

1  **$\delta^{18}\text{O}$  compound-specific stable isotope assessment : an advanced analytical**  
2 **strategy for sophisticated adulterations detection in essential oils -**  
3 **Application to spearmint, cinnamon, and bitter almond essential oils**  
4 **authentication.**

5 **Authors**

6 Aurélien Cuchet<sup>a,b</sup>; Anthony Anchisi<sup>a</sup>; Frédéric Schiets<sup>a</sup>; Elise Carénini<sup>b</sup>; Patrick Jame<sup>a,\*</sup>;  
7 Hervé Casabianca<sup>a</sup>

8 **Affiliations**

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

11 <sup>b</sup>Albert Vieille SAS, 629 Route de Grasse, 06220 Vallauris, France

12 **\*Corresponding author**

13 Patrick Jame, Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, F-69100  
14 Villeurbanne, France

15 Email address: [patrick.jame@isa-lyon.fr](mailto:patrick.jame@isa-lyon.fr)

16 Patrick Jame, Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, F-69100  
17 Villeurbanne, France

18 Email address: [patrick.jame@isa-lyon.fr](mailto:patrick.jame@isa-lyon.fr)

19

20    **Abstract**

21    Natural plant extracts are primarily used as raw materials in the cosmetic and perfumery  
22    industry. However, adulterations with petrochemical products are occurring in the market,  
23    leading to non-100% natural products. Several analytical techniques such as impurity  
24    detection or enantioselective ratio assessments have been previously described as good  
25    indicators to detect any addition of synthetic products, but these techniques are ineffective  
26    with novel type of synthetic pathways such as semisynthesis. In order to improve  
27    authentication, development of advanced analytical strategies such as  $\delta^{18}\text{O}$  stable isotopic  
28    ratios assessment was tested on spearmint, cinnamon and bitter almond essential oils major  
29    metabolites (carvone, (E)-cinnamaldehyde, and benzaldehyde). Natural  $\delta^{18}\text{O}$  mean values  
30    ( $\delta^{18}\text{O}_{\text{Carvone}} = 18.4\text{\textperthousand}$ ;  $\delta^{18}\text{O}_{\text{Cinnamaldehyde}} = 13.9\text{\textperthousand}$ ;  $\delta^{18}\text{O}_{\text{Benzaldehyde}} = 16.5\text{\textperthousand}$ ) were  
31    found to be 29 higher than semisynthetic origin for the 3 studied molecules ( $\delta^{18}\text{O}_{\text{Carvone}} =$   
32     $9.2\text{\textperthousand}$ ;  $\delta^{18}\text{O}_{\text{Cinnamaldehyde}} = 8.8\text{\textperthousand}$ ;  $\delta^{18}\text{O}_{\text{Benzaldehyde}} = 10.9\text{\textperthousand}$ ). These measurements  
33    proved to be efficient to discriminate natural and semisynthetic origins of these components  
34    and therefore potentially lead to a novel way to authenticate natural products.

35    **Keywords**

36    Semisynthesis; Essential oil; Gas chromatography; Isotope ratio mass spectrometry;  
37    Authentication

38

39      **1. Introduction**

40      Natural aromatic raw materials market is growing exponentially nowadays to supply food  
41      industry, perfumery and cosmetics. To satisfy this increasing demand, researches have been  
42      focused on compounds issued from biotechnology processes used to produce aromas and  
43      fragrances from other plant origins. Unfortunately, these new technologies can lead to new  
44      sophisticated frauds. The adulterations found on the natural product market are constantly  
45      evolving and it is nowadays possible to observe additions of biotechnological origin  
46      molecules. These new processes have the capability to create seemingly natural molecules  
47      such as the vanillin, which has been regularly obtained from the ferulic acid contained in rice  
48      [1], or the frambinone obtained from 4-hydroxybenzaldehyde contained in *Escherichia coli*  
49      [2,3].

50      In the regulation field of essential oils, molecules must come from the raw material of the  
51      plant reference to be considered and labelled 100% pure and natural. Molecules produced  
52      from natural reagents, also called semisynthetic compounds, are suspected of being used to  
53      adulterate specific essential oils. Several examples are cited in the literature.  
54      Cinnamaldehyde molecules can be made from bitter almond benzaldehyde and used to  
55      adulterate an essential oil of cinnamon [4]. Greule et al. reported the use of carvacrol,  
56      produced from the carvone of caraway essential oil, to adulterate an essential oil of oregano  
57      [5]. Semisyntheses enable fraudsters to bypass many analytical controls.

58      An essential oil that has undergone an addition of a semisynthetic molecule will be more  
59      difficult to detect than traditional synthetic molecule additions with usual analytical  
60      methods of authentication. One of the most common analysis is the determination of  
61      enantiomeric ratios to control chiral molecules such as linalool, because synthetic chiral

62 molecules are produced in racemic proportion, whereas natural molecules are generally  
63 stereospecific [6]. However, enantiomeric analyses will not be effective in the case of  
64 semisyntheses because the stereochemistry will be preserved. Another common analytical  
65 technique is the determination of radiocarbon activity, which determine the presence of  
66 fossil carbon in the sample [7]. However, a semisynthesis will eventually conduct to a 100%  
67 bio based carbon molecule because no petrochemicals would be used. Finally, stable isotope  
68 analyses of carbon and hydrogen will generally be inefficient to demonstrate this addition,  
69 because the transformations carried out will not generate significant isotopic variations of  
70  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  ratios. Typical examples are the conversion of limonene into carvone or  
71 benzaldehyde into cinnamaldehyde.

72 However, several analytical strategies are possible. A first possibility is the detection of  
73 residual impurities from semisyntheses. This approach has numerous flaws, as many  
74 synthetic pathways do not produce any impurities, and these residual molecules can be  
75 removed with a purification process [7]. A second analytical approach is the Site-Specific  
76 Natural Isotope Fractionation-Nuclear Magnetic Resonance (SNIF-NMR) technique. This  
77 system allows the measurement of isotopic fractionations of specific sites of a molecule,  
78 which can lead to the origin of the product [8]. Several authentication studies have been  
79 conducted using SNIF-NMR [4,9,10]. However, this analytical tool requires that the analysed  
80 molecules are almost entirely pure, which is difficult to achieve on complex matrices such as  
81 essential oils. In addition, there is a risk of isotopic fractionation during the purification step.  
82 Since SNIF-NMR is a relatively insensitive technique, it also requires using a large amount of  
83 sample for analysis.

84 Analysis of oxygen stable isotopes can be an interesting approach. In many cases of  
85 semisynthesis of oxygenated terpenes or phenylpropanoids, the synthesis is characterized by  
86 the addition of an external oxygen atom from the original molecule. The  $\delta^{18}\text{O}$  ratios can  
87 therefore be different, and it seems relevant to measure them. To determine this isotope  
88 ratio, the use of a GC-P-IRMS instrument is necessary to be used on most of the essential  
89 oils. This analytical system to operate is rarely used in authentication on natural products  
90 due to difficulties in the analytical process.

