The final publication is available at

https://doi.org/10.1016/j.foodres.2018.12.054

Additional Information

1 **HS-SPME analysis of the volatiles profile of water celery (*Apium**

2 ***nodiflorum*), a wild vegetable with increasing culinary interest**

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25 **Abstract**

26 Water celery (*Apium nodiflorum*) is a wild plant traditionally harvested in some

27 Mediterranean areas for being consumed raw. Despite its appreciated organoleptic

28 properties, the aromatic profile of the fresh vegetable remains to be studied. In the

29 present study, volatile compounds from five wild populations were extracted by the

30 headspace-solid phase microextraction technique, analysed by gas cromatography-mass

31 spectrometry, and compared to related crops. The wild species had a high number of

32 aromatic compounds. It was rich in monoterpenes (49.2%), sesquiterpenes (39.4%) and

33 phenylpropanoids (9.6%), with quantitative differences among populations, in absolute

34 terms and relative abundance. On average, germacrene D was the main compound

35 (16.6%), followed by *allo*-ocimene (11.9%) and limonene (11.1%). Only in one

36 population, the levels of limonene were greater than those of germacrene D. Among

37 phenylpropanoids, dillapiol displayed the highest levels, and co-occurred with

38 myristicin in all populations except one. These differences may have a genetic

39 component, which would indicate the possibility of establishing selection programmes

40 for the development of water celery as a crop adapted to different market preferences.

41 On the other hand, comparison with related crops revealed some similarities among

42 individual volatiles present in the different crops, which would be responsible of the

43 common aroma notes. However, water celery displayed a unique profile, which was in

44 addition quantitatively richer than others. Thus, this differentiation may promote the use

45 of water celery as a new crop.

## 1. Introduction

Aromatic plants, rich in volatile organic compounds (VOCs), have been highly appreciated by humans since the early beginning of civilization (Evergetis & Haroutounian, 2014). These plants are used in the cuisine to enhance the taste of many dishes, either as fresh herbs or dried spices. There are different botanical families including species rich in VOCs such as *Alliaceae, Apiaceae, Labiateae, Lauraceae, Myrtaceae* or *Rutaceae* (Raut & Karuppayil, 2014). Among these, *Apiaceae* and *Labiateae* families are probably the most relevant for culinary uses as flavour enhancers. Within the *Apiaceae* family it is possible to find species used in cuisine by the aerial parts such as celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.), coriander (*Coriandrum sativum* L.), dill (*Anethum graveolens* L.), fennel (*Foeniculum vulgare* Mill.) or parsley (*Petroselinum crispum* (Mill.) Nyman), or for the seeds such as anise (*Pimpinella anisum* L.) or cumin (*Cuminum cyminum* L.). Due to the importance of the family, it is possible to find several works studying the volatile profiles, specially focused on essential oils (e.g., Cioanca, Hancianu, Mircea, Trifan & Hritcu, 2016; El-Zaeddi et al., 2016; Filly et al., 2014). In addition, the interest on the volatile fraction and/or essential oils from other wild species and neglected vegetables has increased recently, attending mainly to pharmacological uses and, to a lesser extent, culinary purposes (e.g., Dev et al., 2010; Landoulsi et al., 2016; Maggi, Bartolucci & Conti, 2017; Quassinti et al., 2013; Tabanca et al., 2007).

Water celery (*Apium nodiflorum* (L.) Lag., also known as fool's watercress) is a perennial wild herb from the *Apiaceae* family. It is distributed in Central and Southern Europe (especially in the South-West), Northern Africa and Western and Central Asia (Knees, 2003; Molina, Pardo-de-Santayana, & Tardío, 2016), and can be easily found in fresh, shallow water courses such as natural streams or irrigation ditches. The young

73  leaves and tender shoots have been consumed in the Mediterranean cultures in salads

74  and different traditional dishes (Guarrera & Savo, 2016; Licata et al., 2016; Tardío et

75  al., 2016). In the last years wild water celery is marketed in the United Kingdom in

76  mixtures of wild vegetables due to its flavour and crunchy texture (Evans & Irving,

77  2018), and its use may increase if the species becomes cultivated.

