



HAL
open science

Type and magnitude of non-compliance and adulteration in neroli, mandarin and bergamot essential oils purchased on-line: potential consumer vulnerability

Marissa Pierson, Xavier Fernandez, Sylvain Antoniotti

► To cite this version:

Marissa Pierson, Xavier Fernandez, Sylvain Antoniotti. Type and magnitude of non-compliance and adulteration in neroli, mandarin and bergamot essential oils purchased on-line: potential consumer vulnerability. *Scientific Reports*, 2021, 11 (1), 10.1038/s41598-021-90307-2 . hal-03418878

HAL Id: hal-03418878

<https://hal.science/hal-03418878>

Submitted on 17 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Type and magnitude of non-compliance and adulteration in neroli, mandarin and
2 bergamot essential oils purchased on-line: potential consumer vulnerability

3
4 Marissa Pierson,^{1,2} Xavier Fernandez,² Sylvain Antoniotti^{2,3*}

5
6
7 ¹ *European Research Institute on Natural Ingredients (ERINI), 4 Traverse Dupont, 06130*
8 *Grasse, FRANCE.*

9 ² *Université Cote d'Azur, CNRS, Institut de Chimie de Nice, Parc Valrose, 06108 Nice Cedex 2,*
10 *FRANCE.*

11 ³ *Université Cote d'Azur, Institute for Innovation and Partnerships in Flavor, Fragrance,*
12 *Cosmetics, Grasse Biotech, 45 Bd Marcel Pagnol, 06130 Grasse, FRANCE.*

13
14 Correspondence: sylvain.antoniotti@univ-cotedazur.fr
15
16

17
18 **Abstract**

19
20 Thirty-one samples of essential oils used both in perfumery and aromatherapy were
21 purchased to business-to-consumers suppliers and submitted to standard gas
22 chromatography-based analysis of their chemical composition. Their compliance with ISO
23 AFNOR standards was checked and revealed, although ISO AFNOR ranges are relatively loose,
24 that more than 45% of the samples analyzed failed to pass the test and more than 19% were
25 diluted with solvents such as propylene and dipropylene glycol, triethyl citrate, or vegetal oil.
26 Cases of non-compliance could be due to substitution or dilution with a cheaper essential oil,
27 such as sweet orange oil, blending with selected compounds (linalool and linalyl acetate,
28 maybe of synthetic origin), or issues of aging, harvest, or manufacturing that should be either
29 deliberate or accidental. In some cases, natural variability could be invoked. These products
30 are made available to the market without control and liability by resellers and could expose
31 the public to safety issues, in addition to commercial prejudice, in sharp contrast with the
32 ever-increasing regulations applying to the sector and the high demand of consumers for safe,
33 controlled and traceable products in fragrances and cosmetic products.
34

37 Introduction

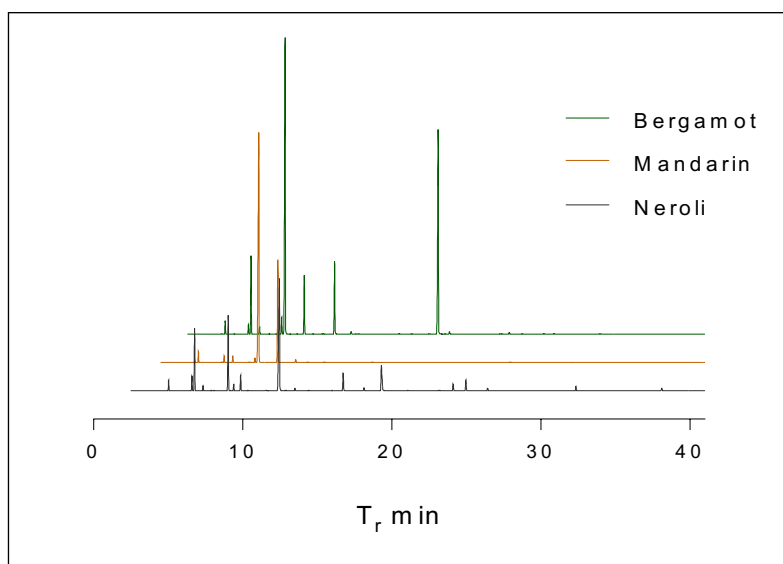
38 Essentials oils (EOs) are complex natural substances obtained by physical treatments of selected parts
39 of plants. In most instances, distillation is used, either through hydro-distillation techniques or, for
40 more sensitive materials, by steam distillation. In the particular case of the *Citrus* genus, cold pressure
41 is generally applied to the pericarp to obtain the EO.

42 The fraction of the metabolome of plants as observed in EOs is mostly composed of terpenes,
43 terpenoids, and phenylpropanoids. Sometimes, linear hydrocarbons are also observed as in rose EOs,
44 or other compounds with sulphur-containing functional groups such as thiols or thiocyanates.
45 Considering the method of obtention, EOs are almost exclusively volatile materials, although in the
46 case of cold-pressed EOs, a non-negligible fraction can be non-volatile.

47 As volatile material, the analytical technique of choice for EOs is gas chromatography (GC) which allows
48 for, when conducted with adequate and validated methods, both qualitative and quantitative
49 characterization.¹ Typically, identity of analytes is obtained by coupling mass spectrometry (MS) with
50 gas chromatography (GC-MS), determining retention indexes and co-injecting pure compounds if
51 necessary, while their proportion in the mixture is determined by using a stable and universal detector
52 such as a flame ionization detector (GC-FID) using the corrected response factor method.^{2,3} The
53 chemical composition of EOs thereby obtained is reproducible, and only subjected to natural variation
54 that could occur depending on geographical, seasonal, or agricultural factors and manufacturing
55 processes.⁴

56 For centuries EOs have attracted the attention of mankind for their biological properties, either as
57 odoriferous material for religious or pagan practice, and perfumery, or for therapeutic applications,
58 either in folk medicine, aromatherapy or pharmaceutical activities.^{5,6} As potentially biologically active
59 material, EOs sometimes contain significant quantities of toxic compounds such as methyleugenol
60 (suspected carcinogen),^{7,8} safrole (weak hepatocarcinogen among other adverse effects), estragole
61 (suspected carcinogen and genotoxic),⁹⁻¹³ furocoumarins (dermatitis inducers),^{14,15} and allergenic
62 compounds, among others.¹⁶

63 In the particular case of *Citrus* EOs, the chemical composition features mostly monoterpenes and
64 monoterpenoids, the prominent metabolite being limonene, either as the *R* or *S* enantiomer
65 depending on the genus, species or even cultivar.^{17,18} *Citrus* EOs such as bergamot (*Citrus bergamia*),
66 mandarin (*Citrus reticulata*), or neroli (*Citrus aurantium* ssp *Amara* or *Bigaradia*, from which only the
67 flowers are steam distilled) EOs play an important role in fine perfumery for their delicate scents; while
68 sweet orange (*Citrus sinensis*) EO, which is almost entirely constituted of (*R*)-limonene, has very little
69 olfactory interest (Figure 1).