91 This article describes the development of the  $\delta^{18}\text{O}$  analysis by GC-P-IRMS to highlight the  
92 additions of semisynthetic-origin compounds (carvone, (E)-cinnamaldehyde, and  
93 benzaldehyde) in essential oils of spearmint, cinnamon and bitter almond.

94

95

96        **2. Material and methods**

97            ***2.1. Materials***

98              ***2.1.1. Essential oils***

99        Forty-eight samples of spearmint *Mentha spicata var crispa*, from India, USA, China, and  
100      France were analysed for this study. From these samples, 11 certified 100% pure and natural  
101      samples were provided by Albert Vieille SAS (Vallauris, France) and Young Living (Lehi, Utah,  
102      USA), and one sample was obtained from hydrodistillation process from fresh spearmint  
103      leaves at the Institute of Analytical Sciences (ISA Lyon, UMR5280 - CNRS, Villeurbanne,  
104      France) laboratory. Other essential oils (n=36) were commercial samples, obtained from  
105      various sources.

106        Fifty-five samples of cinnamon essential oils were analysed for this study. These samples  
107      were obtained from various botanical and geographical origins: *Cinnamomum verum*  
108      essential oils were sourced from Sri Lanka and Madagascar, *Cinnamomum cassia* essential  
109      oils were sourced from China and *Cinnamomum burmanii* (Nees & T.Nees) Blume (Indonesia)  
110      came from Indonesia. Authentic samples (n=11) were provided by Albert Vieille SAS, Astérale  
111      (Madagascar), and Young Living. Moreover, homemade distillations of cinnamon bark were  
112      realized in the ISA Lyon laboratory to obtain cinnamon essential oils from several botanical  
113      origins. Other samples were commercial essential oils (n=44).

114        In addition, 12 pure and natural samples of bitter almond essential oils, obtained from steam  
115      distillation apricots almonds *Prunus armeniaca* L. have also been analysed. These essential  
116      oils were provided by Albert Vieille SAS and are originated from Morocco. For information,  
117      these samples were subjected to a cyanhydric acid disposal process prior to analysis.

119                   **2.1.2. Commercial molecules**

120   Commercial molecules of carvone (R) (n=3), (E)-cinnamaldehyde (n=1), and benzaldehyde  
121   (n=3) were purchased from TCI (Tokyo, Japan), Alfa Aesar (Ward Hill, MA, USA) and Sigma  
122   Aldrich (Saint Louis, MO, USA).

123                   **2.1.3. Syntheses**

124                   **2.1.3.1. Synthesis of carvone (R) from limonene (S)**

125   Several carvone molecules were obtained from limonene conversion. The synthesis of the  
126   carvone has been carried out in 3 steps based on Rothenberger et al. protocol [11] (Figure  
127   1). The first step leads to a first intermediate molecule: the limonene nitrosochloride (1). The  
128   second step leads to the synthesis of carvoxime (2). This compound is then used to produce  
129   the carvone (3).

130   In order to perform this synthesis, the limonene is firstly diluted in isopropanol at a  
131   temperature comprised between 0°C and 10°C. A solution of 37% chloridric acid and  
132   isopropanol is then gradually added to the mixture. The obtained solution is then mixed with  
133   NaNO<sub>2</sub> in deionised water. Limonene nitrosochloride product is then purified using Buchner  
134   vacuum filtration and traces of water were removed using a vacuum desiccator. This product  
135   is then dissolve in dimethylformamide and isopropanol. The mixture is heated to reflux.  
136   Carvoxime product is then extract using diethyl ether and the organic phase is washed with  
137   ultra-pure water until neutral pH. The solution is then dried using anhydrous sodium sulfate,  
138   then filtered. Diethyl ether is then evaporated and the carvoxime is dissolved in an aqueous  
139   solution of oxalic acid (5%), then heated at reflux for 30 minutes. The carvone final product  
140   is then obtained by steam distillation.

142

### **2.1.3.2. Aldol reaction of benzaldehyde**

143 Ten samples of (E)-cinnamaldehyde were produced in laboratory using aldolisation of  
144 benzaldehyde from synthesis origin and natural origin from essential oils of bitter almond  
145 (Figure 2). The synthesis protocol is described below.

146 A volume of 600 µL of benzaldehyde was diluted in 3 mL of ethanol 96%. The mixture was  
147 maintained at 0°C. 1 mL of NaOH 15% was then added and the solution was stirred for 5  
148 minutes. A volume of 200 µL of acetaldehyde (TCI and Sigma Aldrich) was then added at 0°C.  
149 This step is renewed four times every 15 minutes to make sure that the acetaldehyde is  
150 maintained in excess during the reaction entire time. The mixture is then heated at 40°C  
151 during 10 minutes. Highly concentrated hydrochloric acid is then added to acid pH. Diethyl  
152 ether extraction was then carried out to collect the (E)-cinnamaldehyde final product.

153

### **2.1.3.3. Retro aldol reaction of cinnamaldehyde**

154 Five samples of benzaldehyde have been produced in laboratory by retro aldol reaction of  
155 synthetic origin (E)-cinnamaldehyde (Fluka) and natural origin (E)-cinnamaldehyde from  
156 essential oils of *Cinnamomum verum*, *Cinnamomum cassia*, and *Cinnamomum burmanii*.

157 Ten grams of (E)-cinnamaldehyde are firstly diluted in an aqueous solution of sodium  
158 carbonate Na<sub>2</sub>CO<sub>3</sub> (3%). This mixture was heated at reflux during 7 hours. A steam  
159 distillation was then carried out to extract the benzaldehyde.

160

161

162

163           **2.2. Isotopic Analyses**164           **2.2.1. Data Expression**165       Isotopic results are expressed in delta ( $\delta$ ) units according to the following formula [30]:

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

166       where  $i$  is the mass number of the heavy isotope of element  $E$ ,  $R_{Sample}$  is the isotope ratio of  
167       the sample, and  $R_{Reference}$  is the isotope ratio of a relevant internationally recognized  
168       reference material. The  $\delta$  values were multiplied by 1000 and are expressed in units "per  
169       mil" (‰).170       High-purity gases of carbon dioxide (99.998%, 4.8 Messer), hydrogen (99.999%, 5.0 Messer),  
171       and carbon monoxide (99.997%, 4.7 Messer) were used as the working reference gases  
172       injected with each sample. Helium (99.999% purity) was used as a carrier gas.173       All studied metabolites in the essential oils of interest were calibrated with commercial  
174       molecules (carvone, (E)-cinnamaldehyde, and benzaldehyde) previously calibrated against  
175       international standards in elemental analysis – IRMS (EA-IRMS).176       International reference materials IAEA CH7 (polyethylene foil:  $\delta^{13}\text{C} = -32.15\text{\textperthousand}$ ,  $\delta^2\text{H} = -$   
177       100‰), IAEA 601 (benzoic acid:  $\delta^{18}\text{O} = 23.3\text{\textperthousand}$ ), and USGS40 (L-glutamic acid:  $\delta^{13}\text{C} = -$   
178       26.39‰), supplied by the International Agency of Atomic Energy (IAEA, Vienna, Austria),  
179       were used to calibrate each molecule in the mixture, which allowed us to express our  $\delta^{13}\text{C}$   
180       results relative to the Vienna Pee Dee Belemnite (VPDB) scale and our  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  results  
181       relative to the Standard Mean Ocean Water (VSMOW) scale. Each sample was analyzed  
182       versus a monitoring gas for which two pulses were admitted with each analyte. All sample  
183       values were analyzed in duplicate and verified by bracketing the analytical sequence with

184 international reference materials every six analyses.  $\delta^{13}\text{C}$  isotopic analyses of the standards  
185 molecules were conducted using a Flash HT elemental analyzer connected to a Delta V Plus  
186 via a Conflo V interface. The  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  measurements were carried out using a TC-EA  
187 elemental analyzer linked to a Delta V Plus via a Conflo V interface (all Thermo Fisher  
188 Scientific). The method is fully explained in a previous study [7].