78  The taste of water celery has been described as spicy (Guarrera & Savo, 2016) with a

79  flavour that resembles celery, carrot (*Daucus carota* L.) or a mixture of both of them

80  (Heshmati Afshar, Maggi, Iannarelli, Cianfaglione, & Isman, 2017; Nebel, Pieroni, &

81  Heinrich, 2006; "Wild Food UK," 2018). In addition, the volatile fraction has been

82  previously studied in essential oils extracted from dried materials (Benelli, Pavela,

83  Ricciutelli, Lupidi, & Maggi, 2017; Heshmati Afshar et al., 2017; Maxia et al., 2012;

84  Menghini, Leporini, Tirillini, Epifano, & Genovese, 2010). Nevertheless, dried samples

85  do not represent the common way to consume this vegetable. In addition, the extraction

86  of VOCs by hydrodistillation, the common methodology applied for essential oils, has

87  as a disadvantage the occurrence of thermal artifacts, which may modify the real

88  volatile profile, as well as tedious and time-consuming extraction protocols (Rodríguez-

89  Burruezo, Kollmannsberger, González-Mas, Nitz, & Nuez, 2010). As an alternative, the

90  headspace-solid phase microextraction (HS-SPME) technique has been successfully

91  used for the isolation of VOCs in a number of fruits and vegetables (e.g., González-

92  Mas, Rambla, Alamar, Gutiérrez & Granell, 2011; López-Gresa et al., 2017; Mzoughi et

93  al., 2018; Taveira et al., 2009), providing a more accurate profile of volatiles present in

94  fresh vegetables. In the present work, we evaluated the volatile profile of water celery

95  based on the analysis of the fresh edible leaves and tender shoots. Considering that the

96  most common use of water celery is as vegetable eaten raw in salads, HS-SPME

97  technique will provide a more realistic and accurate description of its aroma and flavour

98    factors, as they are perceived by the consumer. We also compared the volatile profile of

99    water celery with related cultivated species (i.e., carrot, celery and parsley) in order to

100   identify qualitative and quantitative differences and similarities, which will be helpful to

101   explain the distinctive features of the aroma and flavour of this species.

102

103   **2. Materials and methods**

104   *2.1. Plant material*

105   Irrigation ditches with regular water flows of the Horta Nord shire of Valencia (Spain)

106   were prospected for water celery populations during the spring of 2015 (Fig. 1). Five

107   isolated populations were sampled: Nod-001 (Puerto de Sagunto, 39º37'30" N, 0º16'49"

108   W), Nod-015 (Foios, 39º31'59" N, 0º20'31" W), Nod-018 (Meliana, 39º31'01" N,

109   0º19'36" W), Nod-023 (Pueblo Nuevo, 39º31'19" N, 0º23'17" W) and Nod-025 (Alfara

110   del Patriarca, 39º32'21" N, 0º23'06" W). The edible shoots were harvested, cleaned and

111   stored at 4 ºC until analysed within the next two days (Fig. 1).

112   In addition, two commercial samples of aerial parts of celery and parsley, and one

113   commercial sample of carrot (including both root and aerial parts) were acquired from

114   local markets and used as reference crops. The samples were also analysed within the

115   next 24 hours after acquisition. For all the materials, three independent replicates were

116   prepared.

117   *2.2. Preparation of samples and extraction of VOCs*

118   Extraction and analysis of volatiles was performed by the HS-SPME technique

119   according to Moreno, Fita, González-Mas and Rodríguez-Burruezo (2012). For that, 1.5

120   g of fresh leaves were weighted, finely chopped with a knife and immediately placed

121   into 20 mL sealed headspace vials. In the case of carrot roots, 1.5 g were weighted and

122   cut in small, regular pieces, then placed into the sealed vials. Pre-incubation of samples

123  was performed at 40ºC during 30 min, then in the extraction step, VOCs were adsorbed

124  on a fibre (50/30 µm DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) for 40 min at

125  the same temperature. The thermal desorption was carried out at 250ºC for 30 s in the

126  splitless mode. Prior to the first analysis, the fibre was conditioned at 270ºC for 1 h and

127  reconditioned after each sample for 30 min at 250ºC to ensure no cross-contamination

128  between samples.

129  *2.3. Analysis of volatiles*

130  VOCs were analysed by gas chromatography-mass spectrometry (GC-MS) using a

131  6890N Network GC System with autosampler coupled to a 5973 Inert Mass Selective

132  Detector (Agilent Techonologies, Santa Clara, CA, USA) and equipped with a HP-5MS

133  J&W silica capillary column (5% phenyl-95% methylpolysiloxane as stationary phase,

134  30 m length x 0.25 mm i.d., 0.25 µm thickness film; Agilent Technologies). Helium was

135  used as carrier gas at a constant flow of 1 mL min$^{-1}$. The temperature of the column was

136  programmed to raise from 100ºC to 250ºC at a rate of 5ºC min$^{-1}$ and then maintained at

137  250ºC for 10 minutes. The transfer line was maintained at 220ºC. The electron impact

138  (EI) mode (70 eV ionization energy, source temperature 225ºC) was used for the

139  detection by the mass spectrometer, and acquisition was performed in scanning mode

140  (mass range *m/z* 35-350 amu).