70

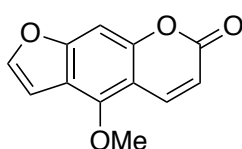
71 **Figure 1.** Examples of total ion chromatograms, offset by 1 minute each, of *Citrus* essential oils
 72 (bergamot, mandarin, and neroli essential oils). Chemical compositions are detailed below.

73

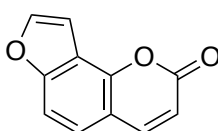
74 *In vitro* and *in vivo* biological activities for *Citrus* EOs have been reported such as antimicrobial, antiviral
 75 and antioxidant properties, insect repellent activities,¹⁹ as well as effects on central nervous system
 76 (anxiety, attention, relaxation, sleep, mood, stress ...) ²⁰ and should be used as natural preservatives.²¹⁻
 77 ²³

78 On a darker side, toxicity issues arise in the presence of furocoumarins, present in the non-volatile
 79 fraction of some *Citrus* EOs obtained by cold-pressure along with the EO (*vide supra*), and
 80 hydroperoxides, such as limonene hydroperoxide due to aging and auto-oxidation under air and light
 81 exposure.²⁴ Furocoumarins and hydroperoxides can induce skin disorders such as contact dermatitis
 82 and other allergenic reactions, and in worse cases exhibit photogenotoxicity for the former (Figure
 83 2).¹⁴ Furocoumarins total content should not exceed 1 ppm in cosmetic products in Europe.²⁵

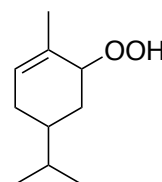
84



85 **Bergapten**



86 **Angelicin**



87 **Limonene 2-hydroperoxide**

85

86 **Figure 2.** Chemical structures of some furocoumarins and limonene 2-hydroperoxide.

87

88 EOs can be obtained from various part of most plants (flowers, leaves, steams, buds, roots, bark ...)
89 resulting in a large diversity of EOs. However, because of the similarities in the early steps of plant
90 metabolism, individual compounds such as monoterpenes are found in the essential oils of many
91 plants. In the case of sweet orange EO, huge quantities are available on the market because it is a co-
92 product of orange juice manufacture resulting in low cost and large availability (30 000 tons in 2004
93 and increasing).²⁶ At the same time, rare EOs of high demand and/or of limited offer such as those
94 obtained from rose (*Rosa damascena*), agarwood (*Aquilaria malaccensis*), orris (*Iris germanica*) or
95 ylang ylang (*Cananga odorata*) are difficult to find and their value can reach several thousands of euros
96 per kilogram. This contrast has resulted in economically motivated adulteration (EMA), a fraudulent
97 practice consisting in adding cheap material to an expensive one to increase profit.²⁷ Adulteration of
98 essential oils is typically observed in four different types:

- 99 - Dilution with a solvent featuring similar physico-chemical properties than EOs such as vegetal
100 oils, or organic solvents (triethylcitrate, (di)propylene glycol, alkyl phthalates, ...);
- 101 - Addition of a cheaper EO more or less close in terms of origin (same genus) or chemical
102 composition (e.g. sweet orange EO (*Citrus sinensis*) in other *Citrus* EOs or palmarosa EO
103 (*Cymbopogon martini*) in rose EOs);
- 104 - Addition of individual compounds, either natural or synthetic, to mimic the olfactory
105 properties, the composition or the chemotype (e.g. linalyl acetate to mimic lavender EO
106 (*Lavendula angustifolia*) or neryl acetate to mimic immortelle EO (*Helichrysum italicum*));
- 107 - Substitution with another EO or mixture of EOs of low value (sweet orange oil, turpentine oil,
108 ...) to which key compounds, either natural or synthetic, are added.

109 These types of deliberate practice should not be agglomerated with accidental events (errors of
110 botanical authentication, inadvertent blending of vegetal material, sometimes handled as powders,
111 contamination during manufacture, storage, labelling, ...) or natural variation that result in non-
112 compliance. Trading of EOs is framed by normative references such as ISO and AFNOR providing
113 industry accepted ranges of concentration for selected compounds in each EO.²⁸

114 In all these cases, analytical chemistry is required to characterize adulteration both qualitatively and
115 quantitatively.²⁹ Adulteration techniques have evolved continuously along with analytical
116 countermeasures to reach a high level of sophistication. Indeed, besides sensory and physico-chemical
117 properties such as density, optical rotation, or refractive index, R&D and QC labs have developed
118 techniques and combinations of techniques to identify even the most subtle cases of adulteration.
119 Worth mentioning but not limited to are isotopic approaches (¹³C/¹²C ratios or ¹⁴C contents),³⁰

120 enantioselective analyses,³¹ multidimensional GC techniques,³² or spectroscopic or spectrometric
121 methods (NMR,³³ fluorescence,³⁴ IR,³⁵ Raman³⁶) coupled with metabolomic approaches.

122 In contrast with these accurate, efficient, and sensitive techniques in constant evolution which enable
123 professionals to detect most cases of adulteration, consumers in business to consumer (B to C)
124 commerce have limited knowledge about issues of adulteration and no analytical capabilities and can
125 only count on the fairness of their suppliers. The inherent risk for consumers handling concentrated,
126 potentially biologically active, molecules with cases of overdose, misuse and intoxication in family
127 practice is compounded upon when these products may not match their labels.³⁷

128 In this context, we embarked into a study of the quality of EOs purchased on-line directly from EOs
129 suppliers web sites or from global shopping platforms operating worldwide. We have chosen to focus
130 on three *Citrus* EOs: mandarin (*Citrus reticulata*), bergamot (*Citrus bergamia*), and neroli (*Citrus*
131 *aurantium* ssp *Amara* or *Bigaradia*).

132

133 **Results and discussion**

134 In the frame of a research program dedicated to the study of authenticity and naturality of essential
135 oils, and considering the increasing interest of consumers for essential oils, natural products, home-
136 made cosmetics and household products, we became interested in sampling essential oils purchased
137 on-line to evaluate their quality. We thus purchased samples of EOs of mandarin (*Citrus reticulata*),
138 bergamot (*Citrus bergamia*), and neroli (*Citrus aurantium* ssp *Amara* or *Bigaradia*). With 23 samples
139 out of ca. 230 made available from a large platform operating on-line, the sampling size was deemed
140 sufficient to have a first estimate ($\pm 20\%$ at the 95% level of confidence, for a normal distribution). To
141 these 23 samples were added 8 additional samples purchased from other retail sources, mostly online
142 as well. To encompass the largest variability possible, we decided to simply start with checking the
143 compliance of these samples with ISO norms using gas chromatography coupled with flame ionization
144 detector (FID) and mass selective analyzer (MS).

145

146 *Analysis of neroli essential oil (NEO)*

147 Nine commercial samples of NEO were analyzed and their peak areas were compared to ISO Norm
148 3517.³⁸ Two samples were acquired from a reputable source and considered premium samples. Of the
149 nine neroli samples, only one sample, N4, met all of the ISO chromatographic specifications. The ISO
150 standard includes acceptable ranges for 13 compounds; α -pinene, β -pinene, sabinene; β -myrcene,

151 limonene, (*E*)- β -ocimene, linalool, α -terpineol, linalyl acetate, neryl and geranyl acetate, (*E*)-nerolidol
152 and (*E,E*)-farnesol.