189                   **2.2.2. Gas Chromatography - Mass Spectrometry - Combustion/Pyrolysis -**  
190                   **Isotope Ratio Mass Spectrometry (GC-MS-C/P-IRMS)**

191 The  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  values of targeted spearmint, bitter almond, and cinnamon essential  
192 oils metabolites were determined by GC-IRMS using a GC Trace 1310 simultaneously  
193 connected to an ISQ MS quadrupole mass spectrometer and to a Delta V Plus isotope ratio  
194 mass spectrometer via a GC Isolink II interface. (Thermo Fisher, Bremen, Germany). This  
195 equipment enabled the identification of compounds via the ISQ MS instrument and specific  
196 compound isotopic ratio measurements of  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  via IRMS.

197 One microliter of diluted essential oil (1  $\mu\text{L}$  into 100  $\mu\text{L}$  of ethanol) was injected using a Tri  
198 Plus RSH autosampler in a Trace 1310 gas chromatograph equipped with an Agilent HP-1  
199 capillary column (50 m \* 0.320 mm \* 0.52  $\mu\text{m}$  internal diameter film thickness) used for  
200 chromatographic separation. The injection port was held at 260°C, fitted with a split liner  
201 containing glass wool and operated in split mode (10:1). The oven temperature program  
202 started at 80°C for 2 min, increased to 150°C at a rate of 10°C/min, increased to 250°C at a  
203 rate of 20°C/min, increased to 325°C at a rate of 20°C/min. Components were separated at a  
204 flow rate of 1.1 mL/min and divided in 2 ways using a Sil Flow connector.

205  $\delta^{13}\text{C}$  analyses requires to perform the combustion of carbon in carbon dioxide. It was  
206 achieved using an alumina tube containing nickel oxide maintained at 1000°C in a furnace.

207 Pyrolysis of the sample for  $\delta^2\text{H}$  measurement in  $\text{H}_2$  was carried out at a high temperature  
208 ( $1400^\circ\text{C}$ ) using an alumina conversion tube (Thermo Fischer Scientific). Undecane,  $\text{C}_{11}\text{H}_{24}$   
209 (Sigma Aldrich), was regularly added to regenerate the alumina pyrolysis tube. For  $\delta^{18}\text{O}$   
210 measurements, it was necessary to ensure the conversion of oxygen atoms into carbon  
211 monoxide. The pyrolysis unit was designed by an inner platinum tub partly filled with nickel  
212 wires incorporated in an outer ceramic tube (Thermo Fisher) hold at  $1280^\circ\text{C}$  and work under  
213 a column flow 1.1 ml/min Helium added by 1.1 ml/min of a mix (98% Helium +2%  $\text{H}_2$ )  
214 necessary in this configuration. Pyrolysis tube was conditioned by injecting 1  $\mu\text{l}$  of high purity  
215 undecane paraffin (sigma Aldrich) in duplicate, allowing to create an inner homogenous  
216 coating into the platinum tube. This layer of carbon created would be necessary bring  
217 enough carbon reactant in the pyrolysis reaction and the production of carbon monoxide.  
218 This coating process has been monitored every week enabling to reconstitute the carbon  
219 layer.

220           ***2.3. Enantiomeric study and impurity detection***

221           ***2.3.1. Carvone enantioselective analysis using GC-FID***

222 Samples of spearmint essential oils have been analysed in Enantio GC-FID to control the  
223 enantioselective ratio of carvone. Enantioselective analyses were performed on a gas  
224 chromatograph 6890N GC System (Agilent Technologies) equipped with a flame ionization  
225 detector (FID) with the detector temperature set at  $250^\circ\text{C}$ . Separation of the carvone  
226 enantiomers were performed on a  $\beta$ -DEX 225 column (30 m \* 0,250 mm \* 0,25  $\mu\text{m}$ , Supelco  
227 Inc.) coated with cyclodextrin stationary phase. The GC conditions were programmed as  
228 follows: 0.2  $\mu\text{L}$  of diluted essential oil (1:100 in ethanol) was injected into the GC in split  
229 mode (100:1) with the injector temperature set at  $250^\circ\text{C}$ . The carrier gas was  $\text{H}_2$  at a  
230 constant flow rate of 1.2 mL/min. The initial oven temperature was held at  $80^\circ\text{C}$  for 5 min,

231 ramped to 130°C at a rate of 2°C/min, then ramped to 180°C at a rate of 5°C/min and held  
232 for 2min.

233

234 **2.3.2. *Impurity detection using GC-MS analysis***

235 GC-MS analyses were performed on a gas chromatograph 6890 N GC System (Agilent  
236 Technologies) interfaced with a 5975 inert XL MSD mass spectrometer with electron impact  
237 ionization (70 eV). 1 µL of pure cinnamon essential oil was injected into the GC in the split  
238 mode (100:1), with the injector temperature set at 250 °C. Helium was used as the carrier  
239 gas with a constant flow rate of 1.0 mL/min. Separation of the volatile compounds in  
240 cinnamon essential oils was achieved using a HP-5 capillary column (30 m\*0.250 mm\*0.25  
241 µm, Agilent Technologies). The initial oven temperature was held at 60 °C for 3 min and then  
242 ramped at a rate of 5°C/min to reach 160°C, then ramped to 300°C at a rate of 10°C/min and  
243 held for 4 min. The SIM parameters were as follows: the targeted mass ions (m/z) were 128,  
244 129 and 158 (molecular ion) to detect 5-phenylpenta-2,4-dienal. NIST library and lab-made  
245 library, combined with retention time, retention indices, literature references, and standards  
246 injections were used to identify the impurity produced during benzaldehyde aldolisation.

247

248

249        **3. Results and discussion**

250            ***3.1. Spearmint Essential Oil***

251            ***3.1.1. Context***

252        Essential oils of spearmint *Mentha spicata* L. are mainly composed of carvone molecule.  
253        These extracts are generally processed with a fractional distillation to obtain more than 80%  
254        of carvone in the composition of the essential oil [12,13]. These products are usually  
255        originated from China, USA, and India.

