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## Authentication of the naturalness of wintergreen (Gaultheria genus) essential oils by gas chromatography, isotope ratio mass spectrometry and radiocarbon assessment

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### ► To cite this version:

Aurélien Cuchet, Patrick Jame, Anthony Anchisi, Frédéric Schiets, Christine Oberlin, et al.. Authentication of the naturalness of wintergreen (Gaultheria genus) essential oils by gas chromatography, isotope ratio mass spectrometry and radiocarbon assessment. *Industrial Crops and Products*, 2019, 142, pp.111873. 10.1016/j.indcrop.2019.111873 . hal-02364897

HAL Id: hal-02364897

<https://hal.science/hal-02364897v1>

Submitted on 20 Jul 2022

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1 **Authentication of the naturalness of wintergreen (*Gaultheria* genus) essential**  
2 **oils by gas chromatography, isotope ratio mass spectrometry and**  
3 **radiocarbon assessment**

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15

16 **Abstract**

17 The essential oil of wintergreen, which is extracted by steam distillation from *Gaultheria* genus  
18 leaves, is mainly used in aromatherapy. However, due to its adulteration easiness with synthetic  
19 material, it is necessary to control samples naturalness with accuracy. The purpose of this work was  
20 to develop a methodology to authenticate the essential oil of wintergreen. Wintergreen essential oil  
21 is composed of more than 99% methyl salicylate. This aromatic ester can be easily synthesized and  
22 used to adulterate wintergreen essential oil. Authentic wintergreen essential oil can be distinguished  
23 from adulterated oils by examining their compositions. The detection of methyl salicylate synthetic  
24 marker compounds (methyl 4-hydroxybenzoate, dimethyl 4-hydroxyisophthalate or dimethyl 2-  
25 hydroxyisophthalate) or the absence of several naturally occurring minor secondary metabolites  
26 (ethyl salicylate and vitispirane) contribute to the authentication. Isotopic values of bulk wintergreen  
27 essential oil have also been determined using an isotope ratio mass spectrometer (IRMS): the  $\delta^{13}\text{C}$   
28 values of authentic samples range from -36.78 to -33.36‰, the  $\delta^2\text{H}$  values range from -173 to -115‰  
29 and the  $\delta^{18}\text{O}$  values range from -1.3 to 5.7‰. However, these analytical methods cannot account for  
30 the natural variability in the essential oils. To determine the boundaries of the natural isotopic  
31 values,  $^{14}\text{C}$  radioactive isotope activity assessment was undertaken, which allows for the  
32 determination of the genuineness of samples that are not assessed using multistable isotope  
33 approaches. Additional studies evaluating  $^{14}\text{C}$  activity of a noncompliant sample using IRMS identified  
34 a  $^{14}\text{C}$ -labeled synthetic methyl salicylate adulteration.

35 **Keywords**

36 **Wintergreen essential oil, Methyl salicylate, Chemical composition, Isotope ratio mass**  
37 **spectrometry, Authentication, Radiocarbon activity**

38 **1. Introduction**

39 Natural raw materials such as essential oils are obtained from the extraction of vegetal products and  
40 are currently widely used in food products, cosmetics (including fragrances), pharmaceuticals, and

41 aromatherapy products due to their medicinal and olfactory properties. These products are  
42 sometimes susceptible to fraud: essential oils sold to consumers do not always possess their original  
43 compositions, despite being labeled 100% pure and natural. Suppliers resort to adulterating their  
44 products by adding low-cost products to their natural materials to improve the product quality, to  
45 increase the raw material volume, or for economic reasons. One of the main adulterations of  
46 essential oils is the addition of less expensive synthetic compounds. Thus, it is necessary to develop  
47 methods of authentication to identify such practices in order to empower consumers to be sure of  
48 the natural quality of their purchases and to enable suppliers to prove the authenticity of their  
49 products.

50 The essential oil of wintergreen can be obtained by the steam distillation of leaves from different  
51 species of the genus *Gaultheria*. These essential oils are produced mainly in high altitude locations in  
52 North America (Canada), China, and Nepal, and the harvest period usually lasts from May until  
53 September. The botanical sources of *Gaultheria* that are most commonly used to produce  
54 wintergreen essential oils are *procumbens* and *fragrantissima*, because these species yield a high  
55 concentration of methyl salicylate. However, wintergreen essential oils obtained from other species  
56 of *Gaultheria* such as *yunnanensis* and *hispidula* are commercialized as well. The steam distillation  
57 yield of leaves is less than 2% (Bantawa et al., 2011). The quantity of essential oils produced in China  
58 is estimated to be between 20 and 70 Mg. Farmers are interested in growing the essential oil plants  
59 only where prices are high enough to make the crop economically viable and falling prices will quickly  
60 lead to a change to the cultivation of other more valuable crops.

61 Due to its very high concentration in the aromatic ester methyl salicylate, wintergreen is notable of  
62 the field of essential oils. Methyl salicylate represents more than 99% of the composition of most  
63 wintergreen species after a bidistillation process (Cong et al., 2015; Nikolić et al., 2013; Singh et al.,  
64 2018). The aromatic and herbaceous wintergreen fragrance can be used in perfumery and in the  
65 cosmetic and food industries, but the main uses are in aromatherapy, oral care, and natural

66 preservatives for cosmetics. Many medical ointments used in aromatherapy are composed of  
67 wintergreen essential oil (Mukhopadhyay et al., 2016) because of its analgesic and anti-inflammatory  
68 properties (Delplancq, 2015; Liu et al., 2013; Michel et al., 2014; Park et al., 2011), which efficiently  
69 relieve pain caused by muscle fever (Ribnicky et al., 2003). Antispasmodic, vasodilators, and liver  
70 stimulant properties of the essential oil have also been reported (Franchomme et al., 2012).  
71 Wintergreen possesses other properties that are useful for various applications. For example,  
72 wintergreen essential oil is used as a biopesticide (Dayan et al., 2009) in food crops and food  
73 packaging because of its antifeedant properties (Jeyasankar, 2012; Prakash and Prakash, 2015), and it  
74 is used as a natural preservative in cosmetic products because of its antioxidant and antibacterial  
75 activities (Pandey et al., 2017).

76 Suppliers desiring to increase their profits illegally might be inclined to adulterate their natural  
77 product by adding synthetic materials that are less expensive. For the 2018 harvest, the price of  
78 essential oil from China was between 50 and 65 euros per kilogram. The most common method of  
79 wintergreen essential oil adulteration is the addition or substitution of synthetic methyl salicylate.  
80 This synthetic molecule can be prepared through several different chemical pathways (Fig. 1.). The  
81 most common pathway starts with salicylic acid that has been obtained by conducting a Kolbe  
82 Schmitt reaction (Luo et al., 2016) using phenol, which can be synthesized from petroleum products  
83 using the Hock process (also known as cumene process). The methylation of salicylic acid leads to  
84 synthetic methyl salicylate (D'Souza and Nagaraju, 2007; Iijima and Yamaguchi, 2008; Su et al., 2009).  
85 Another previously reported method to obtain salicylic acid employs the Lowig oxidation process  
86 with salicylaldehyde as the starting material. Salicylaldehyde can be obtained in several ways, such as  
87 via the Reimer Tiemann reaction, but salicylaldehyde also occurs naturally in meadowsweet flowers  
88 (Olennikov et al., 2016). This process is more expensive than the classical salicylic acid approach, and  
89 an additional step is required. The most economical current method to synthesize methyl salicylate is  
90 the hydrolysis of aspirin (acetylsalicylic acid), which is industrially produced from salicylic acid (Hartel  
91 and Hanna, 2009; Montes et al., 2006).

92 Chiral analysis cannot be performed on methyl salicylate to guarantee the natural authenticity of the  
93 product because the methyl salicylate molecule does not possess an asymmetrical carbon. Previous  
94 studies used isotope ratio mass spectrometry (IRMS) to obtain pertinent isotopic data for methyl  
95 salicylate. Synthetically produced methyl salicylate possesses  $\delta^{13}\text{C}$  values ranging from -28.32 to -  
96 30.84‰ and  $\delta^2\text{H}$  values from -51 to -97‰, whereas natural essential oil values obtained from birch  
97 range from -33.11 to -33.66‰ for  $\delta^{13}\text{C}$  and from -56 to -163‰ for  $\delta^2\text{H}$  (Culp and Noakes, 1992). An  
98 authentication method for methyl salicylate in black tea using  $^{13}\text{C}$  GC-C-IRMS was published in 1999  
99 (Weinert et al., 1999). The most recently developed naturalness authentication method used ERETIC  
100 analysis via SNIF-NMR (Le Grand et al., 2005).

101 The objective of this study was to develop an accurate methodology to ensure the naturalness  
102 authentication of the wintergreen essential oil. Samples from various providers were studied and  
103 compared with commercially produced and laboratory-synthesized methyl salicylate. Different  
104 studies were carried out including a characterization of the essential oils, a determination of the  
105 synthetic precursors and impurities, the analysis of stable isotope ratios of methyl salicylate, and an  
106 assessment of the radiocarbon activity.