153 Samples N6 and N9, were not compliant with all 13 compounds in the ISO range. N9 is low in β -pinene
154 and high in linalyl acetate at $16.19 \pm 0.02\%$ compared to the 3-15% range. N6 had $21.42 \pm 0.02\%$ linalyl
155 acetate, significantly outside the ISO range.

156 N1 and N2 were purchased online from two different labels but shared nearly identical compositions.
157 Both had low values for β -pinene, β -myrcene, limonene, linalool and (*E*)-nerolidol. Linalyl acetate
158 constituted over 26% of the oils' compositions; more than 10% higher than the ISO prescription.
159 Sabinene and α -pinene were not identified in either sample, however the standard range extends to
160 traces of these compounds. Comparing the peak areas to the premium samples shows a gross
161 adulteration by dilution, likely with a vegetal oil or propylene glycol type diluent.

162 N3 and N4 were two of the best samples compared to the ISO requirements. N3 had low β -myrcene
163 and (*E*)-nerolidol content. N4 met all of the prescribed values. N3's adherence is surprising due to its
164 price point at less than \$1/mL compared to N4's \$5.32/mL. This is not to say there is no chance of
165 adulteration in either of these samples, merely that these samples meet, or nearly meet, the advice of
166 the ISO reference.

167 N5 fell out-of-range for 6 out of the 13 compounds.

168 N7 and N8 were severely adulterated with di-propylene glycol and propylene glycol, respectively. The
169 chromatograms clearly show the adulterants as well as the resulting peak reduction of the compounds
170 of interest (see S.I.). The coelution of the adulterant and the analyte peaks makes it difficult to even
171 compare the samples to the ISO reference. This disregard toward the obviousness of adulteration is
172 indicative of the fact that consumers have no real means of verifying the quality of the product. The
173 business knows that the consumer has no access to GC and can adulterate without sophistication.

174 In addition, to boost olfactory properties, N7 contained 4-tert-butylcyclohexyl acetate (mixture of
175 diastereoisomers), a well-known synthetic woody odorant, and N1, N2 and N7 contained nerolin and
176 methyl- β -naphthyl ketone, which are synthetic materials known for decades exhibiting neroli
177 perfume.³⁹

178 By comparing for example N7 and N9^r (Figure 3), broad peaks in N7 are clearly visible and represent
179 dipropylene glycol adulteration. There is a marked difference in linalool concentration at 15 min
180 between the two samples. N7 also has significantly higher concentration of 2-phenylethanol, a
181 naturally occurring compound in neroli found in the reference at about 1.1% but 5.24% in N7.

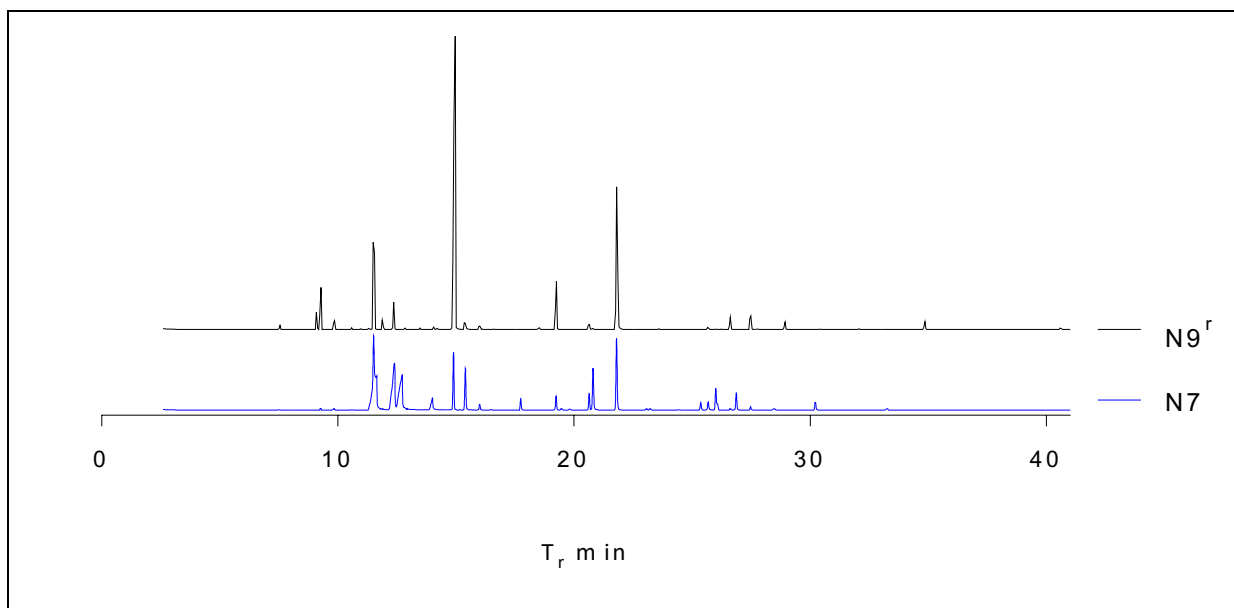


Figure 3. Sample comparison of two neroli samples as seen on GC-FID.

182
183
184
185

186 N10, although considered reference for its olfactory quality, does not meet all specifications.

187

188 *Analysis of mandarin essential oil (MEO)*

189 Mandarin oil is particular in the industry as the fruit is harvested in 3 stages of maturity; green, yellow
190 and red mandarin oils can be sold as distinct oils, although the ISO norm only specifies Italian type
191 mandarin. This range of harvest times affects the chemical composition of the oils but the three
192 mandarin oils are not given individual ISO ranges. This increases the difficulty of determining if the
193 variation of the composition is natural or the result of adulteration. Cold-pressed oils contain
194 compounds too heavy or chemically unlikely to distill like waxes and furocoumarins. Citrus oils have
195 characteristic furocoumarin compositions that can aid in the identification of adulteration. This
196 approach was indeed successfully applied recently using either HPLC with columns with fused-core
197 technology or UPLC-TOF-MS.^{40,41}

198 Eleven commercial samples of MEO were analyzed and their peak areas were compared to ISO Norm
199 3528.⁴² Two samples were acquired from reputable sources and considered premium reference
200 samples. Of the eleven MEO samples, only one sample, M4, met all of the ISO chromatographic
201 specifications. The ISO standard includes acceptable ranges for 7 compounds; α -pinene, β -pinene,
202 myrcene, γ -terpinene, limonene, methyl-N-methylantranilate, and α -sinensal.

203 The premium samples, M12 and M13 did not meet all 7 profiled compounds. M12 fell out of range for
204 4 compounds. M13 was high for β -pinene at $3.13 \pm 0.19\%$ rather than 2.0% max.

205 M1 and M2 were grossly out of specification in very similar fashion. Falling out of range for 6
206 compounds, only passing the myrcene specification. Each have about 95% limonene content which
207 would suggest the oil is actually sweet orange essential oil. Methyl N-methylantranilate was also not
208 detected, an important odorant molecule for mandarin oil.