256        These essential oils are amongst the most important product in the essential oil market.  
257        Therefore, these extracts are highly subjects to be adulterated. Addition of synthetic carvone  
258        is a known adulteration and is easily highlighted with the control of the enantiomeric ratio  
259        [14,15]. An olfactory control is also very efficient, because enantiomers of carvone present  
260        different odours. Carvone (R) has a minty smell and is abundant in spearmint essential oils,  
261        whereas carvone (S) has a spicy aroma and is found in caraway essential oils. However,  
262        carvone (R) isomer can also be synthesized from natural origin limonene (S) from sweet  
263        orange essential oils [16]. This extract is currently the best-selling essential oil in the world  
264        and the cheapest in the market. Limonene represents over than 90% of the composition of  
265        sweet orange essential oil.

266        The semisynthesis of carvone from limonene has the particularity to introduce an oxygen  
267        [11] (Figure 1). An isotopic monitoring of  $\delta^{18}\text{O}$  may potentially be of interest to highlight the  
268        semisynthetic origin of carvone, because this newly introduced oxygen is from a different  
269        source and has not been impacted by the same isotopic fractionations as the original oxygen  
270        from spearmint carvone.

271

272                   **3.1.2. Analysis of results**

273 Enantiomeric and isotopic data are depicted in Table 1 and Figure 3.

274 The determination of the enantiomeric ratio has been carried out in order to control that  
275 carvone essential oils had not been adulterated with petrochemical origin carvone. The  
276 enantiomeric content of carvone (R) is measured between 97.3% and 100.0%. All the  
277 analysed samples present the carvone enantiomer (R) in excess, which is in line with the  
278 literature [14,15]. No significant differences were observed between authentic and  
279 commercial samples.

280  $\delta^{13}\text{C}$  isotopic composition of carvone molecules derived from essential oils of genuine  
281 spearmint ( $-29.35 \pm 1.54\text{\textperthousand}$ ) is similar to the  $\delta^{13}\text{C}$  of carvone molecules from commercial  
282 samples ( $-29.63 \pm 0.87\text{\textperthousand}$ ). These values are in line with the C3 origin [17] of the spearmint  
283 plant. According to the literature, C3 plants presents a  $\delta^{13}\text{C}$  comprised between  $-20\text{\textperthousand}$  and  $-30\text{\textperthousand}$  [18]. The  $\delta^{13}\text{C}$  of the carvone is not efficient to discriminate natural and synthetic  
285 origins of the carvone molecule according to the analyses. Indeed carvone molecules from  
286 petrochemical origins have  $\delta^{13}\text{C}$  values comprised between  $-30.24\text{\textperthousand}$  and  $-26.40\text{\textperthousand}$ , which  
287 coincide with  $\delta^{13}\text{C}$  range value of authentic spearmint essential oils. Same result is observed  
288 with the carvone synthesized from limonene ( $\delta^{13}\text{C} = -29.81\text{\textperthousand}$ ).

289 As regards with  $\delta^2\text{H}$  isotopic values, significant isotopic fractionations are noticed between  
290 samples: authentic samples  $-262 \pm 31\text{\textperthousand}$ ; commercial samples  $-242 \pm 29\text{\textperthousand}$ ). These  
291 differences are probably explained by the different geographical origins, because  $\delta^2\text{H}$  stable  
292 isotope ratios are linked to the geographical location of the harvest [19]. USA spearmint  
293 essential oils have a mean value of  $-282 \pm 28\text{\textperthousand}$ ; China samples mean isotopic value is more  
294 enriched in deuterium ( $-235 \pm 18\text{\textperthousand}$ ); as well as India samples ( $-226 \pm 27\text{\textperthousand}$ ). These natural

295 values are significantly depleted by comparison with  $\delta^2\text{H}$  stable isotopic values of carvone  
296 molecules synthesized from petrochemical products ( $-190 \pm 24\text{\textperthousand}$ ). The study of stable  
297 isotope ratios of hydrogen potentially highlights an origin of synthesis. However, this is  
298 unfortunately not possible in the case of a carvone obtained from limonene conversion,  
299 because the measured  $\delta^2\text{H}$  value ( $-204\text{\textperthousand}$ ) is within the range of authentic sample values.

300 Finally, the study of  $\delta^{18}\text{O}$  isotopic values highlights significant isotopic differences between  
301 authentic and commercial samples. The  $\delta^{18}\text{O}$  value of 100% pure and natural carvone  
302 molecules are comprised between  $14.1\text{\textperthousand}$  and  $22.1\text{\textperthousand}$ . These values are more positives than  
303 the  $\delta^{18}\text{O}$  value measured on the carvone (R) obtained from limonene ( $9.2\text{\textperthousand}$ ). From the  
304 panel of commercial samples, several essential oils presents  $\delta^{18}\text{O}$  values similar to the  
305 measured range determined to authentic samples, but other samples present a lower  $\delta^{18}\text{O}$   
306 value, which potentially indicate that these samples have been adulterated with  
307 semisynthetic carvones.

308           **3.2. *Cinnamon Essential Oil & Bitter Almond Essential Oil***

309           **3.2.1. *Context***

310 Essential oils of cinnamon and bitter almond are amongst the most sold essential oils of the  
311 market. Bitter essential oils are obtained from the extraction of almond (*Prunus amygdalus*  
312 var. *amara* (DC.)) and apricot (*Prunus armeniaca* L.) kernels. This essential oil presents a  
313 composition with more than 95% of benzaldehyde. Cinnamon essential oil comes in two  
314 forms: oil cinnamon essential *Cinnamomum cassia* (L.) J. Presl, mainly from China and from  
315 distillation of wood and / or leaves, and cinnamon essential oil *Cinnamomun verum* J. Presl  
316 (syn. *Cinnamomum zeylanicum* Blume), mainly from Sri Lanka, India, and Madagascar, and

317 made from the distillation of cinnamon bark. These two essential oils are rich in (E) -  
318 cinnamaldehyde.

319 There are many frauds associated with the essential oils of bitter almond and cinnamon.  
320 Since the cassia origin of cinnamon is much cheaper than the Ceylon origin, the mixtures  
321 between these two essential oils are common. However, it is possible to highlight this type  
322 of addition by studying the differences in composition between the two essential oils [20], or  
323 by NIR and FTIR spectroscopic analysis [21,22]. Additions of (E) -cinnamaldehyde and  
324 benzaldehyde are also identifiable by radiocarbon analysis [23]. However, as shown in  
325 literature with the example of methyl salicylate or benzaldehyde [7,24], a labelling of  
326 synthetic molecule makes the measurement of  $^{14}\text{C}$  activity inefficient. Isotopic methods by  
327  $\delta^2\text{H}$  GC-P-IRMS are applicable to highlight the synthetic additions to benzaldehyde (Ruff et  
328 al., 2000) and (E) -cinnamaldehyde [25], as well as SNIF-NMR [4]. The synthesis of (E) -  
329 cinnamaldehyde from benzaldehyde produces a residual impurity, 5-phenylpenta-2,4-dienal  
330 [26], which is identifiable by GC, if the product has not been sufficiently purified.