141  Chromatograms and spectra were processed using the MSD ChemStation D.02.00.275

142  (Agilent Technologies). Identification of compounds was performed by comparing the

143  GC retention time and mass spectra with reference substances (Sigma-Aldrich, Saint

144  Louis, MO, USA), when available, or tentatively by comparing the mass spectra with

145  the NIST 2005 Mass Spectral Library, previous literature and also with a customized

146  library from our laboratory and, when available. Due to the number of volatiles

147  detected, it was not possible to find suitable standards and estimate response factors.

148    Thus, quantification of VOCs was based on the integration of peak areas by the total ion

149    current chromatogram (TIC), as previously reported (e.g., González-Mas et al., 2011;

150    Moreno et al., 2012; Rodríguez-Burruezo et al., 2010). The percentage of each

151    compound was also estimated as the ratio of its peak area relative to the total of

152    compounds identified.

153    *2.4. Statistical analysis*

154    The five populations of water celery data were used for determining the mean value of

155    the species, and coefficients of variation (CV) were calculated. Data were $\log_2$-

156    transformed for normalization. Analysis of variance (ANOVA) was performed and

157    significant differences were calculated with the multiple range Student-Newman-Keuls

158    test. In addition, the relative abundance of each compound in the different samples was

159    calculated as the ratio between the peak area of the compound against the total area of

160    the compounds identified in the sample.

161    Finally, an illustrative comparison of the profiles in the different species was performed

162    by means of both Principal Component Analysis (PCA) and Hierarchical Cluster

163    Analysis, using the ClustVis Tool (Metsalu & Vilo, 2015). Unit variance scaling for the

164    normalized and centred data was applied for PCA. The Hierarchical Cluster Analysis

165    was performed with the distance measures based on Pearson correlations.

166

167    **3. Results**

168    *3.1. VOCs detected among the different species analysed*

169    A total of 64 VOCs were identified among the different species (Table 1). Terpenes

170    represented the largest group in all the materials studied (41 compounds, 16

171    monoterpenes and 25 sesquiterpenes). In addition, terpenoid-derived compounds were

172    also found, including several alcohols (two monoterpene-derived, four sesquiterpene-

173    derived and one diterpene-derived), three monoterpene-derived esters, one

174    monoterpene-derived ketone and one sesquiterpene-derived ether. Phenylpropanoids,

175    including myristicin and the derivatives apiol and dillapiol were also detected. Finally,

176    other compounds detected included two aromatic hydrocarbons, two ketones, one ester,

177    one cyclic alkene, two furanes and one thiazole.

178    The occurrence of the identified compounds differed among species. At a qualitative

179    level, water celery and parsley presented the most diverse volatile fraction, with forty-

180    four compounds identified in each species. On the contrary, celery showed the least

181    diverse volatile composition with only thirty-one VOCs, while carrot (both roots and

182    leaves) showed an intermediate level with thirty-seven VOCs (Table 1).

183    *3.2. Volatile profile of water celery*

184    The volatile profile of water celery was mainly characterized by terpene hydrocarbons.

185    The average relative abundance of monoterpenes ranged between 39.1 (Nod-025) and

186    61.3% (Nod-001); however, the highest absolute levels were determined for Nod-018

187    (Table 2). On the contrary, Nod-025 presented the highest level in sesquiterpenes, more

188    than 2-fold the levels of Nod-001. In relative terms, these contents corresponded to 53.9

189    and 25.8%, respectively.

190    The main terpenes targeted in the species were germacrene D (S17, 16.6% on average),

191    *allo*-ocimene (M16, 11.9%), limonene (M9, 11.1%), β-(*Z*)-ocimene (M11, 9.7%), and

192    terpinolene (M14, 7.3%) (Table 3). However, significant differences in quantitative

193    terms and relative abundance were found among populations (Table 2). Nod-025

194    displayed the highest levels of germacrene D, 2.3-fold higher than Nod-001, and

195    represented 22.5% of the total VOCs within this population. On the contrary, Nod-001

196    displayed the greatest area of limonene (M9), corresponding to a relative abundance of

197    18.0%. Terpinolene (M14) was also relevant in Nod-018, with a content up to 5-fold

198 higher than the other populations; while β-caryophyllene (S10) was accumulated in

199 high, similar content in Nod-015 and Nod-025. By contrast, *allo*-ocimene (M16) and β-

200 (*Z*)-ocimene (M11) had similar levels in the five populations.

201 Phenylpropanoids were also relevant in the species, with a relative abundance between

202 5.2% (Nod-025) and 15.0% (Nod-018) (Table 2). Dillapiol was the main

203 phenylpropanoid in all populations except Nod-001, with levels of expression similar to

204 relevant terpenes. Only in Nod-001, myristicin (P1) was found in higher relative

205 abundance than dillapiol (P2), representing the former 8.4% of total. Interestingly, Nod-

206 025 did not accumulated myristicin (P1).