209 M3 poses an interesting question. Four of the regulated compounds are out of the ISO range, however,
210 they are just slightly beyond the ranges. For example, ISO limits limonene to 65-75% area, this sample
211 has $76.15 \pm 0.04\%$; similarly, γ -terpinene is set from 16-22% with M3 containing $15.35 \pm 0.005\%$. The
212 question then becomes, how hard are the limits of the ISO ranges? Is a sample really “bad” if it falls
213 just beyond the prescribed ranges or should it be considered natural variability?⁴³ Besides the question
214 of maturity of mandarins at the time of harvest mentioned above, linalool and linalyl acetate vary by
215 several percent throughout a growing season of Egyptian neroli oil,⁴⁴ and α -terpineol, terpinen-4-ol,
216 α - & β -phellandrene and camphene change within growing seasons of bergamot crops, with α -
217 terpineol increasing consistently and dramatically throughout the season across different years.⁴⁵

218 M4 and M10 came from the same company. M4 meets all requirements of the ISO mandarin reference.
219 M10 was sold as tangerine and does not meet the requirements of the mandarin norm. There is no
220 official ISO norm for tangerine but literature suggests that a limonene content greater than 90% and a
221 γ -terpinene content near 4% are characteristic.⁴⁶ M10 has a limonene content of 93.4% and γ -
222 terpinene at 2.9% seemingly in agreement with other tangerine oils.

223 M5, M8, and M9 are all very nearly within the specification of the ISO standard. M5 met all specified
224 ranges excluding α -sinensal. M8 is just below the range for methyl N-methylantranilate and α -
225 sinensal was not identified, with all other compounds within the ranges. M9 had a limonene content
226 of $76.12 \pm 0.03\%$ and a γ -terpinene content of $15.64 \pm 0.01\%$. These minor deviations from the ISO
227 profile could be considered natural variations within an oil.

228 M11 only met the specification for limonene, the other 6 compounds falling below the required ranges
229 or not identified at all. Uncharacteristically, this oil also contained linalool and linalyl acetate at over
230 10% each. This could be a sign of adulteration, potentially to improve the scent.

231 Furocoumarins contents were evaluated for the whole set of mandarin samples by reversed-phase
232 HPLC-PDA analysis using 5-methylpsoralen⁴⁷ as analytical reference. M1, M2 and M10 showed the
233 same furocoumarin pattern as sweet orange oil taken as reference. Most samples did not undergo
234 furocoumarin removal process. Orange oil being sold with a label of mandarin oil can more than double
235 the sale price (from \$0.20 to \$0.57 per mL from one source) to consumers that may not have a nose
236 sensitive enough to smell the differences.

237

238 *Analysis of bergamot essential oil (BEO)*

239 Eleven commercial samples of bergamot essential oil were analyzed and their peak areas were
240 compared to ISO Norm 3520.⁴⁸ Two samples were acquired from reputable sources and considered
241 premium reference samples. Of the eleven BEO samples, only one sample, B6, met all of the ISO
242 chromatographic specifications. The ISO standard includes acceptable ranges for 7 compounds: β -
243 pinene, γ -terpinene, limonene, linalool, linalyl acetate, geranial, and β -bisabolene.

244 B1 and B2, purchased at 12.5 cents/mL, were subject to gross dilution. B1 had a large peak identified
245 as triethyl citrate (TEC) comprising 60% of the total peak area. Excluding the TEC peak, B1 meets the
246 relative peak area requirements of the ISO standard. B2 also meets the peak area requirements of the
247 ISO standard, but is heavily diluted with a vegetal oil that is not visible by GC directly. These oils are
248 likely genuine bergamot essential oils that have been diluted with a known perfumery solvent. ¹H-NMR
249 analysis indeed showed in B1 characteristic signals of TEC with 2 quadruplets at 4.8-4.0 ppm, 2
250 doublets with strong coupling constants and roof effect at 2.9-2.5 ppm, and 2 triplets at 1.3-1.2 ppm.
251 In B2, a pattern consisting of ethylenic, allylic and aliphatic signals characteristic of soybean oil was
252 observed (see S.I.).

253 The majority of the bergamot samples nearly met all of the ISO prescription except for either the
254 geranial or β -bisabolene ranges. B3 was just over the range for β -pinene at $9.58 \pm 0.00\%$ and below
255 the 0.3% minimum β -bisabolene level, with all other compounds within the range. B4 and B10 also fell
256 out of range for β -bisabolene. B4, B11 and B12, were lower than the 0.25% minimum geranial
257 specification, B4 being nearly at the limit with $0.24 \pm .004\%$. B10 has a second compound out of range,
258 γ -terpinene, which is $5.83 \pm 0.02 \%$, below the 6% AFNOR value. B9 falls just out of range for β -pinene
259 at $5.43 \pm 0.38\%$, but this standard deviation would suggest the β -pinene can be considered within the
260 range. B7 was in range for all compounds except geranial. B5 has 3 compounds out of range, but to a
261 very small extent. These samples are all very close to being acceptable according to the AFNOR ranges.
262 They are unlikely to be adulterated, but likely graded as lower odor quality by the perfume industry
263 and sold to the essential oil industry.

264 B6 is entirely conformant to the ISO reference. B7, purchased from a French pharmacy, was fully
265 compliant except for geranial again, possibly due to aging, aldehydes such as geranial and neral being
266 lost upon oxidation to the corresponding carboxylic acids or cyclized and dehydrated to *para*-
267 cymene.^{49,50}

268 Reverse-phase HPLC-PDA analysis of bergamot samples revealed the presence of 5-methylpsoralen
269 and a closely related peak, and at significantly lower concentrations in highly diluted samples B1 and

270 B2. Furocoumarin free (FCF) samples; B4, B10, B15, and B16 contained neither of these peaks, but had
271 defaults in their composition in geranial and β -bisabolene.

272

273 *Graphical representation with heat maps*

274 Statistical manipulation follows a modified Z-score analysis. Standard Z-scores are calculated using the
275 statistical mean and standard deviation.