331 In the same way as for mint, (E) -cinnamaldehyde can also be produced by semisynthesis,  
332 from benzaldehyde obtained from the essential oil of bitter almond. The scheme of reaction,  
333 presented in Figure 2, shows that (E)-cinnamaldehyde is obtained by aldolization of  
334 benzaldehyde (1) [4]. During the reaction, the oxygen in the benzaldehyde is removed and  
335 oxygen from acetaldehyde is introduced to form (E) -cinnamaldehyde. A stable isotopic  
336 assessment is therefore again of real interest. As prices fluctuate, essential oil bitter almond  
337 is sometimes cheaper than cinnamon bark essential oil (*Cinnamomum verum*). The fraud can  
338 be reversed. Benzaldehyde can also be hemi-synthesized from (E)-cinnamaldehyde [4],  
339 according to the retro-aldolization reaction (2). This time, the oxygen in the hemi-

340 synthesized benzaldehyde is obtained from water. However, the use of carbonates in the  
341 reaction suggests isotopic exchanges [27].

342 Therefore, adulteration can be realized in either ways, depending on market prices. The  
343 essential oil of cassia is ten times cheaper than the other two; it remains the main source of  
344 fraud.

345 **3.2.2. *Cinnamon Essential Oils***

346 Some reference isotopic values of (E) -cinnamaldehyde have been published. The value  $\delta^{13}\text{C}$   
347 of (E) -cinnamaldehyde from natural cinnamon essential oil is between -28.20 and -28.60 ‰  
348 according to Culp [23,24] and between -24.20‰ and -27.10‰ according to Sewenig [25],  
349 while the values  $\delta^{13}\text{C}$  of synthetic (E) -cinnamaldehyde obtained from the oxidation of  
350 toluene, are between -24.61‰ and -25.70‰. According to the literature, the values  $\delta^2\text{H}$  of  
351 natural (E) -cinnamaldehyde are given between -142‰ and -101‰ [23–25]. (E) –  
352 cinnamaldehyde of petrochemical origin are much more enriched in deuterium, which  
353 results in values  $\delta^2\text{H}$  much higher, between 464‰ and 569‰ after Culp [23,24]. Finally, the  
354 naturally occurring (E)-cinnamaldehyde, a product of the shikimic pathway of biosynthesis  
355 [28], is expected to exhibit value  $\delta^{18}\text{O}$  around 14.9‰ [29].

356 The isotopic values of (E) -cinnamaldehyde of the various essential oils of cinnamon,  
357 determined by GC-C / P-IRMS, are described in Table 2 and Figure 4.

358 The (E) -cinnamaldehydes of essential oils of cinnamon, which did not present impurities,  
359 have isotope ratios  $\delta^{13}\text{C}$  of  $-28.37 \pm 1.14$  ‰ and  $\delta^2\text{H}$  of  $-142 \pm 10$  ‰. These results are in  
360 agreement with the natural isotopic values presented by Culp and Sewenig[23–25].  $\delta^2\text{H}$   
361 stable isotopic values are sometimes more negative however. The mean  $\delta^{18}\text{O}$  value is

362 measured around  $13.9 \pm 2.2\text{\textperthousand}$ , which is close to the value proposed by Schmidt [29]. All of  
363 these values are consistent with a natural origin.

364 Cinnamon essential oils that had impurities present various isotopic values. We can  
365 distinguish those which have  $\delta^2\text{H}$  indicators of one synthetic origin and others. The first ones  
366 have isotope ratios  $\delta^{13}\text{C} -24.70 \pm 1.66\text{\textperthousand}$  and  $\delta^2\text{H}$  between -42 and  $844\text{\textperthousand}$ . These stable  
367 isotopic values demonstrate enrichment in  $^2\text{H}$  and  $^{13}\text{C}$  compared to natural samples. The  
368 highest  $\delta^2\text{H}$  values are indicative of an origin of synthesis from toluene, while the lower  
369 values seem to correspond to benzyl chloride origin [25]. The measured  $\delta^{18}\text{O}$  values are very  
370 variable, and fluctuate from 1.2 to  $21.8\text{\textperthousand}$ . Isotope analyses confirm the detection of  
371 synthetic residues: these samples have undergone a partial or total addition of (E) -  
372 cinnamaldehyde of synthetic or semisynthetic origin.

373 However, other samples, which nevertheless had traces of 5-phenylpenta-2,4-dienal, exhibit  
374 naturally occurring (E) -cinnamaldehyde isotopic values ( $\delta^{13}\text{C} -27.54 \pm 0.65\text{\textperthousand}$  and  $\delta^2\text{H} -147 \pm$   
375  $12\text{\textperthousand}$ ). However, we can see that the values in  $\delta^{18}\text{O}$  are lower ( $4.0 \pm 3.3\text{\textperthousand}$ ). The oxygen  
376 values do not correspond to a natural origin from of the phenylpropanoid biosynthetic  
377 pathway from shikimic acid. The oxygen of (E) -cinnamaldehyde was probably altered, from  
378 a naturally occurring reagent: acetaldehyde from ethanol oxidation.

379 To verify this hypothesis, several semisyntheses of (E) -cinnamaldehyde were carried out, by  
380 aldolization of benzaldehydes, from different samples of almond essential oils bitter.  
381 Isotopic values  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  obtained correspond to a natural origin of (E) -cinnamaldehyde,  
382 but this is not the case with  $\delta^{18}\text{O}$ , which is measured between  $7.9\text{\textperthousand}$  and  $9.5\text{\textperthousand}$ . These values  
383 are lower than the values measured previously on essential oils of cinnamon natural, nor  
384 does it correspond to the value given by Schmidt [29].

385 The oxygen introduced during the aldolization of benzaldehyde comes from acetaldehyde  
386 used as reagent. It is the latter that will influence the final value of  $\delta^{18}\text{O}$  of (E) -  
387 cinnamaldehyde produced by this semisynthesis. To verify this claim, the two commercial  
388 acetaldehyde samples used to produce the samples by aldolization, are analyzed by EA-P-  
389 IRMS, by direct introduction. The measure of their value  $\delta^{18}\text{O}$  is  $8.7 \pm 0.1\text{\textperthousand}$ . By comparison,  
390 the mean value  $\delta^{18}\text{O}$  measured on the 7 samples of (E) -cinnamaldehyde obtained by  
391 aldolization of benzaldehyde natural is  $8.8 \pm 0.6\text{\textperthousand}$ .

392 These results confirm the origin of the oxygen molecule introduced during the synthesis  
393 from benzaldehyde. If the  $\delta^{18}\text{O}$  value of acetaldehyde is more negative, the (E) -  
394 cinnamaldehydes produced during aldolization will also have more negative  $\delta^{18}\text{O}$  values,  
395 which may explain the values very low measured on some suspect samples. Indeed,  
396 acetaldehyde is produced industrially from agricultural ethanol, whose values  $\delta^{18}\text{O}$  are low  
397 (between  $3.0\text{\textperthousand}$  and  $8.1\text{\textperthousand}$  after Schmidt [29]). It is therefore possible that syntheses can be  
398 carried out with acetaldehydes weakly enriched in  $^{18}\text{O}$ .