## 107 **2. Material and Methods**

### 108 **2.1. *Samples information***

109 Twenty-one *Gaultheria procumbens* L. essential oils samples were provided by Albert Vieille  
110 (Vallauris, France), originating from China and Nepal. Thirty samples of *Gaultheria procumbens* L.,  
111 *Gaultheria fragrantissima* Wall., and *Gaultheria leucocarpa* var. *yunnanensis* (Franch.) T.Z. Hsu & R.C.  
112 Fang. essential oils were obtained from different suppliers and originated from China and Canada.  
113 Eleven commercial samples were purchased from a pharmacy. Two authentic samples of *Gaultheria*  
114 *fragrantissima* Wall. and *Gaultheria leucocarpa* var. *yunnanensis* (Franch.) T.Z. Hsu & R.C. Fang.  
115 essential oils, originating in China (Yunnan province) and Nepal, were supplied by Young Living (Lehi,  
116 UT, USA). Five synthetic methyl salicylate commercial samples were purchased from several suppliers

117 (Sigma Aldrich (Saint Louis, MO, USA), Thermo Fisher Scientific (Waltham, MA, USA), and TCI (Tokyo,  
118 Japan)). Several methyl salicylates were made in our laboratory via different synthetic pathways. One  
119 sample was obtained from aspirin (Sigma Aldrich), three were synthesized with salicylic acid (TCI and  
120 Cooper, Melun, France) as a starter material, and one sample was made from salicylaldehyde (Sigma  
121 Aldrich).

## 122 **2.2. Gas chromatography (GC-FID) analysis**

123 GC analyses were carried out using a gas chromatograph 6890N (Agilent Technologies, Santa Clara,  
124 CA, USA) equipped with a flame ionization detector (FID), with the detector temperature set at  
125 250°C. Separation of the volatile compounds was performed on a HP-1 capillary column (50 m\*0.320  
126 mm\*0.52 µm, Agilent Technologies) and a HP-Innowax capillary column (60 m\*0.320 mm\*0.50 µm).  
127 The GC conditions for the HP-1 column were programmed as follows: 1 µL of pure sample was  
128 injected into the GC in the split mode (120:1) with the injector temperature set at 250°C. The carrier  
129 gas was helium at a constant flow rate of 1.5 mL/min. The initial oven temperature was held at 80°C  
130 for 2 min, ramped to 220°C at a rate of 2°C/min, and then to 295°C at a rate of 10°C/min and held for  
131 30 min. The GC conditions for the Innowax column were programmed as follows: 1 µL of pure sample  
132 was injected into the GC in the split mode (100:1) with the injector temperature set at 250°C. The  
133 carrier gas was helium at a constant flow rate of 1.3 mL/min. The initial oven temperature was held  
134 at 60°C for 2 min, ramped to 245°C at a rate of 2°C/min and held for 35 min. Identification of the  
135 volatile compounds was achieved using the Kovats Index determined with relative retention indices  
136 from C5 to C30 alkanes.

## 137 **2.3. Gas chromatography-mass spectrometry (GC-MS) analysis**

138 GC-MS analyses were performed on a gas chromatograph 6890N GC System (Agilent Technologies)  
139 interfaced with a 5975 inert XL MSD mass spectrometer with electron impact ionization (70 eV). Each  
140 1 µL pure sample was injected into the GC in the split mode (120:1), with the injector temperature  
141 set at 250°C. Helium was used as the carrier gas with a constant flow rate of 1.2 mL/min. Separation

142 of the volatile compounds was achieved using a HP-1 capillary column (50 m\*0.200 mm\*0.50  $\mu$ m,  
143 Agilent Technologies). The initial oven temperature was held at 60°C for 2 min and then ramped at a  
144 rate of 3°C/min to reach a final temperature of 320°C and then held for 10 min. The mass scanning  
145 range was from 35 to 350 m/z in TIC. The SIM parameters were as follows: the targeted mass ions  
146 (m/z) were 91 (tropylium ion), 121 (M - CH<sub>3</sub>O) and 152 (molecular ion) from 36 to 44 min to detect  
147 methyl 4-Hydroxybenzoate and 120 (ortho rearrangement), 147 (double loss of CH<sub>3</sub>OH (methanol)  
148 and CH<sub>3</sub>O), 178 (loss of CH<sub>3</sub>OH), and 210 (molecular ion) from 44 to 50 min to detect dimethyl 4-  
149 hydroxyisophthalate and dimethyl 2-hydroxyisophthalate. NIST library and lab-made library,  
150 combined with retention time, retention indices, literature references, and standards injections were  
151 used to identify the molecules.

#### 152 **2.4. Elemental Analysis –Isotope Ratio Mass Spectrometry (EA-IRMS)**

153 High purity carbon dioxide (CO<sub>2</sub>) 99.998% (4.8 Messer), hydrogen (H<sub>2</sub>) 99.999% (5.0 Messer), and  
154 carbon monoxide (CO) 99.997% (4.7 Messer) were used as the working reference gases. Helium flow  
155 at 99.999% was used as the carrier gas.

156 International standards IAEA CH7 (polyethylene foil:  $\delta^{13}\text{C}$  (‰) -31.82,  $\delta^2\text{H}$  (‰) -100.3), USGS40 (L-  
157 glutamic acid:  $\delta^{13}\text{C}$  (‰) -26.39), and IAEA-601 (Benzoic acid:  $\delta^{18}\text{O}$  (‰) +23.3) supplied by the IAEA  
158 (International Agency of Atomic Energy, Vienna, Austria) were used for the calibration of all the  
159 studied samples. All sample values were obtained in duplicate and were verified by bracketing the  
160 analytical sequence with the international standards every sixth analysis.

161 The carbon isotopic analyses ( $\delta^{13}\text{C}$  value) of the synthetic standards were carried out using a Flash HT  
162 elemental analyzer connected to a Delta V Plus via a Conflo V interface (Thermo Fisher Scientific,  
163 Bremen, Germany). The samples were weighted in tin capsules (5 X 8, Sántis Analytical AG, Teufen,  
164 Switzerland) and dropped into the combustion unit, which was maintained at 915°C in a stream of  
165 helium with a flow rate of 90 mL.min<sup>-1</sup> and pulsed with oxygen. The flash combustion (approximately  
166 1800°C) caused the complete oxidation of carbon to CO<sub>2</sub>. The gases flowed through the reduction



167 furnace, which was held at 600°C and included a copper wire where the excess oxygen was trapped.  
168 The reduction furnace was followed by a water trap containing anhydrous magnesium perchlorate.  
169 The Flash HT elemental analyzer was connected to the isotope ratio mass spectrometer Thermo  
170 Delta V Plus via an online interface (Conflo IV). This intermediary device provided for automatic  
171 adjustment of the sample dilution and generation of the reference gas pulses, which facilitated the  
172 isotopic determination.

173 The  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  measurements of the synthetic standards were carried out using a TC-EA elemental  
174 analyzer linked to a Delta V Plus via a Conflo V interface (all Thermo Fisher Scientific). Samples  
175 weighed in silver capsules (3.3 X 5 mm, Säntis Analytical AG, Teufen, Switzerland) were dropped into  
176 the pyrolysis unit, which was held at 1400°C. This high-temperature reactor consisted of an inner  
177 glassy carbon tube half-filled with glassy carbon granules integrated into an external ceramic tube.  
178 Hydrogen and oxygen were converted to  $\text{H}_2$  and  $\text{CO}$  and separated on a gas chromatography column  
179 prior to introduction into the IRMS via the same interface (Conflo IV). The interface, as previously  
180 described, provided steady and regular reference gas pulses.

181 The  $\delta^{13}\text{C}$  values (‰) were calibrated relative to Vienna Pee Dee Bee Belemnite (VPDB) using two  
182 multipulse injections of the  $\text{CO}_2$  reference gas.  $\text{H}_2$  and  $\text{CO}$  reference gases were used in the same way  
183 for the determination of the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values (‰), and the results were calibrated to Standard  
184 Mean Ocean Water (SMOW).

185 Isotopic results are expressed in delta unit ( $\delta$ ) according to the following formula:

186 
$$\delta(^{13}\text{C}/^{12}\text{C}) = \left[ \frac{R(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{R(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right]$$

187 where the  $\delta^{13}\text{C}$  values are given within a maximum standard deviation (SD) of  $\pm 0.21\%$ ,  $\delta^2\text{H}$  within  
188  $\text{SD}=\pm 5\%$ , and  $\delta^{18}\text{O}$  within  $\text{SD}=\pm 0.6\%$ .