207 *3.3. Comparison of the volatile profile of water celery with related crops*

208 Water celery was determined as the species with the highest values of total VOCs,

209 followed by parsley, while celery and the carrot samples presented low contents (Table

210 3). As in water celery, terpenoids were also the main group in all related species, with

211 monoterpenes relative abundances between 49.8% and 75.3% (carrot leaves and

212 parsley, respectively). Sesquiterpenes represented also the second major class in celery

213 and carrot materials, although the total absolute levels were significantly lower

214 compared to water celery. By contrast, the relative abundance of phenylpropanoids in

215 parsley was greater than the sesquiterpenes fraction (16.2% and 7.3% on average,

216 respectively) (Table 3).

217 In parsley, the main monoterpenes were 1,3,8-*p*-menthatriene (M15, 24.0% on average),

218 β-phellandrene (M10, 15.1%) and terpinolene (M14, 13.6%) (Table 3). As

219 phenylpropanoids, the species presented myristicin (P1) and apiol (P3) at relative

220 abundances of 7.6 and 9.0%, respectively. Celery also had great amounts of terpenes,

221 specially limonene (M9, 31.0%), and β-caryophyllene (S10, 23.6%) (Table 3). β-

222 Selinene (S18) was the third in abundance (12.0%). Phenylpropanoids were not found

9

223  in celery, and other compounds such as terpenoid derived compounds were present in

224  low amounts, representing only 1.8 % of the VOCs profile of this species. On the other

225  hand, the leaves and roots of carrot, even belonging to the same species, had important

226  differences (Table 3). The most abundant VOC in leaves was β-myrcene (M5),

227  representing 26.3% of its aroma. Other terpenes of relevance were germacrene D (S17,

228  13.2%), β-caryphyllene (S10, 9.7%) and α-pinene (M2, 9.0%). By contrast, terpinolene

229  was the main terpene of carrot roots (M14, 31.0%), followed by β-caryophyllene and α-

230  pinene (23.7 and 6.9%, respectively). Myristicin was the unique phenylpropanoid found

231  in the species, and accounted for 11.4% of total in carrot root but was only present as

232  traces in carrot leaf (Table 3).

233  The hierarchical cluster analysis showed the differences and similarities among species

234  in an illustrative way. The analysis separated two main clusters: A and B, including

235  different subclusters (Fig. 2). Subcluster A1 grouped the sesquiterpenes found in water

236  celery, which were at the highest contents compared to the related crops. Germacrene D

237  (S17), main compound of water celery, was found in this subcluster. Levels of this VOC

238  were on average 4-fold higher than in carrot leaves, although representing similar

239  relative abundances, and even higher compared to the other materials (Table 3). In this

240  subcluster was also included the phenylpropanoid myristicin (P1), determined at similar

241  levels in water celery, parsley and carrot roots but accounting for different relative

242  abundances (Table 3). Within the subcluster A2, A2.1 included the most relevant

243  monoterpenes in water celery: limonene (M9), found at similar content in celery but

244  representing in the latter a higher relative abundance, and *allo*-ocimene (M16), present

245  in all materials but displaying the highest content in water celery (Fig. 2, Table 3).

246  Subcluster A2.2 grouped the isomers α-caryophyllene (S14) and β-caryophyllene (S10)

247  and the derived caryophyllene oxide (SE1) (Fig. 2), whose content varied among

248    samples. In particular, β-caryophyllene (S10) had similar levels to carrot leaves and

249    celery although the relative abundance in water celery was lower (Table 3). Finally,

250    subcluster A2.3, which clearly separated the volatile profile of water celery from the

251    rest of species, included among others, the compounds reported as unique for water

252    celery (1,2-benzothiazole, T1; spathulenol, A4; dillapiol, P2; 4-hydroxy-3-methyl

253    acetophenone, K3; and α-gurjunene, S8) (Fig. 2).

254    Subclusters in cluster B grouped compounds of relevance for the other species (Fig. 2).

255    Most compounds grouped in subcluster B1 were exclusive of celery, such as β-selinene

256    (S18), one of the main VOCs of celery, and α-selinene (S20); or found in the species at

257    the highest content, like α-cedrene (S9), also present in parsley but at significant lower

258    levels (Table 3). On the contrary, subcluster B2.1 grouped some of the main compounds

259    of parsley (Fig. 2). In this subcluster was included the compound 1,3,8-*p*-menthatriene

260    (M15), on average 280-fold higher than in water celery; but also those exclusive for the

261    species including β-phellandrene (M10), with similar levels to limonene (M9) in water

262    celery,  and apiol (P3), close to the levels of dillapiol (P2) in water celery (Table 3).

263    Finally, subcluster B2.2 grouped compounds unique from carrot (e.g. β-

264    sesquiphellandrene, S12; and (*Z*)-β-farnesene) together with others found also in parsley

265    at considerable levels like α-pinene (M2), which level in these species was higher than

266    in water celery.