399           **3.2.3. Bitter Almond Essential Oil**

400 For benzaldehyde, the isotopic values of natural origin indicated in the literature are  
401 between  $-27.06\text{\textperthousand}$  and  $-29.58\text{\textperthousand}$  for  $\delta^{13}\text{C}$ , and between  $-145\text{\textperthousand}$  and  $-84\text{\textperthousand}$  for  $\delta^2\text{H}$  from the  
402 study by Culp and Noakes [23,24], and between  $-113\text{\textperthousand}$  and  $-148\text{\textperthousand}$  for  $\delta^2\text{H}$  from Ruff et al  
403 [30], for a bitter almond origin. Schmidt et al. [29] present a value  $\delta^{18}\text{O}$  for one naturally  
404 occurring benzaldehyde from the shikimic route, around  $19.3\text{\textperthousand}$ . Ruff's work and al. [30] also  
405 focused on samples of natural benzaldehyde ex-*Cinnamomum cassia*, the values  $\delta^2\text{H}$  data is  
406 between  $-83$  and  $-144\text{\textperthousand}$ , which merges with the range of values natural benzaldehyde from  
407 bitter almond essential oil. The isotopic values of Synthetic benzaldehyde are different

408 depending on the method of synthesis. A benzaldehyde from the acid or basic hydrolysis of  
409 (dichloromethyl) benzene has values between -28.52‰ and -29.99‰ for  $\delta^{13}\text{C}$  [23,24],  
410 between -47 and -85‰ for  $\delta^2\text{H}$  [23,24,30], and around 6.8‰ for  $\delta^{18}\text{O}$ . While a benzaldehyde  
411 obtained by oxidation of toluene will have a value  $\delta^{13}\text{C}$  between -24.70 and -26.50‰  
412 according to Culp and Noakes [23,24], a strongly positive  $\delta^2\text{H}$  isotopic value, between 420 ‰  
413 and 720‰ approximately, and a value  $\delta^{18}\text{O}$  around 14.1‰ according to the publication by  
414 Schmidt et al. [29].

415 The isotopic values of benzaldehyde in various bitter almond essential oils analyzed,  
416 determined by GC-C / P-IRMS, are described in Table 3.

417 Bitter almond essential oils have isotopic values corresponding to an origin natural  
418 benzaldehyde, isotopic values varying from -31.81‰ to -29.80‰ for  $\delta^{13}\text{C}$ , from -131‰ to -  
419 97‰ for  $\delta^2\text{H}$ , and from 13.4‰ to 19.4‰ for  $\delta^{18}\text{O}$ .

420 These values differ from those measured on benzaldehydes of petrochemical origin. Indeed,  
421 it is possible to observe more positive isotopic values on the three elements analyzed, which  
422 puts in evidence a greater enrichment in  $^{13}\text{C}$ ,  $^2\text{H}$ , and  $^{18}\text{O}$  in the synthetic benzaldehydes.  
423 These values are characteristic of a synthesis from toluene.

424 Finally, various samples of benzaldehydes, obtained by retro-aldolization of (E) –  
425 cinnamaldehydes from different origins were analyzed. Semisyntheses, carried out from (E)  
426 –cinnamaldehydes natural oils derived from cassia and verum cinnamon essential oils, have  
427 isotopic ratios stable  $\delta^{13}\text{C}$  ( $-29.92 \pm 0.54\text{‰}$ ) and  $\delta^2\text{H}$  ( $-108 \pm 11\text{‰}$ ) similar to the values  
428 obtained on oils bitter almond essentials. However, the isotopic values  $\delta^{18}\text{O}$  are lower (10.9  
429  $\pm 0.4\text{‰}$ ) than those of natural essential oils.

430 The oxygen introduced during the retroaldolization reaction of (E) -cinnamaldehyde comes  
431 from water. However, isotopic exchanges occur between water and the carbonate used in  
432 the synthesis ( $\text{Na}_2\text{CO}_3$ ), at 100°C [27]. The  $\delta^{18}\text{O}$  value of benzaldehyde obtained by  
433 retroaldolization is therefore dependent on the isotopic value of the carbonate used during  
434 the synthesis, which explains why we find similar isotopic values on the different  
435 benzaldehydes produced by retroaldolization, despite the use as a starting reagent from  
436 different origins of (E) -cinnamaldehydes (synthetic or natural from essential oils of bitter  
437 almond).

438 The  $\delta^{18}\text{O}$  of tap water, used for this synthesis, cannot be measured in EA-P-IRMS. To be able  
439 to carry out the measurement, we used an OA-ICOS system (Off Axis - Integrated cavity  
440 output spectroscopy) [31–33]. The  $\delta^{18}\text{O}$  of water is measured at -11 ‰, while carbonates are  
441 generally highly enriched in  $^{18}\text{O}$  (between 25‰ and 30‰, [27]). The isotopic exchange of  
442 oxygen between the carbonate ion  $\text{CO}_3^{2-}$  and  $\text{H}_2\text{O}$  lead to an intermediate value, which  
443 corresponds to the  $\delta^{18}\text{O}$  values that we measure on benzaldehydes produced by retro-  
444 aldolization.

445

446

447 **Conclusion**

448 The results presented in this study are promising, as there are currently no analytical  
449 solutions to highlight evidence of semisynthetic product additions with  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$   
450 measurements, radiocarbon assessment, and enantiomeric ratio determination. In addition,  
451 biotechnologies will continue to grow with production of molecules from various origins  
452 using novel processes.  $\delta^{18}\text{O}$  isotopic measurements will be of greater interest depending of  
453 technological advances.

454 Other leads of investigation can be considered, such as the authentication of Oregano  
455 *Origanum vulgare* essential oils, primarily constituted of carvacrol. This molecule is easily  
456 synthesized from limonene (predominantly compound of sweet orange essential oil) and / or  
457 carvone (primarily found in spearmint and caraway essential oils)[34]. Another future  
458 prospect could be the study of the semisynthesis of (E)-anethol (Star anise *Illicium verum*,  
459 Anise *Pimpinella anisum*, and fennel *Foeniculum vulgare* essential oils) from methyl chavicol  
460 (basil *Ocimum basilicum*, tarragon *Artemisia dracunculus*, and chervil *Anthriscus cerefolium*  
461 essential oils).

462 Furthermore,  $\delta^{18}\text{O}$  isotopic study is not restricted to semisynthetic frauds. Many applications  
463 are potentially applicable, depending of the matrix of interest.  $\delta^{18}\text{O}$  compound specific  
464 analyses using GC-P-IRMS can potentially be a new complementary tool for authentication,  
465 especially since many oxygenated constituents are present in the composition of essential  
466 oils, and that most of these metabolites have not yet been studied in  $\delta^{18}\text{O}$  compound  
467 specific GC-P-IRMS analysis. As reported in previous studies [7],  $\delta^{18}\text{O}$  metabolite specific  
468 values are efficient to highlight a petrochemical origin. In addition, this measure can provide

469 geographical origin authentication, as the isotopic fractionations of oxygen are linked to the  
470 atmospheric cycles of water, similar to those of hydrogen.