276

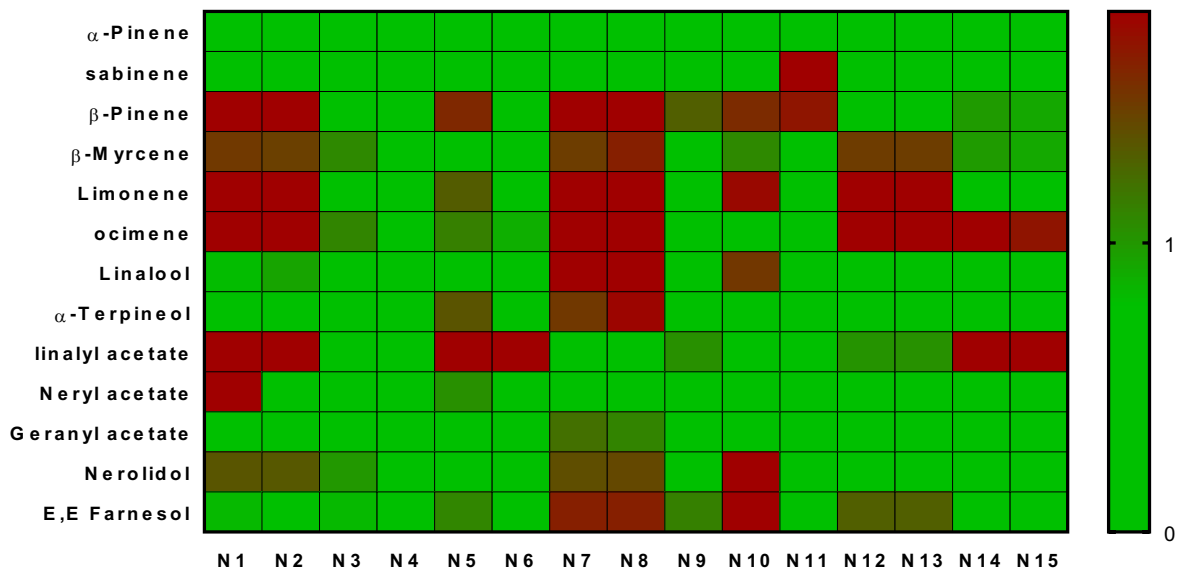
277
$$z = \frac{x - \mu}{\sigma}$$

278 The score relays the relative distance of a data point to the mean, a Z-score less than one would place
279 the data point within the first standard deviation of the data set.

280 In this analysis, the Z-score calculation was modified to reflect the AFNOR/ISO guidelines. The mid-
281 point of the range replaces the statistical average (μ), and the distance between the mid-point and the
282 edge of the range becomes the standard deviation (σ). This results in a score of the distance of each
283 compound from the accepted value, normalized to the magnitude of the range. This allows for easier
284 comparison of compounds present at varying levels and with different specifications.

285 Heat maps are generated by applying the modified Z-score calculation to each sample's compounds of
286 interested per the AFNOR standard. A score less than or equal to one is green, signifying the acceptable
287 level of the compound. Scores over 1 are (gradient from green to red) with the intensity reflecting the
288 severity of the distance from the accepted value. This visual representation shows quickly and
289 intuitively if a sample is compliant or far from the AFNOR guidelines (Figures 4-6).

290

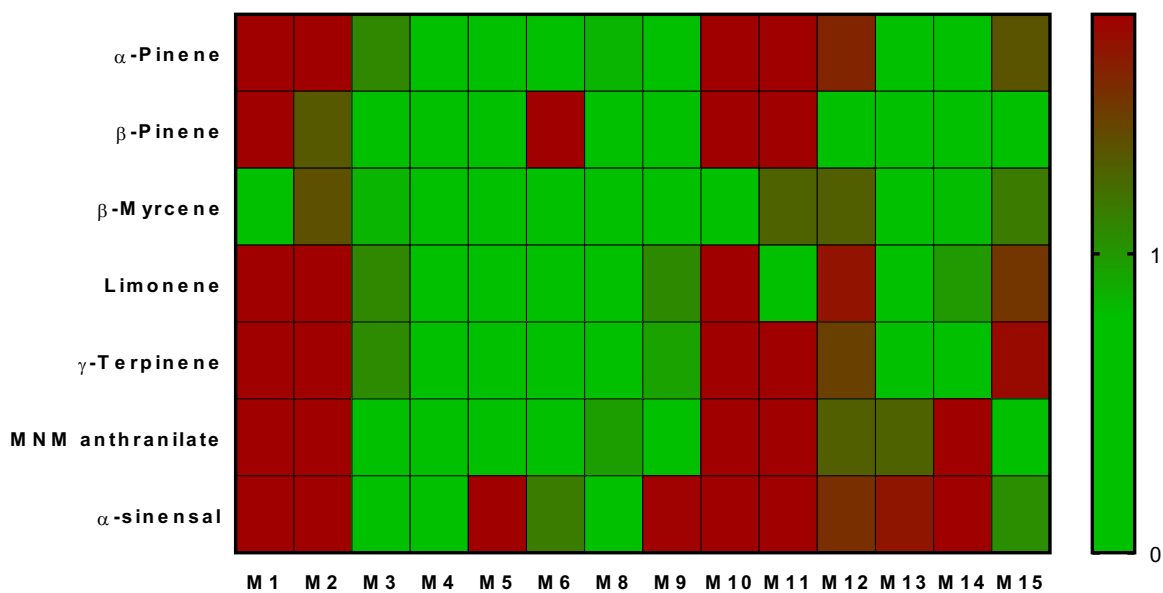


291

292

293

Figure 4. Heat map of relative scores of neroli essential oils for relevant compounds according to ISO 3517. N9 and N10 were obtain from a reputable source.



294

295

296

Figure 5. Heat map of relative scores of mandarin essential oils for relevant compounds according to ISO 3528. M12 and M13 were obtain from reputable source.