267    The PCA score plot clearly separated the materials evaluated according to the

268    qualitative and quantitative differences described among them (Fig. 3). The first two

269    principal components accounted for 63.0% of the variance (PC1 37.8%, PC2 25.2%),

270    which increased to 79.5% when the third principal component was considered. Water

271    celery and celery were clearly separated in the PC1, while the samples of parsley and

272    leaves of carrot overlapped. By contrast, the second principal component grouped the

273  materials by species, overlapping the roots and aerial parts of carrot and with water

274  celery and celery being very close to each other. Finally, the third principal component

275  separated the roots of carrot from the other leafy materials (Fig. 3).

276

277  **4. Discussion**

278  Water celery has been traditionally gathered from the wild and included in the culinary

279  tradition of several countries, especially among the Mediterranean cultures, that lived in

280  close connection with the nature. Despite the popularity of this wild vegetable,

281  described as an aromatic herb with spicy, intense flavour (Guarrera & Savo, 2013,

282  2016), there are few works focused on the aromatic profile of the species, based in the

283  analysis of the extracted essential oil from dried leaves and with pharmacological and

284  environmental purposes (Benelli et al., 2017; Heshmati Afshar et al., 2017; Maxia et al.,

285  2012; Menghini et al., 2010). Here we provide for the first time the volatile profile of

286  the unprocessed water celery shoots using the HS-SPME technique instead of other

287  extracting methods.

288  Previous works reported this species as rich in monoterpene hydrocarbons (20.9 to

289  58.7%) and phenylpropanoids (33.9 to 70.8%) (Benelli et al., 2017; Heshmati Afshar et

290  al., 2017; Maxia et al., 2012; Menghini et al., 2010), while the sesquiterpene fraction

291  represented less than 7.0% of total volatiles. By contrast, in our study, sesquiterpenes

292  ranged from 25.7 to 53.9%. These differences probably derived from the extraction

293  method and analysis employed. In this way, Stashenko, Jaramillo and Martínez (2004)

294  found that the HS-SPME technique significantly increased the percentage of total

295  sesquiterpenes, in comparison to other techniques. On the other hand, drying methods

296  may also affect the extraction of VOCs, although differences in these terms are not clear

297  and apparently depend on the spice, drying method and compound considered (Díaz-

12

298  Maroto, Pérez-Coello & Cabezudo, 2002; Pirbalouti, Mahdad & Craker, 2013).

299  Consequently, monoterpenes and phenylpropanoids, specially limonene, dillapiol and

300  myristicin were previously described as the main VOCs (Benelli et al., 2017; Heshmati

301  Afshar et al., 2017; Maxia et al., 2012; Menghini et al., 2010). By contrast, the HS-

302  SPME technique allowed in our work to identify the sesquiterpene germacrene D as the

303  main VOC in fresh leaves of water celery, while the relative abundance of

304  phenylpropanoids were not higher than 15% in any of the populations.

305  Our results showed that the volatile profile of water celery is quantitative and

306  qualitatively very rich, at the same or even higher level than parsley. The main aroma

307  constituents of water celery were germacrene D (S17), which provides weak spicy and

308  fruity flavour, and limonene (M9), with citrus and fresh notes (Acree and Arn, 2004;

309  Jirovetz, Buchbauer, Ngassoum & Geissler, 2002; "The Good Scents Company", 2018);

310  as well as *allo*-ocimene (M16), terpinolene (M14), and β-caryophyllene (S10), which

311  provide different woody, spicy and sweet notes (Acree and Arn, 2004; Jirovetz et al.,

312  2002; "The Good Scents Company", 2018). Also, the great content in dillapiol (P2)

313  contributed to the spicy and woody notes that can be detected in this vegetable.

314  Moreover, the specific aroma of a species is due not only to the main components, but

315  also the relative abundance of them (Auda, Pineau, Mestdagh, Poisson, & Rytz, 2016).

316  The aroma of water celery has been described as a mixture of carrot and celery

317  (Heshmati Afshar et al., 2017), with notes that related it to parsley. The aromatic profile

318  described here reinforces this idea. On the one hand, all of them were rich in terpenes.

319  This family of VOCs has been described as the main group in the essential oil in

320  *Apiaceae* species, together with the phenylpropanoids (e.g., El-Zaeddi et al., 2016;

321  Jawdat, Al-Faoury, Odeh & Al-Safadi, 2015; Valente et al., 2013). Many sesquiterpenes

322  determined here provide herbal and woody notes (Acree and Arn, 2004; Jirovetz et al.,

13

323    2002; "The Good Scents Company", 2018), whose presence would characterise the

324    common, basal aroma in all the materials. Moreover, some of the most prominent VOCs

325    of water celery were present in a similar relative abundance in celery, carrot and, to a

326    lesser extent, in parsley. For instance, limonene (M9), β-caryophyllene (S10), *allo-*

327    ocimene (M16), β-(Z)-ocimene (M11) and terpinolene (M14) were the predominant

328    compounds of celery and were in high relative abundances also in water celery. All

329    these VOCs are probably the reason for similarities in the aroma of both vegetables. The

330    same happened with the presence of β-caryophyllene (S10) and terpinolene (M14) in

331    carrot samples. And probably the notes of parsley come from the phenylpropanoid

332    myriscitin (P1) present in parsley and water celery. On the contrary, the presence of

333    other unique compounds, such as dillapiol (P2) or spathulenol (A4), and the specific

334    combinations of VOCs present, would contribute further to differentiate the aroma of

335    water celery.