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477 **Declaration of Competing Interests**

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

479

480

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589 **Table 1:** Carvone compound-specific  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  isotopic values and enantiomeric ratios measured in spearmint authentic and  
 590 commercial essential oils, and carvone from petrochemical and from limonene conversion origins (n=number of samples).

Samples	Enantiomeric values of carvone				Compound-specific isotopic values of carvone (GC-C/P-IRMS)											
	% Carvone (R)				$\delta^{13}\text{C}$				$\delta^2\text{H}$				$\delta^{18}\text{O}$			
	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
Spearmint essential oils – Authentic samples (n=12)	99.7	98.7	100.0	0.4	-29.35	-33.34	-27.24	1.54	-262	-294	-211	31	18.4	14.1	22.1	2.6
Commercial spearmint essential oils (n=36)	99.1	97.3	100.0	0.7	-29.63	-30.96	-28.15	0.87	-242	-321	-201	29	9.0	-1.5	18.4	5.6
Carvone (R) – Petrochemical origin (n=3)	100.0	100.0	100.0	0.0	-27.94	-30.24	-26.40	2.03	-190	-217	-176	24	13.2	10.9	14.5	2.0
Carvone – Limonene conversion origin (n=1)	99.4	NA	NA	NA	-29.81	NA	NA	NA	-204	NA	NA	NA	9.2	NA	NA	NA

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595 **Table 2:** (E)-Cinnamaldehyde compound-specific  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  isotopic values in cinnamon authentic and commercial essential oils, and  
 596 (E)-Cinnamaldehyde from petrochemical and from benzaldehyde aldol origins (n=number of samples). Indication of the eventual detection of  
 597 the 5-phenylpenta-2,4-dienal aldol reaction impurity using GC-MS and compliance of  $\delta^2\text{H}$  value (maximal value of -101‰ based on [23–25]).

Samples	$\delta^2\text{H}$ complied?	Impurity detected?	Compound-specific isotopic values of (E)-cinnamaldehyde (GC-C/P-IRMS)											
			$\delta^{13}\text{C}$			$\delta^2\text{H}$				$\delta^{18}\text{O}$				
			Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
Cinnamon essential oil – Authentic samples (n=11)	Yes	No	-28.37	-27.60	-26.13	1.14	-142	-156	-123	10	13.9	10.9	16.9	2.2
Cinnamon essential oil - Suspected hemi synthetic (E)-Cinnamaldehyde adulteration	Yes	Yes	-27.54	-28.68	-26.00	0.65	-147	-168	-125	12	4.0	-2.8	10.5	3.3
Cinnamon essential oil - Synthetic (E)-Cinnamaldehyde adulteration	No	Yes	-24.70	-27.57	-23.29	1.66	146	-42	844	391	11.7	1.2	21.8	7.6
(E)-Cinnamaldehyde – Petrochemical origin (n=1)	No	Yes	-25.96	NA	NA	NA	540	NA	NA	NA	16.1	NA	NA	NA
(E)-Cinnamaldehyde – natural benzaldehyde (bitter almond oil) aldol reaction origin (n=7)	Yes	Yes	-26.23	-28.51	-24.03	1.68	-125	-138	-109	9	8.8	7.9	9.5	0.6
(E)-Cinnamaldehyde – synthetic benzaldehyde aldol reaction origin (n=3)	Yes	Yes	-26.10	-26.69	-25.79	0.51	389	379	407	15	9.4	8.8	9.8	0.5

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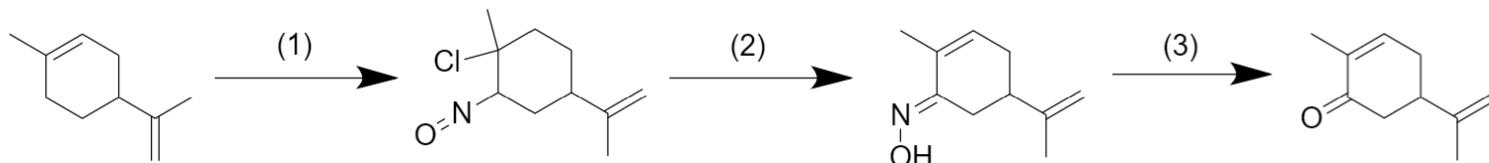
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601 **Table 3:** Benzaldehyde compound-specific  $\delta^{13}\text{C}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  isotopic values in bitter almond authentic essential oils, and benzaldehyde from  
 602 petrochemical and from (E)-cinnamaldehyde retro aldol origins (n=number of samples).

Samples	Compound-specific isotopic values of benzaldehyde (GC-C/P-IRMS)											
	$\delta^{13}\text{C}$				$\delta^2\text{H}$				$\delta^{18}\text{O}$			
	Mean	Min	Max	SD	Mean	Min	Max	SD	Mean	Min	Max	SD
Bitter almond essential oil – Authentic samples (n=12)	-30.72	-31.81	-29.80	1.00	-117	-131	-97	12	16.5	13.4	19.4	2.1
Benzaldehyde – Petrochemical origin (n=3)	-26.17	-26.91	-25.26	0.84	494	389	546	91	20.9	19.2	23.7	2.4
Benzaldehyde – Natural (E)-cinnamaldehyde (cinnamon oil) retro-aldol reaction origin (n=4)	-29.92	-30.42	-29.18	0.54	-108	-120	-99	11	10.9	10.3	11.2	0.4
Benzaldehyde – Synthetic (E)-cinnamaldehyde retro-aldol reaction origin (n=1)	-26.57	NA	NA	NA	705	NA	NA	NA	11.2	NA	NA	NA

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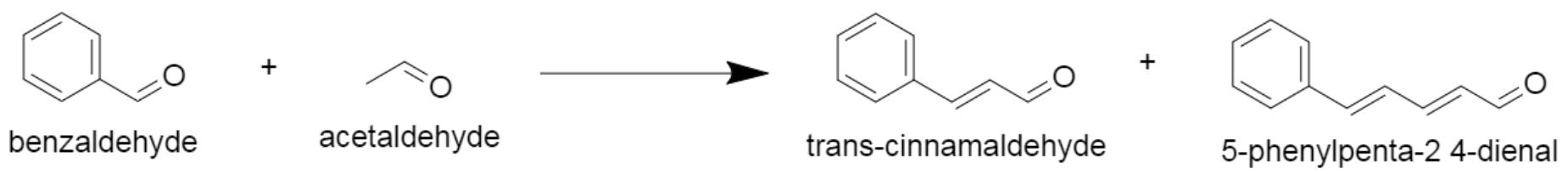
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606 **Figure 1:** Synthesis of carvone from limonene in 3 steps: (1) Synthesis of limonene nitrosochloride from limonene. (2) Production of carvoxime.