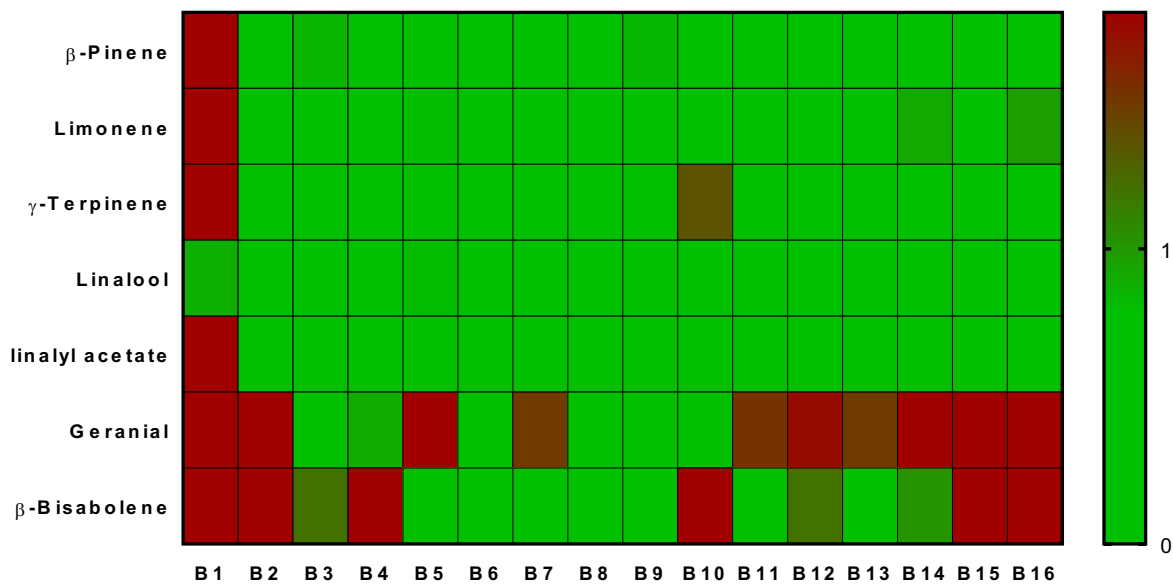


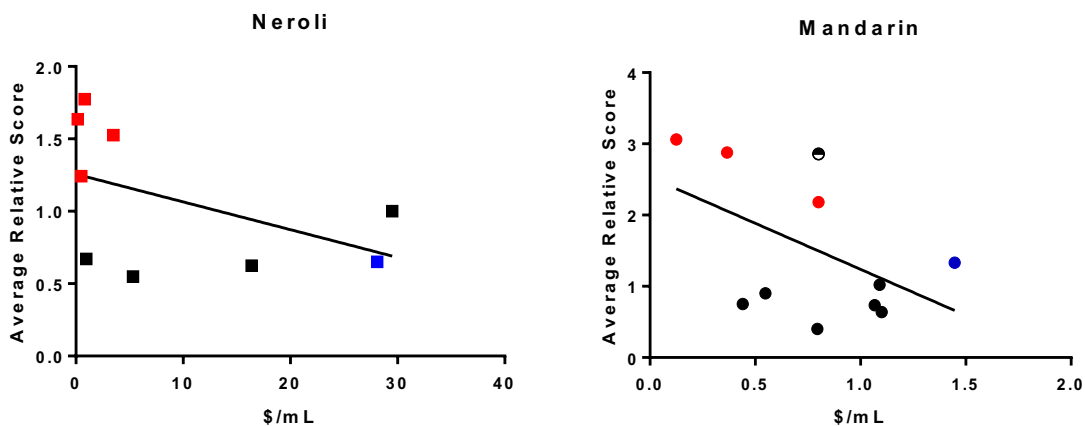
Figure 6. Heat map of relative scores of bergamot essential oils for relevant compounds according to ISO 3520. B11 and B12 were obtained from reputable sources.

Price/quality relationship

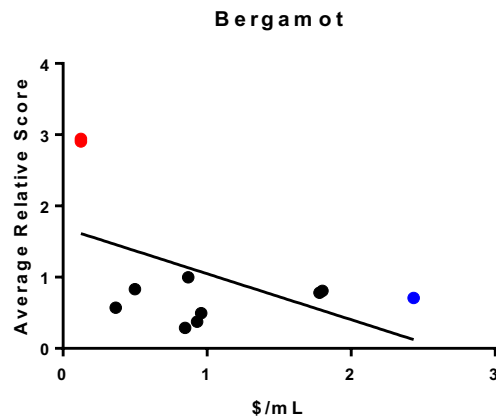
Price per milliliter of oil was calculated based on the purchase price in USD, excluding tax, and the container volume per label. Samples were chosen from online retailers with the intention of obtaining a range of price points. The hypothesis being that adulteration would be more prevalent in samples with a lower price/mL. Economically motivated adulteration is the unethical practice of adding material to a product to increase profits. To add diluent to an oil sold well below the market value would allow the seller to make a profit even at a reduced price, a buyer could predict that the quality of the product may be compromised when buying at a deep discount. To add diluent to an oil sold at or above market value would significantly increase the profits of the seller while falsely assuring the buyer that the oil is of the quality expected at the higher price point.

Because of neroli's high price, it is of particular interest in this study. Neroli is the steam distilled oil of bitter orange flowers, floral EOs are often expensive due to the delicate harvest procedures and low oil yields.⁵¹ Additionally, because neroli blossoms become oranges if left to be pollinated on the tree, there is often a limit on neroli harvest in order to not impact the later bitter orange harvest. The price per milliliter of neroli in our sample set ranged from \$0.17/mL to \$29.50/mL. Sample N4, the only sample to meet all of the ISO guidelines for neroli EO was purchased at \$5.31/mL, falling about the median price of our sample list while the most expensive sample, N5, failed to meet 6 out of 13 of the requisite values (Figure 7).

319



320



321

322

323 **Figure 7.** The average relative score of each sample is plotted against the price in USD per
324 milliliter of essential oil (red: adulterated; black: acceptable; blue: reference).

325

326 Mandarin is a considerably less expensive oil. The price range of the samples analyzed here was \$0.12-
327 \$1.45/mL, with the average price/mL equal to \$0.78.

328 This data does not give a clear prescriptive for purchasing a quality essential oil. The problem is not
329 resolved by simply buying the most expensive oil. One could imagine that advice being quite dangerous
330 to the market, flooding it with overpriced low-quality oils. The transparency and the quality control
331 standards of businesses then become the consumers' guides for purchasing.

332 **Conclusion**

333 Regulatory bodies will struggle to police the online world of consumer products because of the
334 magnitude of the industry. It would be impossible to test every essential oil in the online market the

335 way we have for these 31 products. Policing the market becomes even more difficult when a company
336 that exists only online can change names or raw materials with little to no traceability. Label control
337 seems non-existent with nearly all labels including some version of the words 100% pure, natural, or
338 therapeutic grade essential oil. Most labels include verbiage to dilute essential oils as they are highly
339 concentrated and can irritate skin, even on samples that had been heavily diluted already. There is
340 also the question of who is at fault when an oil is sold adulterated. Is the adulteration happening at
341 the raw material stage and the seller is only guilty of not verifying their raw materials? Or are the
342 sellers buying quality raw materials and adulterating the products themselves? The surest choice for
343 one consumer at the end of this complex value chain seems to be to purchase essential oils from known
344 suppliers with a brand strategy, generally running rigorous quality control.

345

346 **Materials and Methods**

347 Commercial EO samples were obtained through internet retailers and French pharmacies. Premium
348 samples were obtained from reputable fragrance houses. Nine (9) bergamot (*Citrus bergamia*)
349 essential oil samples were purchased from online retailers, and 1 was purchased in a French pharmacy.
350 Eight (8) mandarin (*Citrus reticulata*) essential oil samples were purchased online, 1 from a French
351 pharmacy. One (1) Tangerine sample was also purchased online to compare to the mandarin samples.
352 Eight (8) neroli (*Citrus aurantium*) essential oil samples were purchased from online retailers.

353 An Agilent Technologies gas chromatograph (6890) was used with a flame ionization detector (FID),
354 and an Agilent J&W HP-5 column with dimensions 30m x 0.32mm ID, with 0.25µm film thickness. 1 µL
355 of diluted essential oil (20 µL sample and 20 µL of 5mg/mL methyl octanoate internal standard diluted
356 to 1.0 mL with ethyl acetate) was injected in split mode at 10:1. Oven temperature was programmed
357 at 50 C for 3 minutes, then to 265 C at 3 C/min, a final ramp to 300 C at 15 C/min and 5 min hold.
358 Injector and detector temperatures were set at 265 °C. Hydrogen was the carrier gas at a constant flow
359 of 35 mL/min. Linear retention indices were calculated with reference to n-alkanes (C7-C30).