## 189 **2.5. Radiocarbon analysis via Liquid Scintillation Counting (LSC)**

190 All liquid samples were burned in a pyrocombustion mineralization device (Mineralyseur ERALY) to  
191 obtain CO<sub>2</sub>. This device consists of a quartz combustion tube and two furnaces in series. An  
192 automatic syringe pump type device makes the injection of liquid samples possible. The first furnace,  
193 mounted at 400°C, is swept by a flow of nitrogen and triggers the vaporization of the liquid  
194 produced. The second furnace, maintained at 750°C, is crossed by an oxygen flow and ensures the  
195 oxidation of the gas resulting from the vaporization. The carbon dioxide formed is collected and  
196 chemically transformed into benzene (C<sub>6</sub>H<sub>6</sub>). It is in this liquid form that its carbon-14 content will be  
197 measured by counting in liquid scintillation. The samples are measured in liquid scintillation counters  
198 with very low background with TRI-CARB 1500, 2000CA/LL, 2260XL, 2560TR/XL, 3170TR/SL, and  
199 2900TR Packard and Perkin Elmer counters. A sample is counted four or five times during 1000 min in  
200 different counters each time. The counting rate of the background noise of the counter is subtracted  
201 (net count rate) from the counting rate of the sample. The <sup>14</sup>C activity is obtained by normalizing the  
202 net count rate from the count rate of the reference standard (NBS Oxalic Acid). All the values of  
203 modern carbon are in percent modern and are corrected from isotopic fractionation, using the data  
204 of stable isotopes (ratio <sup>13</sup>C/<sup>12</sup>C) measured on CO<sub>2</sub> obtained from the combustion of the sample.  
205 Values are given within a maximum SD of ± 0.6%.

### 206 **3. Results and discussion**

#### 207 **3.1. *Essential oil composition via GC-FID and GC-MS***

208 The compositions of wintergreen essential oils and commercial and laboratory-made synthetic  
209 methyl salicylates are listed in Table 1 with relative Kovats indices. The major compounds are also  
210 represented in Fig. 2.

211 The synthetic methyl salicylates exhibited a high purity, nearly 99%, but several impurity peaks can  
212 be seen in their compositions, which can be attributed to precursors and secondary products of the  
213 methyl salicylate synthesis. Seven compounds were identified. Phenol (**16**) was the initial synthesis  
214 precursor of the Kolbe Schmitt reaction. Ethyl salicylate (**2**), methyl o-cresotinate (**18**), methyl 2-

215 methoxybenzoate (**17**), and methyl 4-hydroxybenzoate (**13**) were all produced during the  
216 methylation of salicylic acid. Dimethyl 4-hydroxyisophthalate (**15**) and dimethyl 2-  
217 hydroxyisophthalate (**14**) were secondary products of a Kolbe Schmitt reaction impurity. The other  
218 minor compounds were primarily from residual solvents. The ex-salicylaldehyde sample was the only  
219 synthetic methyl salicylate without the presence of dimethyl 4-hydroxyisophthalate, which was the  
220 second major component in the other six samples. The absence of this compound was expected,  
221 since it did not come from the carboxylation of phenol, which was identified in trace amounts in the  
222 ex-salicylaldehyde sample.

223 In the set of wintergreen essential oils, a total of 62 compounds were identified, covering more than  
224 99% of the total peak area of the 64 samples analyzed. The methyl salicylate represented, among the  
225 natural essential oils, the majority of the volatile compounds and represented 97.36 to 99.71% of the  
226 total amount. Most of the other components were found in trace amounts and were not  
227 systematically present in the wintergreen oils. Natural variability, due to botanical varieties and  
228 geographical origins, induces important differences among natural wintergreen compositions. The  
229 minority compounds that were the most prevalent were limonene (**1**) (Mean value  $\pm$  SD= 0.04  $\pm$   
230 0.06%), ethyl salicylate (**2**) (0.09  $\pm$  0.07%), linalool (**3**) (0.05  $\pm$  0.02%), alpha-pinene (**4**) (0.01  $\pm$  0.02%),  
231 geraniol (**5**) (0.02  $\pm$  0.02%), benzyl alcohol (**6**) (0.02  $\pm$  0.01%), vitispirane (**7**) (0.02  $\pm$  0.01%), eugenol  
232 (**8**) (0.02  $\pm$  0.02%), beta pinene (**9**) (0.01  $\pm$  0.01%), safrole (**10**) (0.01  $\pm$  0.04%), para cymene (**11**) (0.01  
233  $\pm$  0.01%), trans-beta caryophyllene (**12**) (0.01  $\pm$  0.01%), and phenol (**16**) (0.01  $\pm$  0.00%).

234 Methyl 4-hydroxybenzoate, dimethyl 4-hydroxyisophthalate, and dimethyl 2-hydroxyisophthalate  
235 have only been identified in several adulterated essentials oils, in which many secondary metabolites  
236 found in authentic wintergreens are in low abundance, such as ethyl salicylate (0.02  $\pm$  0.02%) and  
237 vitispirane (0.00  $\pm$  0.01%), whereas these samples have similar methyl salicylate concentrations  
238 compared with those of natural oils. These three molecules can potentially be used as synthesis  
239 marker compounds, but they have not always been detected, probably because of an addition of

240 impurity-free methyl salicylate or another remedy. A single-ion monitoring (SIM) method was  
241 developed for GC-MS in order to monitor trace amounts of these adulteration marker components in  
242 all the essential oils in this study.

### 243 **3.2. Stable-Isotopes analysis**

244 The determination of minor peaks establishing that the process of production is synthesis is not so  
245 easy to show, when natural wintergreen is partly diluted. In this scheme, IRMS will be relevant to  
246 conclude. The isotopic measurement is performed in 5 min allowing fast and cheap analyses when  
247 the assessment of raw materials need to be done.

248 The  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  isotopic values of the 64 wintergreen essential oils samples are summarized  
249 in Table 2 and shown in a 3D-plot representation (Fig. 3.). Because methyl salicylate constitutes more  
250 than 99% of the wintergreen essential oil, isotopic measurements have been conducted using an  
251 elemental analyzer connected to an isotope ratio mass spectrometer (EA-IRMS) for bulk stable  
252 isotope analysis (BSIA).

253 The natural methyl salicylates exhibited significantly different values of the three isotope ratios  
254 investigated,  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$ , compared with those of the synthetic samples. The 3D plot of the  
255 isotopic values clearly shows two distinct areas: one for the authentic wintergreen samples and the  
256 other one for the synthetic methyl salicylates and adulterated wintergreens. The samples with at  
257 least one identified synthetic marker compound demonstrated values close to those of the synthetic  
258 methyl salicylates. Conversely, the essential oils without markers possessed isotopic deltas in the  
259 same range as those of the natural essential oils. Only two samples were found in the synthetic range  
260 that did not have any synthetic markers, but they were located far away from the natural essential  
261 oils: an adulteration was clearly demonstrated. All three isotopes were highly correlated, as shown in  
262 Fig. 4.

263 According to Culp et al. (1992), natural methyl salicylates have  $\delta$ -values, measured in five samples,  
264 ranging between -33.11 and -33.66‰ for  $^{13}\text{C}$  and from -56 to -163‰ for  $^2\text{H}$ . The results obtained in  
265 this study of 41 authentic wintergreen oils indicated that the authentic range is tighter for  $\delta^2\text{H}$ , with  
266 an average value of -135‰ and a maximal value of -115‰, and different  $\delta^{13}\text{C}$  natural values, with an  
267 upper mean value of -34.98‰. The synthetic normal range of  $\delta^2\text{H}$  is wider than the deltas of the  
268 other isotopes, notably depending on the synthetic origin of the methyl salicylates, as the  $\delta^2\text{H}$   
269 isotopic fractionation is larger. Clear differentiation is distinguished between natural and synthetic  $\delta$ -  
270 values: synthetic methyl salicylates show more positive  $\delta$ -values, especially on the  $\delta^{18}\text{O}$ , which is  
271 consistent with previous work on isotopic patterns of phenylpropanoids (Schmidt et al., 2001).  
272 Isotopic ratios of adulterated essential oils have been determined to be between natural and  
273 synthetic ones.

### 274 **3.3. Radiocarbon activity assessment**

#### 275 *3.3.1. Analysis of the wintergreen $^{14}\text{C}$ activity*

276 The analysis of the  $^{14}\text{C}$  radiocarbon activity was conducted to determine the percentages of  
277 biosourced carbon in the samples. A natural product is composed of 100% biosourced carbon, which  
278 is equivalent to an activity of  $13.7 \pm 0.1$  dpm/gC in 2017 (Disintegrations per minute per gram of  
279 carbon), whereas a synthetic compound from petrochemistry does not have any  $^{14}\text{C}$  activity. An  
280 adulteration with synthetic methyl salicylate will result in a mixture of natural and synthetic  
281 products, which will result in an intermediate radiocarbon activity. This analysis can be carried out  
282 with liquid scintillation counting (LSC) or with accelerator mass spectrometry (AMS) methods.

283 The determination of the percentage of biosourced carbon accurately establishes the percentage of  
284 adulteration in analyzed samples. Furthermore, this percentage helps confirm the naturalness of  
285 samples in order to verify the results from GC-FID, GC-MS, and EA-C/P-IRMS and to determine more  
286 precise authenticity ranges.