336    Also, our findings showed that the volatile fraction of water celery was quantitatively

337    richer compared to the related species evaluated, particularly in the levels of

338    sesquiterpenes. An increasing content in these compounds would be reflected in a more

339    intense aroma. In fact, the aroma and taste of wild edible vegetables is frequently cited

340    as one of the sociocultural reasons behind the consumption of these plants (Serrasolses

341    et al., 2016); and, in the case of water celery, this is a critical point for its consumption

342    since the vegetable is described as an aromatic ingredient and, therefore, is used for

343    adding flavour to several dishes (Guarrera and Savo, 2013, 2016).

344    Finally, differences among the five water celery populations were described, in terms of

345    absolute GC areas and for relative abundance of individual compounds. The genotype

346    can be responsible in the production and accumulation of volatile compounds (Darriet,

347    Andreani, De Cian, Costa, & Muselli, 2014). Thus, our results may be used in future

348  works of selection and adaptation for developing a new crop adapted to the consumers'

349  preferences. For instance, Nod-001 presented the lowest levels in sesquiterpenes, so the

350  intensity of the woody and herbal odour that many of these compounds provide, would

351  be the lowest. On the contrary, this population may be selected for developing a variety

352  with more intense citrus and minty notes, due to the higher content and relative

353  abundance in limonene (M9) of this accession. By contrast, Nod-025 could be selected

354  for the high content combined with relative abundance in germacrene D (S17) and β-

355  caryophyllene (S10), two main sesquiterpenes that would provide woody and spicy

356  notes with high intensity due to their content. Moreover, based on the variation found in

357  our materials and considering that only populations of the Horta Nord shire of Valencia

358  were studied, our results suggest that more diversity on the volatile composition for this

359  species could be found in other areas. This suggestion is supported by the differences

360  that Maxia et al. (2012) found between specimens coming from Italy or Portugal.

361  Therefore, further surveys are advisable in order to widen the genetic pool available for

362  breeding based on the organoleptic quality of this species. In addition, the use of

363  controlled conditions for growing materials in the subsequent steps of the breeding

364  program would be useful for minimising the environmental effect.

365

366  **5. Conclusion**

367  As a whole, the volatile profile of water celery was determined as rich in terpenes, but

368  also the phenylpropanoids dillapiol and myristicin presented relevant contents in the

369  fresh leaves of this vegetable. In contrast to previous studies, the sesquiterpene fraction

370  was accumulated in higher percentage in our water celery materials, differences that

371  may be related to the extraction protocol and analysis of the VOCs. In fact, the HS-

372  SPME technique employed in the current study allowed identifying germacrene D as

373    the main compound of water celery, together with limonene and *allo*-ocimene.

374    Differences found among the populations for these contents and relative abundance may

375    present a genetic component, thus allowing the selection of materials according to

376    consumers' preferences.

377    The particular aroma of water celery, although unique, presented similarities in the

378    relative abundance of different VOCs with their relatives. These similarities would

379    account for the similarities in the aroma of water celery and the other species. In

380    addition, the sesquiterpene family of VOCs was accumulated in higher concentration in

381    water celery, which would be reflected in a more intense aroma with herbal, spicy and

382    citrus notes. Therefore, this distinct aroma quality may be useful for the differentiation

383    and enhancement of water celery as new salad ingredient, enhancing its value as a

384    potential new crop for vegetable diversification.

385

386    **Declarations of interest**

387    None.

388

389    **Acknowledgments**

394

395    **Funding sources**

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542    **Table 1.** List of volatile organic compounds (VOC) identified in the present study with

543    its retention index (RI), identification method (Id) and their presence in the materials

544    studied: water celery (WaCel), carrot leaf (CtL), carrot root (CtR), celery (Cel) and

545    parsley (Par).

