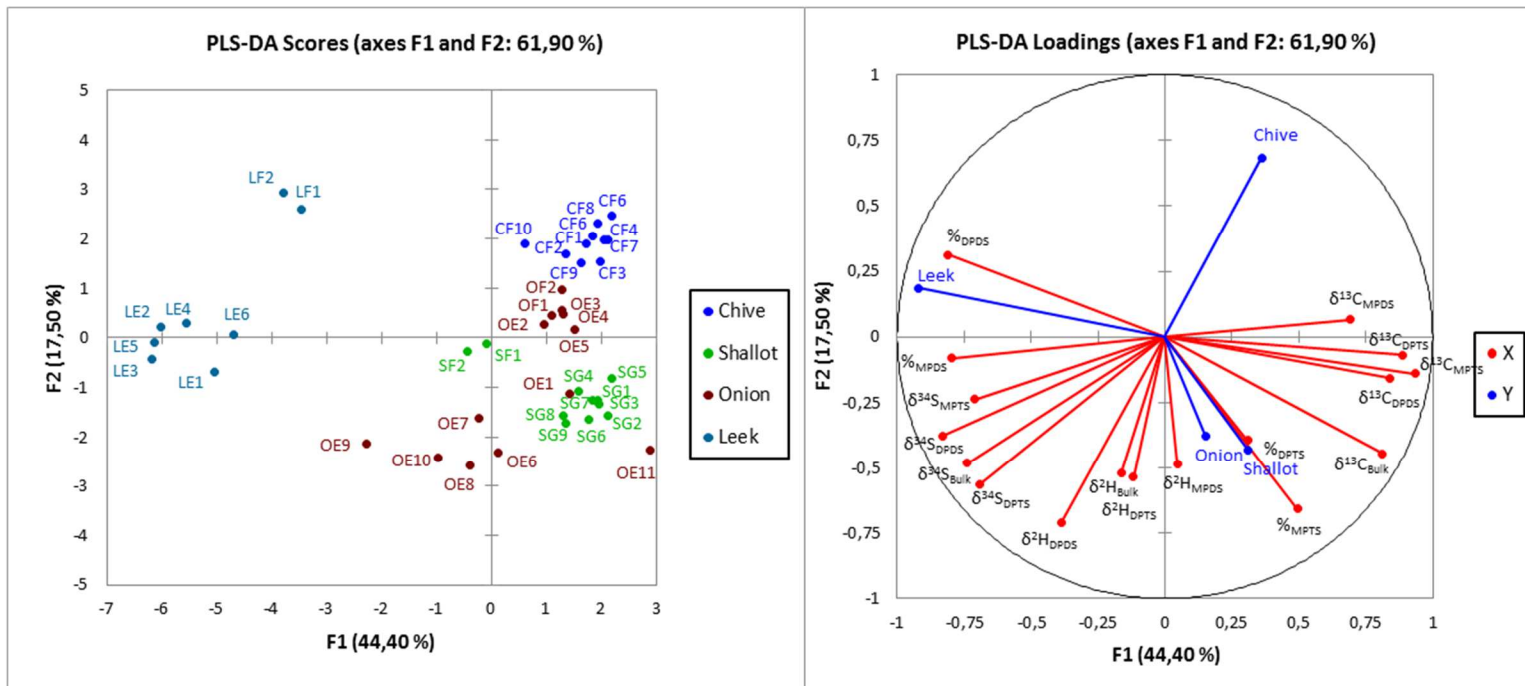
665



667

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

681

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%

685