287 Twenty samples from the analytical panel were chosen to be analyzed with LSC. Biosourced carbon  
288 percentages of the 20 samples are listed in Table 3. The naturalness range of the analyzed samples  
289 was large, from the 100% synthetic-free to the 100% nonnatural. The synthetic commercial methyl  
290 salicylate (Sample 20) had no  $^{14}\text{C}$  activity, as expected, because it is usually prepared from petroleum  
291 products. Wintergreen essential oils that have a higher activity than natural products are probably a  
292 mixture of branches and leaves of the *Gaultheria*, because woody tissues are generally older vegetal  
293 raw materials, resulting in a higher  $^{14}\text{C}$  activity due to higher radioactivity in the past years. These  
294 values have been rounded to 100%. Surprisingly, Sample 17 showed 100% biosourced carbon,  
295 whereas stable isotopic data classified this sample in the synthetic origin category. Further  
296 investigations were carried out to explain this anomaly.

### 297 3.3.2. Correlation study

298 A linear correlative study (pairwise correlation) was conducted to determine which variables from  
299 the 3 isotopes and the 73 secondary metabolites that constituted the wintergreen essential oils were  
300 the best correlated with the percentage of biosourced carbon. Sample 17 was excluded from this  
301 statistical test.

302 Results determined for the 20 samples analyzed in LSC show that  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  are the isotope  
303 values that best described the naturalness of the samples with a Pearson correlation coefficient  
304 greater than 0.95 in absolute value. Although  $\delta^2\text{H}$  is also strongly correlated with  $^{14}\text{C}$  activity, its  
305 higher measurement uncertainty and its more significant isotopic dispersion in the various synthetic  
306 samples resulted in a lower linear correlation coefficient. Fig. 5. shows the correlation between  $\delta^{18}\text{O}$   
307 and  $\delta^{13}\text{C}$  and the biosourced carbon contents.

308 The metabolite study confirmed that the presence of synthetic impurities is correlated with the  
309 naturalness of the samples. Methyl 4-hydroxybenzoate and dimethyl 4-hydroxyisophthalate both had  
310 a Pearson correlation coefficient higher than 0.90 in absolute value. Furthermore, some alternative  
311 metabolites were directly correlated with the percentage of biosourced carbon (with  $r > |0.85|$ ). A

312 small amount of vistispirane (<0.20%) and ethyl salicylate (<0.06%) could potentially indicate the  
313 addition of a synthetic material. However, the concentration of these compounds can be extremely  
314 variable from one wintergreen to another, as geographical origin and variety affect the composition  
315 of essential oils.

### 316 3.3.3. <sup>14</sup>C-labeled-compound addition

317 Sample 17 demonstrated a natural radiocarbon activity, despite being clearly adulterated, as its  $\delta^{13}\text{C}$ ,  
318  $^2\text{H}$  and  $^{18}\text{O}$  values were in the synthetic area. One hypothesis is that this sample had been  
319 adulterated with a <sup>14</sup>C marked compound. <sup>14</sup>C labeled phenol, methanol, salicylic acid, and methyl  
320 salicylate are available on the market. The methyl salicylate molecule is composed of 8 carbons. Each  
321 of these carbons can potentially be <sup>14</sup>C labeled. To demonstrate this adulteration, a saponification  
322 was conducted to remove the methyl and produce salicylic acid. This chemical reaction was carried  
323 out on samples 1 and 17 with the addition of sodium hydroxide and methanol by heating under  
324 reflux. After methanol removal and acidification, a white powder of salicylic acid was obtained.  
325 Radiocarbon activity was assessed on both salicylic acids and compared with the parent wintergreen.  
326 The ex-sample 1 (1S) salicylic acid <sup>14</sup>C activity was not altered by the saponification, whereas ex-  
327 sample 17 (17S) salicylic acid exhibited a biosourced carbon percentage greater than 115%. These  
328 observations indicate that the removed carbon did not have any radioactive activity and that the  
329 produced salicylic acid had a higher radioactive activity than any natural material. Therefore, this  
330 wintergreen essential oil was indeed adulterated with the addition of synthetic methyl salicylate and  
331 some <sup>14</sup>C-labeled methyl salicylate. A 1 mL solution of 250 microcurie 6C-ring labeled methyl  
332 salicylate can mimic approximately 40500 kg of 100% synthetic methyl salicylate to appear as a 100  
333 % biosourced content, despite its synthetic origin. Use of <sup>14</sup>C-labeled salicylic acid or methanol is also  
334 a feasible approach to adulterating synthetic methyl salicylate during the Kolbe Schmitt reaction. This  
335 fraud could not be detected with radiocarbon activity measurement methods, and only the use of  
336 IRMS highlighted this adulteration possibility.

337 **4. Conclusion**

338 The multianalytical approach presented in this study demonstrated several methods to clearly  
339 authenticate the naturalness of wintergreen essential oils.

340 An abnormally low concentration of some secondary metabolites in wintergreen, such as ethyl  
341 salicylate or vitispirane, can indicate that adulteration has occurred. However, this marker is only an  
342 indicator, as compositions differ between each species. The detection of resultant impurities from  
343 the synthesis of methyl salicylate (methyl 4-hydroxybenzoate, dimethyl 4-hydroxyisophthalate, and  
344 dimethyl 2-hydroxyisophthalate) is another indicator of fraud. The presence of these impurities is  
345 proof of the addition of synthetic material.

346 Multielement isotopic analysis with IRMS can also assess the compliance of wintergreen. The  
347 advantages of this method are its rapidity and its inexpensive price. The minimal values of  
348 acceptability of the 3 isotope deltas were determined by applying an empirical law (mean + 3\*SD)  
349 considering that the values of the 41 authentic essential oils followed a unimodal normal  
350 distribution. The results of these calculations showed that 99.87% of the authentic essential oils  
351 exhibited  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  isotopic values lower than -32.87‰, -101‰, and 5.9‰, respectively.  
352 Wintergreen essential oils exhibiting a higher value for at least one stable isotope are likely to have  
353 been adulterated with synthetic material. However, natural isotopic variation is important, because  
354 the authenticity area of one isotope can be obscured by slightly adulterated essential oils.

355 Consequently, multielement isotopic analysis is needed to ensure the naturalness of the product.

356 The  $^{14}\text{C}$  radioactive activity is a powerful tool to precisely assess the authenticity of natural products,  
357 but this study also demonstrated the complementarity of using both stable and radioactive isotope  
358 techniques to authenticate wintergreen essential oils, as  $^{14}\text{C}$  assessment cannot detect  $^{14}\text{C}$ -labeled  
359 product additions, which have been only detected with IRMS.

360 **Acknowledgments**



361 This work is provided in partial fulfillment of the PhD requirements for Aurélien Cuchet who has  
362 received a grant from the Association Nationale de la Recherche et de la Technologie (ANRT, Paris,  
363 France). The research was supported by a contract between Albert Vieille SAS and ISA (Institut des  
364 Sciences Analytiques, Villeurbanne, France). The authors are grateful to Young Living for providing  
365 authentic samples.

## 366 **References**

- 367 Bantawa, P., Da Silva, J.A.T., Ghosh, S.K., Mondal, T.K., 2011. Determination of essential oil contents  
368 and micropropagation of *Gaultheria fragrantissima*, an endangered woody aromatic plant of  
369 India. J. Hortic. Sci. Biotechnol. 86, 479–485.  
370 <https://doi.org/10.1080/14620316.2011.11512792>
- 371 Cong, F., Joshi, K.R., Devkota, H.P., Watanabe, T., Yahara, S., 2015. Dhasingreoside: new flavonoid  
372 from the stems and leaves of *Gaultheria fragrantissima*. Nat. Prod. Res. 29, 1442–1448.  
373 <https://doi.org/10.1080/14786419.2014.1003932>
- 374 Culp, R.A., Noakes, J.E., 1992. Determination of synthetic components in flavors by  
375 deuterium/hydrogen isotopic ratios. J. Agric. Food Chem. 40, 1892–1897.  
376 <https://doi.org/10.1021/jf00022a033>
- 377 Dayan, F.E., Cantrell, C.L., Duke, S.O., 2009. Natural products in crop protection. Bioorg. Med. Chem.,  
378 Modern Trends in Agrochemistry 17, 4022–4034. <https://doi.org/10.1016/j.bmc.2009.01.046>
- 379 Delplancq, C., 2015. Dérivés salicylés issus de *Gaultheria yunnanensis* : activités anti-inflammatoires  
380 et analgésiques, toxicité et données pharmacocinétiques.
- 381 D'Souza, J., Nagaraju, N., 2007. Esterification of salicylic acid with methanol/dimethyl carbonate over  
382 anion-modified metal oxides. Indian J. Chem. Technol. 9.
- 383 Franchomme, P., Jollois, R., Pénéol, D., 2012. L'aromathérapie exactement : encyclopédie de  
384 l'utilisation thérapeutique des huiles essentielles : fondements, démonstration, illustration et  
385 applications d'une science médicale naturelle. Roger Jollois, Limoges.
- 386 Hartel, A.M., Hanna, J.M., 2009. Preparation of Oil of Wintergreen from Commercial Aspirin Tablets.  
387 A Microscale Experiment Highlighting Acyl Substitutions. J. Chem. Educ. 86, 475.  
388 <https://doi.org/10.1021/ed086p475>
- 389 Iijima, T., Yamaguchi, T., 2008. K<sub>2</sub>CO<sub>3</sub>-catalyzed direct synthesis of salicylic acid from phenol and  
390 supercritical CO<sub>2</sub>. Appl. Catal. -Gen. - APPL CATAL -GEN 345, 12–17.  
391 <https://doi.org/10.1016/j.apcata.2008.03.037>
- 392 Jeyasankar, A., 2012. Antifeedant, insecticidal and growth inhibitory activities of selected plant oils  
393 on black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). Asian Pac. J. Trop. Dis.  
394 2, S347–S351. [https://doi.org/10.1016/S2222-1808\(12\)60179-0](https://doi.org/10.1016/S2222-1808(12)60179-0)