360 GC-MS analyses were performed on a 7890A GC system coupled to a 5975C VL mass spectrometer
361 detector (Agilent Technologies) equipped with an Agilent J&W HP-5 column with dimensions 30m x
362 0.32mm ID, with 0.25µm film thickness. 1 µL of diluted essential oil (20 µL sample and 20 µL of 5mg/mL
363 methyl octanoate internal standard diluted to 1.0 mL with ethyl acetate) was injected in split mode at
364 10:1. The GC-MS experimental conditions developed in the laboratory were the same as GC-FID
365 analysis except for injector and detector temperatures (200 °C); carrier gas (helium); ionization voltage
366 70 eV; electron multiplier, 1 kV. Compound identification was accomplished through comparison of

367 their mass spectra to NIST05 libraries as well as by comparison of their retention indices literature
368 data. EOs compositions are given as relative area percentages. See SI for more details.

369

370 References

- 371 1 Rubiolo, P., Sgorbini, B., Liberto, E., Cordero, C. & Bicchi, C. Essential oils and volatiles: sample
372 preparation and analysis. A review. *Flavour Fragr. J.* **25**, 282-290, doi:10.1002/ffj.1984 (2010).
- 373 2 Bicchi, C., Chaintreau, A. & Joulain, D. Identification of flavour and fragrance constituents. *Flavour*
374 *Fragr. J.* **33**, 201-202, doi:10.1002/ffj.3445 (2018).
- 375 3 Cachet, T. *et al.* IOFI recommended practice for the use of predicted relative-response factors for the
376 rapid quantification of volatile flavouring compounds by GC-FID. *Flavour Fragr. J.* **31**, 191-194,
377 doi:10.1002/ffj.3311 (2016).
- 378 4 N. Dhami, A. D. M. Phytochemical variation: How to resolve the quality controversies of herbal
379 medicinal products? *J. Herbal Med.* **5**, 118-127 (2015).
- 380 5 Baser, K. H. C. & Buchbauer, G. Handbook of Essential Oils: Science, Technology, and Applications. 2nd
381 edn, pp1128 (CRC Press, 2015).
- 382 6 Puškárová, A., Bučková, M., Kraková, L., Pangallo, D. & Kozics, K. The antibacterial and antifungal
383 activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. *Sci. Rep.* **7**, 8211,
384 doi:10.1038/s41598-017-08673-9 (2017).
- 385 7 Sipe, H. J., Lardinois, O. M. & Mason, R. P. Free Radical Metabolism of Methyleugenol and Related
386 Compounds. *Chem. Res. Toxicol.* **27**, 483-489, doi:10.1021/tx400256b (2014).
- 387 8 Auerbach, S. S. *et al.* Predicting the hepatocarcinogenic potential of alkenylbenzene flavoring agents
388 using toxicogenomics and machine learning. *Toxicol. Appl. Pharmacol.* **243**, 300-314 (2010).
- 389 9 Rietjens, I. M. C. M. *et al.* Flavonoids and alkenylbenzenes: Mechanisms of mutagenic action and
390 carcinogenic risk. *Mutat. Res.-Fund. Mol. M.* **574**, 124-138,
391 doi:<https://doi.org/10.1016/j.mrfmmm.2005.01.028> (2005).
- 392 10 Abdo, K. M. *et al.* 14-Week toxicity and cell proliferation of methyleugenol administered by gavage to
393 F344 rats and B6C3F1 mice. *Food Chem. Toxicol.* **39**, 303-316, doi:[https://doi.org/10.1016/S0278-](https://doi.org/10.1016/S0278-6915(00)00143-5)
394 [6915\(00\)00143-5](https://doi.org/10.1016/S0278-6915(00)00143-5) (2001).
- 395 11 Johnson, J. D. *et al.* Two-Year Toxicity and Carcinogenicity Study of Methyleugenol in F344/N Rats and
396 B6C3F1 Mice. *J. Agric. Food Chem.* **48**, 3620-3632, doi:10.1021/jf000364a (2000).
- 397 12 Burkey, J. L., Sauer, J.-M., McQueen, C. A. & Glenn Sipes, I. Cytotoxicity and genotoxicity of
398 methyleugenol and related congeners — a mechanism of activation for methyleugenol. *Mutat. Res.-*
399 *Fund. Mol. M.* **453**, 25-33, doi:[https://doi.org/10.1016/S0027-5107\(00\)00070-1](https://doi.org/10.1016/S0027-5107(00)00070-1) (2000).
- 400 13 Chan, V. S. W. & Caldwell, J. Comparative induction of unscheduled DNA synthesis in cultured rat
401 hepatocytes by allylbenzenes and their 1'-hydroxy metabolites. *Food Chem. Toxicol.* **30**, 831-836,
402 doi:[https://doi.org/10.1016/0278-6915\(92\)90047-0](https://doi.org/10.1016/0278-6915(92)90047-0) (1992).
- 403 14 Melough, M. M., Cho, E. & Chun, O. K. Furocoumarins: A review of biochemical activities, dietary
404 sources and intake, and potential health risks. *Food Chem. Toxicol.* **113**, 99-107,
405 doi:<https://doi.org/10.1016/j.fct.2018.01.030> (2018).
- 406 15 Sage, E. *et al.* Oxidative DNA damage photo-induced by 3-carbethoxypsoralen and other
407 furocoumarins: Mechanisms of photo-oxidation and recognition by repair enzymes. *J. Mol. Biol.* **209**,
408 297-314, doi:[https://doi.org/10.1016/0022-2836\(89\)90278-7](https://doi.org/10.1016/0022-2836(89)90278-7) (1989).
- 409 16 Sarkic, A. & Stappen, I. Essential Oils and Their Single Compounds in Cosmetics—A Critical Review.
410 *Cosmetics* **5**, doi:10.3390/cosmetics5010011 (2018).
- 411 17 Asztemborska, M. & Ochocka, J. R. in *Studies in Natural Products Chemistry* Vol. 27 (ed Rahman Atta
412 ur) 361-391 (Elsevier, 2002).
- 413 18 Delort, E. *et al.* Comparative analysis of three Australian finger lime (*Citrus australasica*) cultivars:
414 Identification of unique citrus chemotypes and new volatile molecules. *Phytochemistry* **109**, 111-124,
415 doi:<https://doi.org/10.1016/j.phytochem.2014.10.023> (2015).
- 416 19 Adorjan, B. & Buchbauer, G. Biological properties of essential oils: an updated review. *Flavour Fragr. J.*
417 **25**, 407-426, doi:10.1002/ffj.2024 (2010).
- 418 20 Dobetsberger, C. & Buchbauer, G. Actions of essential oils on the central nervous system: An updated
419 review. *Flavour Fragr. J.* **26**, 300-316, doi:10.1002/ffj.2045 (2011).

420 21 E.M. Mustafa, N. Citrus Essential Oils: Current and Prospective Uses in the Food Industry. *Recent Pat.*
421 *Food Nutr. Agric.* **7**, 115-127 (2015).

422 22 Hąc-Wydro, K., Flasiński, M. & Romańczuk, K. Essential oils as food eco-preservatives: Model system
423 studies on the effect of temperature on limonene antibacterial activity. *Food Chem.* **235**, 127-135,
424 doi:<https://doi.org/10.1016/j.foodchem.2017.05.051> (2017).

425 23 Mahato, N. *et al.* Citrus essential oils: Extraction, authentication and application in food preservation.
426 *Crit. Rev. Food Sci. Nutr.* **59**, 611-625, doi:10.1080/10408398.2017.1384716 (2019).