| VOC | Code | RI | Id[d] | Wa Cel | CtL | CtR | Cel | Par |
|---|---|---|---|---|---|---|---|---|
| *Alcohols* | | | | | | | | |
| *p*-cymen-8-ol[a] | A1 | 1197 | MS | X | | X | X | |
| (Z)-carveol[a] | A2 | 1206 | MS | X | | | X | |
| germacrene D-4-ol[b] | A3 | 1660 | MS | X | | | | X |
| spathulenol[b] | A4 | 1536 | MS | X | | | | |
| α-cadinol[b] | A5 | 1580 | MS | X | | | | X |
| α-bisabolol[b] | A6 | 1625 | R | X | X | X | | |
| phytol[c] | A7 | 2045 | MS | X | X | | X | X |
| | | | | | | | | |
| *Esters* | | | | | | | | |
| (Z)-3-hexenyl acetate | E1 | 992 | MS | | X | | | X |
| bornyl acetate[a] | E2 | 1277 | MS | | | X | | X |
| carvyl acetate[a] | E3 | 1346 | MS | | | | X | |
| α-terpinyl acetate[a] | E4 | 1432 | MS | | | X | | |
| | | | | | | | | |
| *Furanes* | | | | | | | | |
| 4,7-dimethyl benzofuran | F1 | 1244 | MS | | | | | X |
| 3-butylidenephtalide | F2 | 1655 | MS | | | X | | |
| | | | | | | | | |
| *Aromatic hydrocarbons* | | | | | | | | |
| 1-Methyl-4-sec-butylbenzene | H1 | 1141 | MS | | | | | X |
| β-methylnaphthalene | H2 | 1345 | R | X | X | X | X | X |
| | | | | | | | | |
| *Ketones* | | | | | | | | |
| *p*-methylacetophenone | K1 | 1142 | MS | | | | | X |
| carvone[a] | K2 | 1190 | R | X | | | X | |
| 4-hydroxy-3-methyl acetophenone | K3 | 1363 | MS | X | | | | |
| | | | | | | | | |
| *Monoterpenes* | | | | | | | | |
| α-thujene | M1 | 902 | MS | | | | | X |
| α-pinene | M2 | 948 | R | X | X | X | X | X |
| camphene | M3 | 943 | R | | X | X | X | X |
| β-pinene | M4 | 943 | R | X | X | X | X | X |
| β-myrcene | M5 | 958 | R | X | X | X | X | X |
| α-phellandrene | M6 | 969 | R | X | X | X | X | X |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| α-terpinene | M7 | 998 | R | X | X | X | X | X |
| *p*-cymene | M8 | 1042 | R | X | X | X | X | X |
| limonene | M9 | 1018 | R | X | X | X | X | X |
| β-phellandrene | M10 | 964 | MS | | | | | X |
| β-(*Z*)-ocimene | M11 | 976 | R | X | X | X | X | X |
| β-(*E*)-ocimene | M12 | 976 | R | X | X | X | X | X |
| γ-terpinene | M13 | 998 | R | X | X | X | X | X |
| terpinolene | M14 | 1052 | R | X | X | X | X | X |
| 1,3,8-*p*-menthatriene | M15 | 1029 | MS | X | | X | X | X |
| *allo*-ocimene | M16 | 993 | R | X | X | X | X | X |

*Phenylpropanoids*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| myristicin | P1 | 1516 | R | X | X | X | | X |
| dillapiol | P2 | 1705 | MS | X | | | | |
| apiol | P3 | 1705 | MS | | | | | X |

*Sesquiterpenes*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| δ-elemene | S1 | 1367 | MS | X | X | X | | X |
| α-cubebene | S2 | 1344 | MS | X | X | X | | X |
| ylangene | S3 | 1221 | MS | X | X | X | | X |
| α-copaene | S4 | 1221 | R | X | X | X | X | X |
| β-bourbonene | S5 | 1339 | MS | X | X | | | X |
| β-cubebene | S6 | 1339 | MS | X | X | X | X | X |
| β-elemene | S7 | 1398 | R | X | X | X | X | X |
| α-gurjunene | S8 | 1419 | R | X | | | | |
| α-cedrene | S9 | 1403 | R | | | | X | X |
| β-caryophyllene | S10 | 1494 | R | X | X | X | X | X |
| α-bergamotene | S11 | 1430 | R | X | X | X | X | X |
| β-sesquiphellandrene | S12 | 1446 | MS | | X | X | | |
| γ-muurolene | S13 | 1435 | MS | X | X | | | X |
| α-caryophyllene | S14 | 1579 | R | X | X | X | X | X |
| (*Z*)-β-farnesene | S15 | 1440 | R | | X | X | | |
| α-curcumene | S16 | 1524 | MS | | | | X | |
| germacrene D | S17 | 1515 | R | X | X | X | | X |
| β-selinene | S18 | 1469 | MS | | | | X | |
| α-zingiberene | S19 | 1451 | MS | | | X | | |
| α-selinene | S20 | 1474 | MS | | | | X | |
| β-bisabolene | S21 | 1500 | MS | | X | X | | X |
| γ-cadinene | S22 | 1435 | MS | X | X | X | | X |
| δ-cadinene | S23 | 1469 | MS | X | X | | X | |
| cadala-(1,10)-3,8-triene | S24 | 1423 | MS | X | X | X | | X |
| cadalene | S25 | 1706 | MS | X | X | | | |