- 395 Le Grand, F., George, G., Akoka, S., 2005. Natural Abundance <sup>2</sup> H-ERETIC-NMR Authentication of the  
396 Origin of Methyl Salicylate. J. Agric. Food Chem. 53, 5125–5129.  
397 <https://doi.org/10.1021/jf050385a>
- 398 Liu, W.-R., Qiao, W.-L., Liu, Z.-Z., Wang, X.-H., Jiang, R., Li, S.-Y., Shi, R.-B., She, G.-M., 2013.  
399 *Gaultheria*: Phytochemical and Pharmacological Characteristics. Molecules 18, 12071–12108.  
400 <https://doi.org/10.3390/molecules181012071>
- 401 Luo, J., Preciado, S., Xie, P., Larrosa, I., 2016. Carboxylation of Phenols with CO<sub>2</sub> at Atmospheric  
402 Pressure. Chem. - Eur. J. 22, 6798–6802. <https://doi.org/10.1002/chem.201601114>
- 403 Michel, P., Dobrowolska, A., Kicel, A., Owczarek, A., Bazylo, A., Granica, S., Piwowarski, J.,  
404 Olszewska, M., 2014. Polyphenolic Profile, Antioxidant and Anti-Inflammatory Activity of  
405 Eastern Teaberry (*Gaultheria procumbens* L.) Leaf Extracts. Molecules 19, 20498–20520.  
406 <https://doi.org/10.3390/molecules191220498>
- 407 Montes, I., Sanabria, D., García, M., Castro, J., Fajardo, J., 2006. A Greener Approach to Aspirin  
408 Synthesis Using Microwave Irradiation. J. Chem. Educ. 83, 628.  
409 <https://doi.org/10.1021/ed083p628>
- 410 Mukhopadhyay, M., Bantawa, P., Mondal, T.K., Nandi, S.K., 2016. Biological and phylogenetic  
411 advancements of *Gaultheria fragrantissima*: Economically important oil bearing medicinal  
412 plant. Ind. Crops Prod. 81, 91–99. <https://doi.org/10.1016/j.indcrop.2015.11.042>
- 413 Nikolić, M., Marković, T., Mojović, M., Pejin, B., Savić, A., Perić, T., Marković, D., Stević, T., Soković,  
414 M., 2013. Chemical composition and biological activity of *Gaultheria procumbens* L. essential  
415 oil. Ind. Crops Prod. 49, 561–567. <https://doi.org/10.1016/j.indcrop.2013.06.002>
- 416 Olennikov, D., Kashchenko, N., Chirikova, N., 2016. Meadowsweet Teas as New Functional Beverages:  
417 Comparative Analysis of Nutrients, Phytochemicals and Biological Effects of Four *Filipendula*  
418 Species. Molecules 22, 16. <https://doi.org/10.3390/molecules22010016>
- 419 Pandey, B.P., Thapa, R., Upreti, A., 2017. Chemical composition, antioxidant and antibacterial  
420 activities of essential oil and methanol extract of *Artemisia vulgaris* and *Gaultheria*  
421 *fragrantissima* collected from Nepal. Asian Pac. J. Trop. Med. 10, 952–959.  
422 <https://doi.org/10.1016/j.apjtm.2017.09.005>
- 423 Park, I.-K., Seo, S.-M., Kim, J., 2011. Nematicidal activity of plant essential oils and components from  
424 *Gaultheria fragrantissima* and *Zanthoxylum alatum* against the pine wood nematode,  
425 *Bursaphelenchus xylophilus*. Nematology 13, 87–93.  
426 <https://doi.org/10.1163/138855410X504907>
- 427 Prakash, K., Prakash, B., 2015. Assessment of Toxicity, Antifeedant Activity, and Biochemical  
428 Responses in Stored-Grain Insects Exposed to Lethal and Sublethal Doses of *Gaultheria*  
429 *procumbens* L. Essential Oil. J. Agric. Food Chem. 63, 10518–10524.  
430 <https://doi.org/10.1021/acs.jafc.5b03797>
- 431 Ribnicky, D.M., Poulev, A., Raskin, I., 2003. The Determination of Salicylates in *Gaultheria*  
432 *procumbens* for Use as a Natural Aspirin Alternative. J. Nutraceuticals Funct. Med. Foods 4,  
433 39–52. [https://doi.org/10.1300/J133v04n01\\_05](https://doi.org/10.1300/J133v04n01_05)
- 434 Schmidt, H.-L., Werner, R.A., Roßmann, A., 2001. 18O Pattern and biosynthesis of natural plant  
435 products. Phytochemistry 58, 9–32. [https://doi.org/10.1016/S0031-9422\(01\)00017-6](https://doi.org/10.1016/S0031-9422(01)00017-6)

- 436 Singh, V., Gunjan, Ali, M., 2018. Isolation of volatile constituents and biological studies of aerial parts  
437 of *Gaultheria procumbens* L. Int. J. Green Pharm. IJGP 11.  
438 <https://doi.org/10.22377/ijgp.v11i04.1359>
- 439 Su, X., Li, J., Xiao, F., Wei, W., Sun, Y., 2009. Esterification of Salicylic Acid with Dimethyl Carbonate  
440 over Mesoporous Aluminosilicate. Ind. Eng. Chem. Res. 48, 3685–3691.  
441 <https://doi.org/10.1021/ie801148v>
- 442 Weinert, B., Ulrich, M., Mosandl, A., 1999. GC-IRMS analysis of black Ceylon, Assam and Darjeeling  
443 teas. Z. Für Leb. -Forsch. A 208, 277–281. <https://doi.org/10.1007/s002170050417>
- 444
- 445

446 **Table 1.** Chemical compositions of presumed authentic wintergreen essential oils, adulterated ones,  
 447 synthetic and lab-made methyl salicylates