607 (3) Synthesis of carvone.

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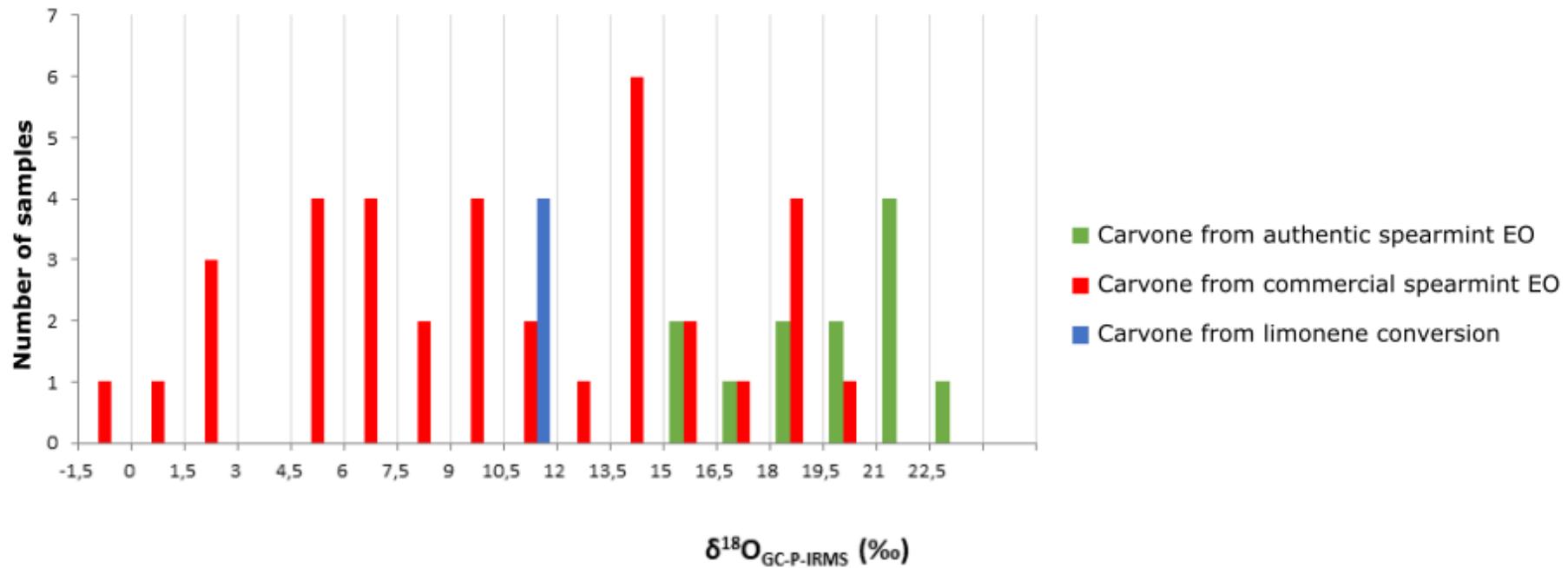
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612 **Figure 2:** Synthesis of (E)-cinnamaldehyde from aldolisation of benzaldehyde. This synthesis leads to the production of an impurity : 5-  
613 phenylpenta-2,4-dienal

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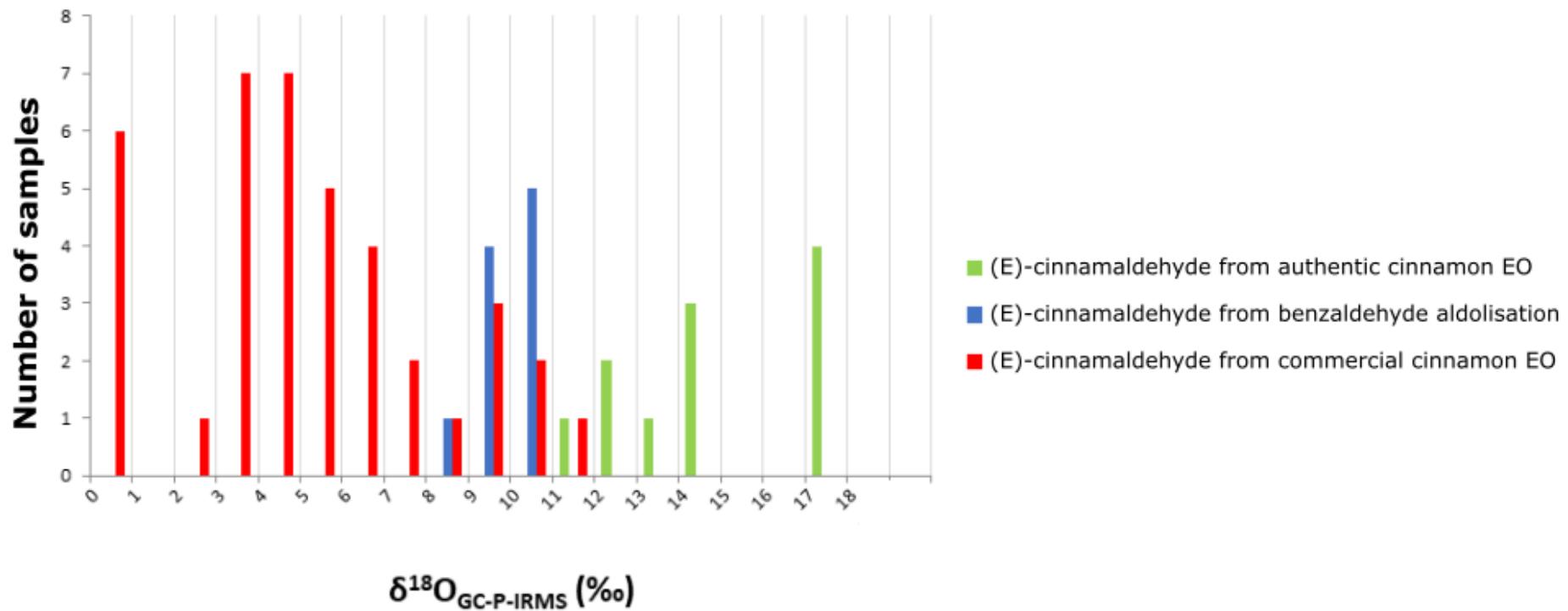
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617 **Figure 3:** Distribution of  $\delta^{18}\text{O}_{\text{GC-P-IRMS}}$  carvone isotopic values measured on 48 spearmint essential oils and 4 semisynthetic origin carvones from  
 618 sweet orange limonene.

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623 **Figure 4:** Distribution of  $\delta^{18}\text{O}_{\text{GC-P-IRMS}}$  ( $\text{E}$ )-cinnamaldehyde isotopic values measured on 50 *Cassia* and *Verum* cinnamon essential oils and 10 ( $\text{E}$ )-  
624 cinnamaldehyde obtained from benzaldehyde aldolisation (Bitter almond and petrochemical origins).

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