427 24 de Groot, A. Limonene Hydroperoxides. *Dermatitis* **30**, 331-335, doi:10.1097/der.0000000000000465
428 (2019).

429 25 Commission, E. Scientific Committee on Consumer Products (SCCP) Opinion on Furocoumarins in
430 Cosmetic Products. Report No. SCCP/0942/0905 (2005),
431 https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_036.pdf, downloaded 27th
432 April 2021.

433 26 Swift, K. A. D. Catalytic transformations of the major terpene feedstocks. *Top. Catal.* **27**, 143-155
434 (2004).

435 27 Do, T. K. T., Hadji-Minaglou, F., Antoniotti, S. & Fernandez, X. Essential oil authenticity: a challenge for
436 the analytical chemist *Trends Anal. Chem.* **66**, 146–157 (2015).

437 28 ISO, *Essential oils*. in Standards catalogue, ICS 71.100.60.

438 29 T. K. T. Do, F. H.-M., S. Antoniotti, X. Fernandez. Essential oil authenticity. *Trends Anal. Chem.* **66**, 146–
439 157 (2015).

440 30 Gershon, H., Lykkeberg, A., Goren, F. & Mason, S. Identifying Fraudulent Natural Products: A
441 Perspective on the Application of Carbon-14 Analysis. *J. Agric. Food Chem.* **67**, 13393-13399,
442 doi:10.1021/acs.jafc.9b01821 (2019).

443 31 Cagliero, C. *et al.* Enantioselective Gas Chromatography with Derivatized Cyclodextrins in the Flavour
444 and Fragrance Field. *Isr. J. Chem.* **56**, 925-939, doi:10.1002/ijch.201600091 (2016).

445 32 Lebanov, L., Tedone, L., Kaykhaii, M., Linford, M. R. & Paull, B. Multidimensional Gas Chromatography
446 in Essential Oil Analysis. Part 2: Application to Characterisation and Identification. *Chromatographia*
447 **82**, 399-414, doi:10.1007/s10337-018-3651-9 (2019).

448 33 Cerceau, C. I., Barbosa, L. C. A., Alvarenga, E. S., Maltha, C. R. A. & Ismail, F. M. D. ¹H-NMR and GC for
449 detection of adulteration in commercial essential oils of *Cymbopogon ssp.* *Phytochem. Anal.* **31**, 88-97,
450 doi:10.1002/pca.2869 (2020).

451 34 Mbogning Feudjio, W. *et al.* Fluorescence Spectroscopy Combined with Chemometrics for the
452 Investigation of the Adulteration of Essential Oils. *Food Anal. Methods* **10**, 2539-2548,
453 doi:10.1007/s12161-017-0823-4 (2017).

454 35 Bounaas, K. *et al.* Essential oil counterfeit identification through middle infrared spectroscopy.
455 *Microchem. J.* **139**, 347-356, doi:<https://doi.org/10.1016/j.microc.2018.03.008> (2018).

456 36 Vargas Jentzsch, P., Gualpa, F., Ramos, L. A. & Ciobotă, V. Adulteration of clove essential oil: Detection
457 using a handheld Raman spectrometer. *Flavour Fragr. J.* **33**, 184-190, doi:10.1002/ffj.3438 (2018).

458 37 Lee, K. A., Harnett, J. E. & Cairns, R. Essential oil exposures in Australia: analysis of cases reported to
459 the NSW Poisons Information Centre. *Med. J. Aust.* **212**, 132-133, doi:doi: 10.5694/mja2.50403 (2020).

460 38 Neroli. <https://www.iso.org/fr/standard/45645.html>, downloaded 17th April 2020.

461 39 West, T. F. C. Synthetic perfumes. *Sci. Prog.* **36**, 38-54 (1948).

462 40 Russo, M. *et al.* Reduced time HPLC analyses for fast quality control of citrus essential oils. *J. Essent.*
463 *Oil Res.* **27**, 307-315 (2015).

464 41 Masson, J. *et al.* Oxygenated heterocyclic compounds to differentiate Citrus spp. essential oils through
465 metabolomic strategies. *Food Chem.* **206**, 223-233 (2016).
466 <https://www.iso.org/fr/standard/53970.html>, downloaded 17th April 2020.

467 43 König, W. A., Fricke, C., Saritas, Y., Momeni, B. & Hohenfeld, G. Adulteration or natural variability?
468 enantioselective gas chromatography in purity control of essential oils. *J. High Resolut. Chromatogr.*
469 **20**, 55-61, doi:10.1002/jhrc.1240200202 (1997).

470 44 Bonaccorsi, I. *et al.* Composition of Egyptian Neroli Oil. *Nat. Prod. Commun.* **6**, 1934578X1100600723
471 (2011).

472 45 Dugo, G. *et al.* Characterization of cold-pressed and processed bergamot oils by using GC-FID, GC-MS,
473 GC-C-IRMS, enantio-GC, MDGC, HPLC and HPLC-MS-IT-TOF. *J. Essent. Oil Res.* **24**, 93-117 (2012).

474 46 Reeve, D., Treatt, R., Arthur, D. & Treatt, F. Riding the citrus trail: when is a mandarin a tangerine ?
475 *Perfumer & Flavorist* **27**, 20-22 (2002).

- 476 47 Dugrand-Judek, A. *et al.* The Distribution of Coumarins and Furanocoumarins in Citrus Species Closely
477 Matches Citrus Phylogeny and Reflects the Organization of Biosynthetic Pathways. *PLoS One* **10**,
478 e0142757-e0142757 (2015).
479 48 <https://www.iso.org/fr/standard/8893.html>, downloaded 17th april 2020.
480 49 Turek, C. & Stintzing, F. C. Stability of Essential Oils: A Review. *Compr. Rev. Food Sci. F.* **12**, 40-53
481 (2013).
482 50 Jakab, E. *et al.* Thermo-oxidative decomposition of lime, bergamot and cardamom essential oils. *J.*
483 *Anal. Appl. Pyrolysis* **134**, 552-561 (2018).
484 51 Sarrou, E., Chatzopoulou, P., Dimassi-Theriou, K. & Therios, I. Volatile Constituents and Antioxidant
485 Activity of Peel, Flowers and Leaf Oils of Citrus aurantium L. Growing in Greece. *Molecules* **18**, 10639-
486 10647, doi:10.3390/molecules180910639 (2013).
487
488
489

490 **Acknowledgements**

492 This work was supported by Université Cote d'Azur and the CNRS. We are grateful to François Gray for
493 training M. Pierson at her arrival in the lab.
494

495 **Author Contributions**

496 M.P. performed the analytical experimentation. M.P. and S.A. wrote the article. M.P., X.F. and S.A.
497 participated in drafting the article. All authors designed the study, discussed the results and
498 commented on the manuscript.
499

500 **Additional Information**

501 A part of this study was supported by a research grant from Pharmapur UG (Düsseldorf, Germany) in
502 the frame of a research program on naturality and authenticity of essential oils. Pharmapur had no
503 input or impact on the design of experiments, or on analysis and presentation of the results.