No	Compounds	CAS	KI HP1 <sup>a</sup>	KI Innowax <sup>b</sup>	% Total peak area		
					Authentic wintergreen essential oils (n=41)	Synthetic methyl salicylate (n=10)	Adulterated wintergreen essential oils (n=23)
					Min-Max	Min-Max	Min-Max
	Ethanol	64-17-5	480	937	0.00-1.37	0.00-0.03	0.00-2.74
	Acetone	67-64-1	489	827	0.00-0.03	0.00-0.02	0.00-0.02
	Hexanal	66-25-1	772	1097	0.00-0.01	nd	0.00-tr
	(E)-2-Hexenal	6728-26-3	822	1241	0.00-0.01	nd	0.00-tr
	(Z)-3-Hexenol-1	928-96-1	831	1397	0.00-0.09	0.00-tr	0.00-0.04
	(E)-2-Hexenol	928-95-0	840	1415	0.00-0.01	nd	0.00-0.01
	1-Hexanol	11-27-1	843	1360	0.00-0.04	0.00-tr	0.00-0.01
	Heptanal	11-71-7	875	1206	0.00-0.01	nd	0.00-tr
	2-Heptanol	543-49-7	879	1320	0.00-0.04	nd	0.00-0.02
	Benzaldehyde	100-52-7	929	1546	0.00-0.01	0.00-tr	0.00-tr
(4)	$\alpha$ -Pinene	80-56-8	932	1037	0.00-0.05	0.00-tr	0.00-0.59
	Camphene	79-92-5	945	1088	0.00-0.01	nd	0.00-0.01
	1-Heptanol	111-70-6	948	1463	0.00-tr	nd	0.00-0.02
(16)	Phenol	108-95-2	950	1981	0.00-0.02	0.00-0.12	0.00-0.06
	Sabinene	3387-41-5	966	1138	0.00-0.01	nd	0.00-0.03
(9)	$\beta$ -Pinene	127-91-3	972	1129	0.00-0.02	nd	0.00-0.15
	Myrcene	123-35-3	981	1177	0.00-0.01	nd	0.00-0.01
	(Z)-3-Hexenyl Acetate	3681-71-8	984	1334	0.00-0.01	nd	0.00-tr
	$\alpha$ -Phellandrene	99-83-2	999	1185	0.00-0.01	nd	0.00-tr
(6)	Benzyl Alcohol	100-51-6	1004	1881	tr-0.04	nd	0.00-0.02
	$\alpha$ -Terpinene	99-86-5	1011	1202	0.00-0.01	nd	0.00-0.01
(11)	p-Cymene	99-87-6	1013	1295	0.00-0.05	nd	0.00-0.05
(1)	Limonene	138-86-3	1023	1219	0.00-0.33	0.00-tr	0.00-0.80
	1,8-Cineole + $\beta$ - Phellandrene	470-82-6 555-10-2	1023	1229	0.00-0.01	nd	Nd
	$\gamma$ -Terpinene	99-85-4	1050	1269	0.00-0.03	nd	0.00-0.01
	1-Octanol	111-87-5	1051	1565	0.00-0.06	nd	0.00-0.01
	Terpinolene	586-62-9	1080	1308	0.00-0.01	nd	0.00-0.01
(3)	Linalool	78-70-6	1083	1561	tr-0.11	nd	0.00-0.46
	Camphor	76-22-2	1121	1556	0.00-0.01	nd	0.00-0.02
	(E)-Pinocarveol	1674-08-4	1124	1683	0.00-0.01	nd	0.00-0.01
	Isopulegol	7786-67-6	1131	1598	0.00-0.01	nd	0.00-0.02
	Ethyl Benzoate	93-89-0	1144	1688	0.00-0.01	nd	0.00-0.18
	Terpinen-4-ol	562-74-3	1162	1622	0.00-0.02	nd	0.00-tr
	Methyl Salicylate	119-36-8	1169	1809	97,36-99.71	96,36-99.93	94.94-99.76
	$\beta$ -Cyclocitral	432-25-7	1199	1640	0.00-0.01	nd	0.00-0.01
	Nerol	106-25-2	1210	1810	0.00-0.05	nd	0.00-0.01
	Neral	106-26-3	1215	1708	0.00-0.08	nd	0.00-0.03
(5)	Geraniol	106-24-1	1233	1866	0.00-0.06	nd	0.00-0.01
	Geranial	141-27-5	1242	1760	0.00-0.06	nd	0.00-tr
(2)	Ethyl Salicylate	118-61-6	1245	1821	0,05-0.50	0.00-0.04	0.00-0.05
(10)	Safrole	94-59-7	1263	1908	0.00-0.16	nd	0.00-0.01
(18)	Methyl o-Cresotinate	23287-26-5	1267	-	0.00-tr	0.00-tr	0.00-0.01
	Bornyl acetate	200-694-4	1270	1612	0.00-0.08	nd	Nd
(7)	Vitispirane	65416-59-3	1271	1504	0.02-0.03	nd	0.00-0.01
	Isobornyl acetate	125-12-2	1274	1618	0.00-0.02	nd	0.00-0.01

	Nagina Ktone (Dehydro Elshotzia Ketone)	6138-88-1	1276	1884	0.00-0.01	nd	Nd
(17)	Methyl 2-Methoxybenzoate	606-45-1	1295	2070	0.00-0.01	0.00-0.08	0.00-0.01
(8)	Eugenol	97-53-0	1327	2196	0.00-0.12	nd	0.00-0.04
	$\alpha$ -Terpenyl Acetate	80-26-2	1333	1724	0.00-0.03	nd	0.00-tr
	Methyl 2,4-Dihydroxybenzoate	2150-47-2	1346	-	0.00-0.01	0.00-0.02	0.00-tr
	$\alpha$ -Copaene	223-364-4	1377	1517	0.00-0.01	nd	0.00-tr
(13)	Methyl 4-Hydroxybenzoate	202-785-7	1410	-	Nd	0.00-0.06	0.00-0.04
(12)	(E)- $\beta$ -Caryophyllene	87-44-5	1419	1628	0.00-0.04	nd	0.00-0.10
	Aromadendrene	25246-27-9	1439	1634	0.00-0.05	nd	0.00-tr
	$\alpha$ -Humulene	6753-98-6	1452	1704	0.00-0.01	nd	0.00-0.01
	Eugenyl Acetate	93-28-7	1493	2287	0.00-0.03	nd	0.00-0.01
	$\Delta$ -Cadinene	478-76-1	1515	1785	0.00-0.03	nd	0.00-0.02
	(Z)-3-Hexenyl Benzoate	3681-71-8	1542	2160	0.00-0.01	nd	0.00-tr
(15)	Dimethyl 4-Hydroxyisophthalate	5985-24-0	1573	2468	Nd	0.00-0.19	0.00-0.68
(14)	Dimethyl 2-Hydroxyisophthalate	36669-06-04	1611	2583	Nd	0.00-tr	0.00-0.03
	Benzyl Salicylate	118-58-1	1834	2757	0.00-0.05	nd	0.00-0.04
	Total identified				99.89 $\pm$ 0.20	99.06 $\pm$ 1.33	99.85 $\pm$ 0.39

448 Compositions of essential oils have been determined with GC-FID and GC-MS analysis. Compounds are listed in order of  
449 their elution from HP1 column. Total identification data are reported as mean  $\pm$  standard deviation.

450 <sup>a</sup> KI: Kovats Index on HP1 column

451 <sup>b</sup> KI: Kovats Index on HP-Innowax column

452 n: Number of samples, nd: Non-detected, tr: Traces (Compounds detected below 0.01%)

453

454 **Table 2.**  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  values of methyl salicylate from various origins determined by EA-C/P-  
 455 IRMS

Isotopic values	Authentic wintergreen essential oils (n=41)				Synthetic methyl salicylate (n=10)				Adulterated wintergreen essential oils (n=23)			
	Min (‰)	Max (‰)	Mean (‰)	SD (‰)	Min (‰)	Max (‰)	Mean (‰)	SD (‰)	Min (‰)	Max (‰)	Mean (‰)	SD (‰)
$\delta^{13}\text{C}$	-36,78	-33,36	-34.98	0.70	-32,09	-27,71	-29.49	1.49	-32.37	-27,45	-29.42	1.36
$\delta^2\text{H}$	-173	-115	-135	11	-87	63	-54	44	-99	7	-56	28
$\delta^{18}\text{O}$	-1,3	5.7	1.3	1.5	19,5	32.0	23.3	4.0	8.6	29,2	19.0	4.4

456 n: Number of samples

457 SD: Standard deviation

458 EA-C/P-IRMS: Elemental Analysis – Combustion / Pyrolysis – Isotope Ratio Mass Spectrometry

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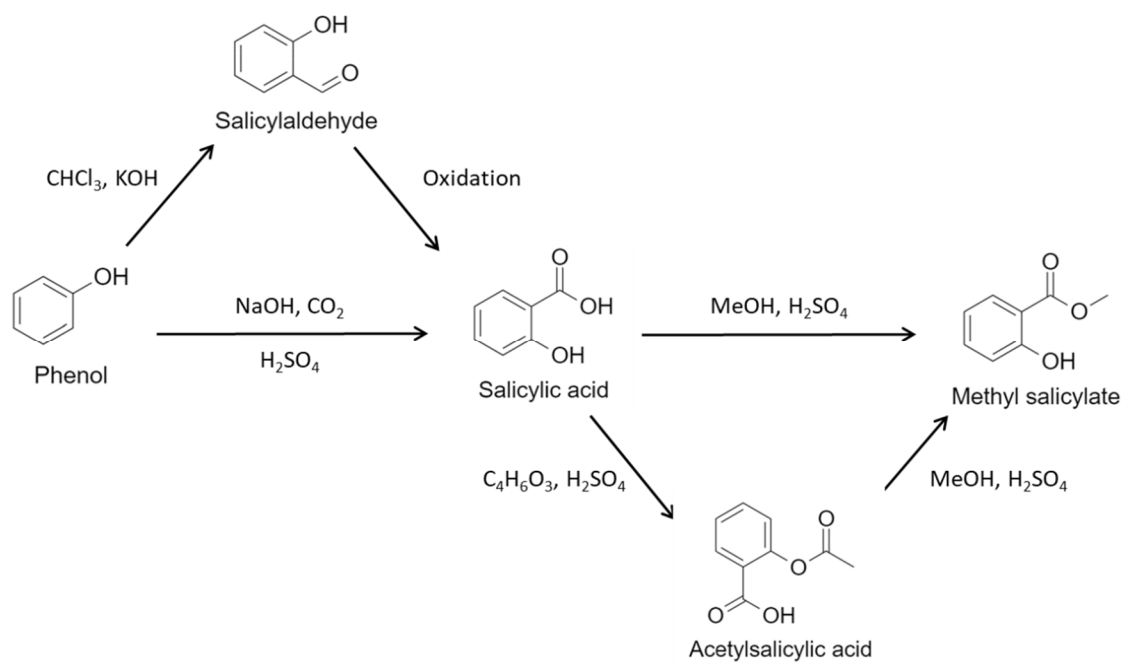
461 **Table 3.** Percentage of bio-sourced carbon determined by LSC, along with  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$  values  
462 determined by EA-C/P-IRMS, and GC-FID relative peak area percentage of ethyl salicylate, vitispirane,  
463 methyl 4-hydroxybenzoate and dimethyl 4-hydroxyisophthalate on 19 samples of wintergreen  
464 essential oils (Sample numbers 1 to 19) and one commercial methyl salicylate (Sample number 20).  
465 Pairwise correlation values between bio-sourced carbon % and other variables are also referenced.  
466 Percentage of bio-sourced carbon determined by LSC on salicylic acid samples (1S and 17S) obtained  
467 from samples 1 and 17.

Sample number	Product	LSC	EA-C/P-IRMS			GC-FID (% relative peak area)			
		Biosourced carbon (%)	$\delta^{13}\text{C}$ (‰)	$\delta^2\text{H}$ (‰)	$\delta^{18}\text{O}$ (‰)	Ethyl salicylate	Vitispirane	Methyl-4-hydroxybenzoate	Dimethyl 4-hydroxyisophthalate
1	Wintergreen	92.9	-34.63	-133	1.6	0.09	0.02	0.00	0.00
1S	Salicylic Acid	92.8	-	-	-	-	-	-	-
2	Wintergreen	96.4	-34.99	-119	0.7	0.09	0.02	0.00	0.00
3	Wintergreen	36.2	-29.80	-92	15.0	0.03	0.01	0.01	0.17
4	Wintergreen	100.0	-34.95	-134	0.0	0.06	0.02	0.00	0.00
5	Wintergreen	0.0	-29.18	-43	20.4	0.01	0.00	0.03	0.23
6	Wintergreen	7.4	-29.39	7	20.0	0.02	0.00	0.02	0.14
7	Wintergreen	14.0	-29.75	-13	17.9	0.02	0.00	0.03	0.24
8	Wintergreen	49.1	-31.89	-59	12.8	0.03	0.01	0.02	0.15
9	Wintergreen	28.7	-30.10	-23	16.5	0.02	0.00	0.03	0.21
10	Wintergreen	0.0	-28.41	-46	19.8	0.01	0.01	0.02	0.11
11	Wintergreen	93.5	-34.16	-128	4.4	0.06	0.02	0.00	0.00
12	Wintergreen	100.0	-34.39	-129	1.0	0.10	0.02	0.00	0.00
13	Wintergreen	14.6	-29.17	-17	18.9	0.02	0.00	0.03	0.24
14	Wintergreen	26.8	-30.26	-30	17.1	0.02	0.00	0.02	0.11
15	Wintergreen	27.9	-30.18	-34	17.0	0.02	0.00	0.02	0.12
16	Wintergreen	100.0	-35.63	-137	2.8	0.08	0.02	0.00	0.00
17	Wintergreen	104.7	-28.29	-78	20.0	0.01	0.00	0.00	0.00
17S	Salicylic Acid	116.5	-	-	-	-	-	-	-
18	Wintergreen	100.0	-35.84	-135	3.0	0.12	0.02	0.00	0.00
19	Wintergreen	100.0	-34.65	-135	1.2	0.15	0.02	0.00	0.00
20	Methyl Salicylate	0.0	-28.33	-71	21.7	0.01	0.00	0.02	0.19
Pairwise correlation (Pearson correlation coefficient <i>r</i> )			-0.99	-0.90	-0.99	0.89	0.93	-0.92	-0.90

468 LSC: Liquid Scintillation Counting

469 EA-C/P-IRMS: Elemental Analysis – Combustion / Pyrolysis – Isotope Ratio Mass Spectrometry

470

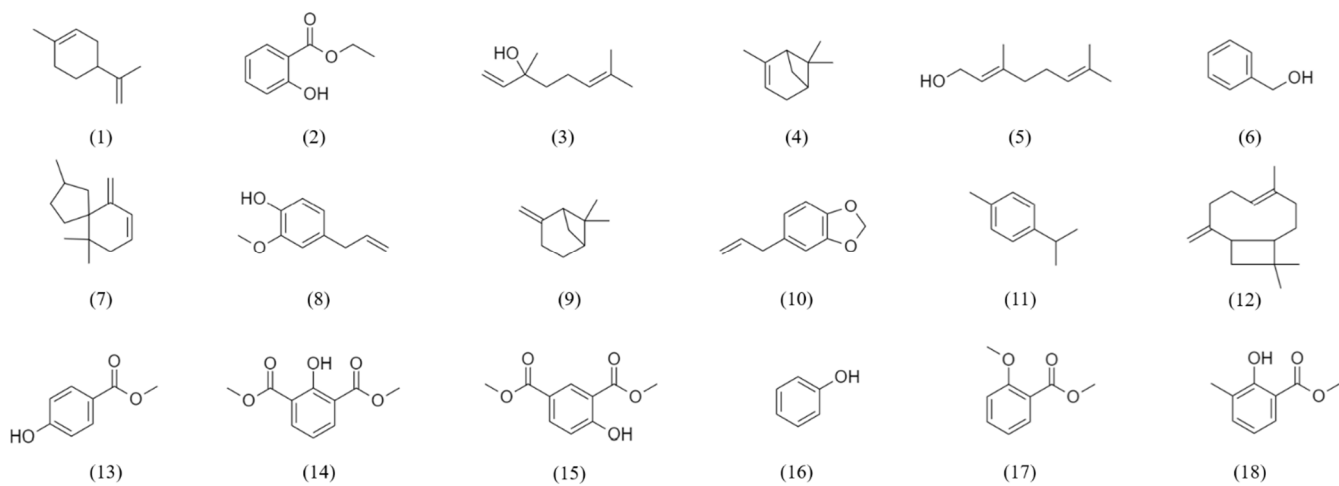


471

472 **Fig. 1.** Principal synthetic paths to synthesize methyl salicylate

473





474

475 **Fig. 2.** Structure of major compounds of the essential oil of wintergreen and of resultant impurities

476 from methyl salicylate synthesis. (1) Limonene; (2) ethyl salicylate; (3) linalool; (4)  $\alpha$ -pinene; (5)

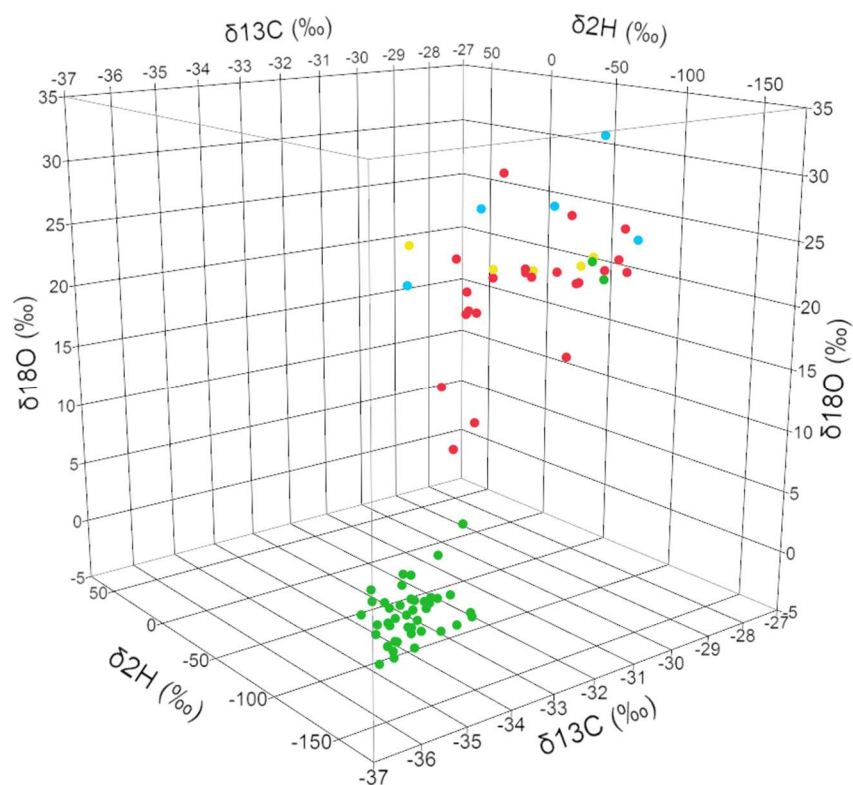
477 geraniol; (6) benzyl alcohol; (7) vitispirane; (8) eugenol; (9)  $\beta$ -pinene; (10) safrole; (11) p-cymene; (12)

478 (E)- $\beta$ -caryophyllene; (13) methyl 4-hydroxybenzoate; (14) dimethyl 2-hydroxyisophthalate; (15)

479 dimethyl 4-hydroxyisophthalate; (16) phenol; (17) methyl 2-methoxybenzoate; (18) methyl o-

480 cresotinate

481

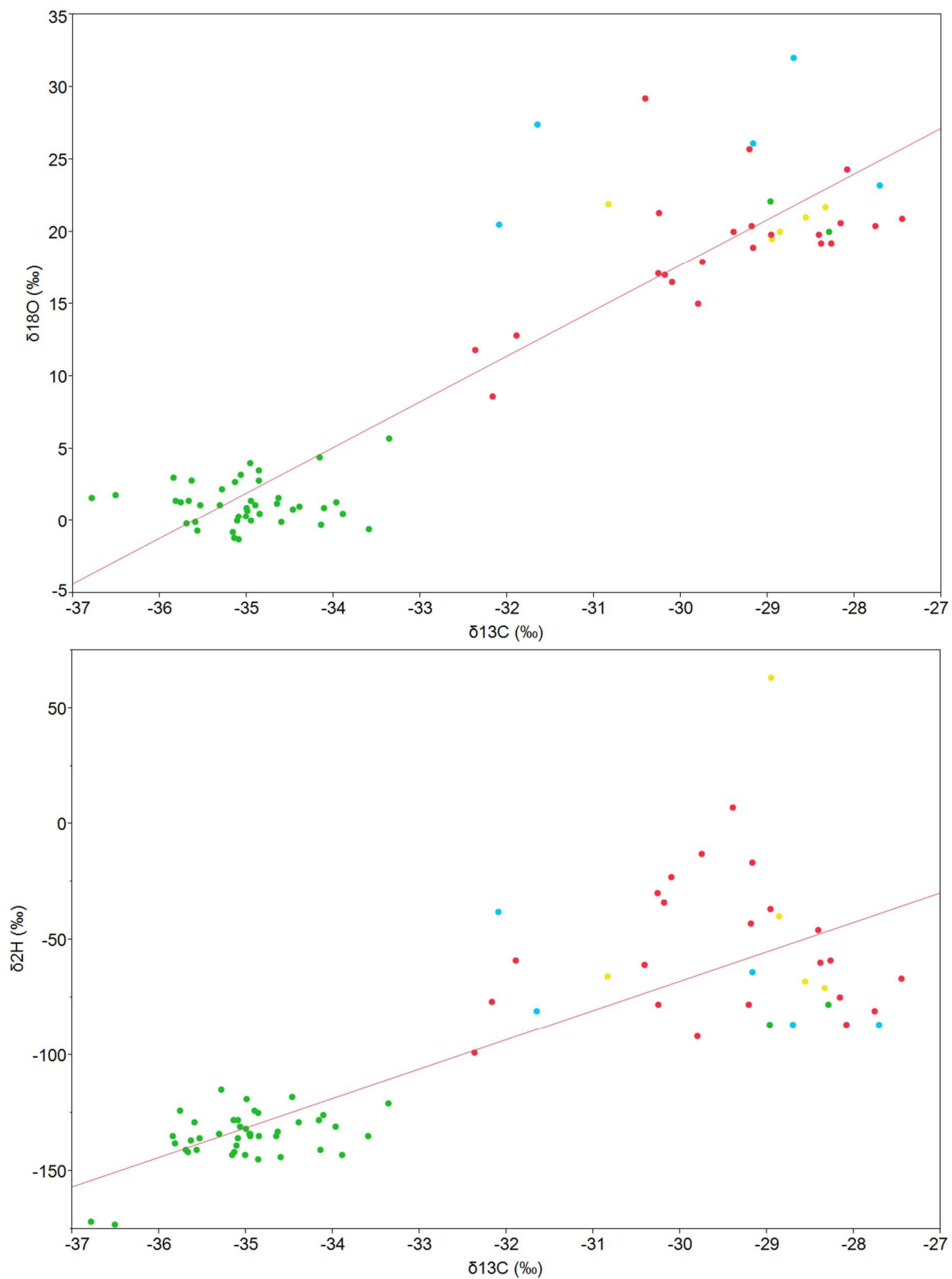


482

483 **Fig. 3.** EA-C/P-IRMS (Elemental Analysis – Combustion / Pyrolysis – Isotope Ratio Mass Spectrometry)

484 multi-element isotopic analysis of methyl salicylate from different origins: lab-made methyl  
 485 salicylates (in blue); commercial methyl salicylates (in yellow); wintergreen essential oils with at least  
 486 one synthetic marker compound (in red); wintergreen essential oils with no synthetic impurities (in  
 487 green). Each sample has been analyzed at least in duplicate:  $\delta$  values are a mean result of both  
 488 determined results.

489



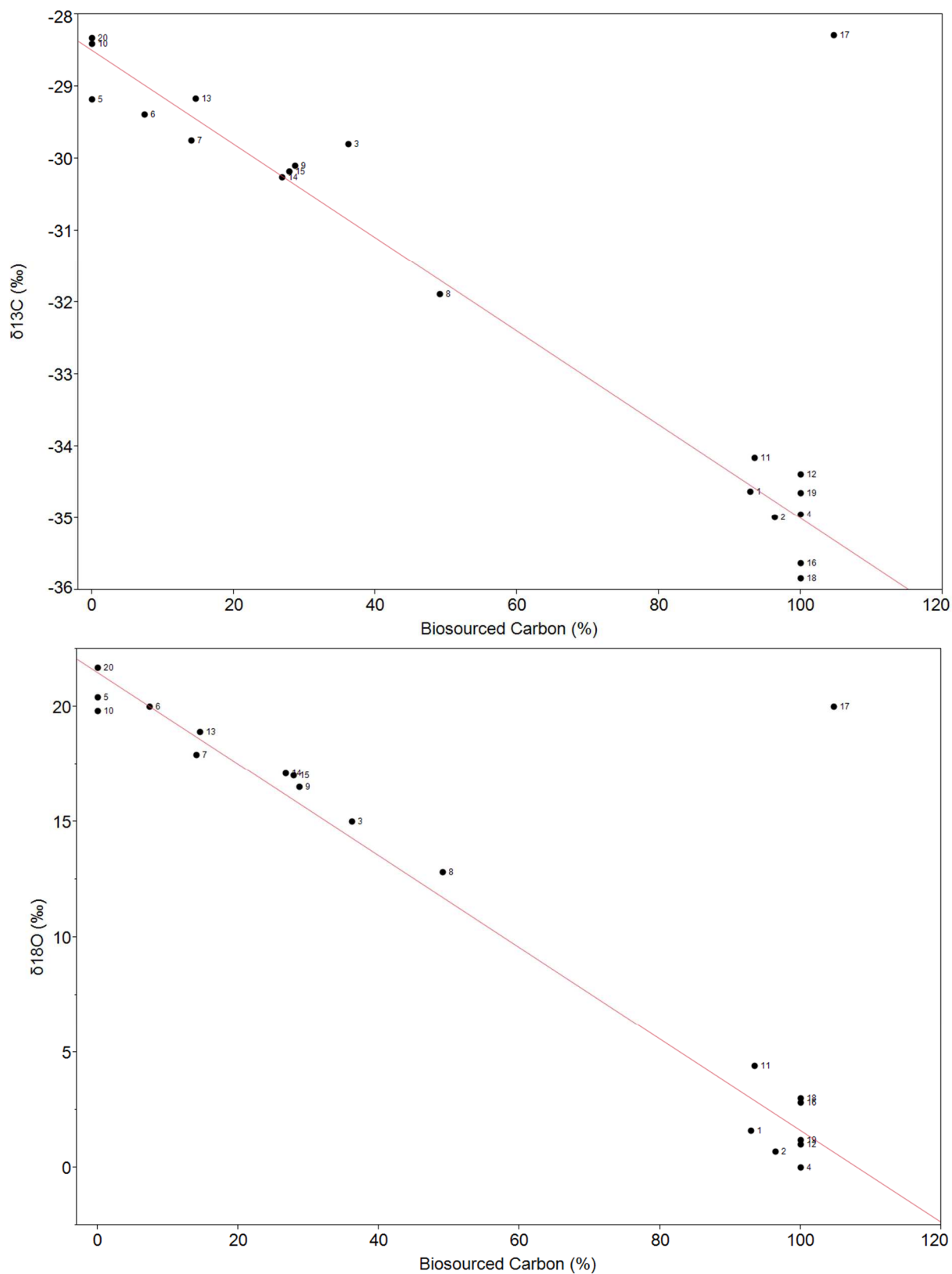
490

491 **Fig. 4.** Correlation between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values ( $r^2=0.85$ ) and between  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  values ( $r^2=0.67$ )

492 of methyl salicylates from different origins: lab-made methyl salicylates (in blue); commercial methyl

493 salicylates (in yellow); wintergreen essential oils with at least one synthetic marker compound (in  
494 red); wintergreen essential oils with no synthetic impurities (in green).

495



496

497 **Fig. 5.** Bivariate fit of  $\delta^{13}\text{C}$  (‰) and  $\delta^{18}\text{O}$  (‰) by % of bio-sourced carbon measured on 19  
 498 wintergreen essential oils and one commercial synthetic methyl salicylate (Sample 20). In red: Linear  
 499 regression fit of the data (excluding sample 17). Determination coefficient:  $r^2=0.97$  and  $r^2=0.98$ .