*Ethers*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| caryophyllene oxide[b] | SE1 | 1507 | R | X | X | X | X | X |

*Thiazols*

| 1,2-benzothiazole | T1 | 1208 | MS | X |

546  [a] Monoterpenoid derived compound

547  [b] Sesquiterpenoid derived compound

548  [c] Diterpenoid derived compound

549  [d] The identification of the VOCs is indicated by: R, if the VOC matched to the GC

550  retention time and MS with substances of reference; or MS, if the MS matched with the

551  NIST 2005 Mass Spectral library and considering the literature available and our

552  customized library

553　**Table 2.** Mean values (n=3) and coefficient of variation (CV) of the individual VOCs targeted in the five populations of water celery (Nod-001,

554　Nod-015, Nod-018, Nod-023, and Nod-025), expressed as GC peak area (x10$^6$). The relative abundance is also indicated, in parentheses, as

555　percentage from each VOC against the total identified.

| VOC | Nod-001 | Nod-015 | Nod-018 | Nod-023 | Nod-025 | CV |
|---|---|---|---|---|---|---|
| *p*-cymen-8-ol | 1.42 a | 0.43 a | 4.71 b | 1.64 a | 1.23 a | 0.82 |
| | (0.0) | (0.0) | (0.0) | (0.0) | (0.0) | |
| (Z)-carveol | 1.39 a | tr[a] | tr | tr | tr | 2.09 |
| | (0.0) | | | | | |
| germacrene D-4-ol | 3.73 a | 6.80 b | 6.31 b | 4.97 ab | 6.69 b | 0.28 |
| | (0.0) | (0.1) | (0.1) | (0.1) | (0.1) | |
| spathulenol | 8.08 a | tr | 8.62 a | 7.07 a | 3.51 a | 0.71 |
| | (0.1) | | (0.1) | (0.1) | (0.0) | |
| α-cadinol | 6.31 a | 5.14 a | - | 3.24 a | 4.90 a | 0.78 |
| | (0.1) | (0.1) | | (0.0) | (0.1) | |
| α-bisabolol | 31.72 a | 27.36 a | 28.57 a | 17.99 a | 20.63 a | 0.35 |
| | (0.4) | (0.3) | (0.3) | (0.2) | (0.2) | |
| phytol | 93.35 a | 85.89 a | 74.39 a | 83.67 a | 97.99 a | 0.23 |
| | (1.2) | (1.0) | (0.7) | (1.0) | (1.1) | |
| β-methylnaphthalene | 1.56 a | 1.43 a | 0.96 a | 28.90 c | 3.49 b | 1.56 |
| | (0.0) | (0.0) | (0.0) | (0.3) | (0.0) | |
| carvone | 1.48 a | 2.05 a | 0.73 a | 1.68 a | 1.00 a | 0.65 |
| | (0.0) | (0.0) | (0.0) | (0.0) | (0.0) | |
| 4-hydroxy-3-methyl acetophenone | 13.72 a | 17.06 ab | 11.32 a | 22.01 b | 14.16 a | 0.29 |
| | (0.2) | (0.2) | (0.1) | (0.3) | (0.2) | |

| | | | | | |
|---|---|---|---|---|---|
| α-pinene | 43.93 b (0.5) | 34.21 ab (0.4) | 33.35 ab (0.3) | 31.45 ab (0.3) | 22.22 a (0.2) | 0.28 |
| β-pinene | 612.22 c (7.4) | 506.07 bc (5.6) | 306.07 ab (2.8) | 349.34 abc (3.9) | 247.75 a (2.7) | 0.41 |
| β-myrcene | 130.54 a (1.6) | 108.38 a (1.2) | 137.54 a (1.3) | 120.44 a (1.3) | 111.20 a (1.2) | 0.26 |
| α-phellandrene | 28.10 ab (0.3) | 21.31 a (0.2) | 40.20 b (0.4) | 30.78 ab (0.3) | 25.71 ab (0.3) | 0.25 |
| α-terpinene | tr | 2.85 a (0.0) | 2.16 a (0.0) | 1.83 a (0.0) | tr | 1.14 |
| $p$-cymene | 33.30 a (0.4) | 65.37 b (0.7) | 45.64 a (0.4) | 36.05 a (0.4) | 31.67 a (0.3) | 0.36 |
| limonene | 1476.22 d (18.0) | 1002.70 bc (11.0) | 804.87 ab (7.5) | 1132.49 c (12.7) | 767.76 a (8.4) | 0.27 |
| β-(Z)-ocimene | 874.69 a (10.6) | 858.05 a (9.4) | 1190.80 a (11.0) | 910.65 a (9.8) | 722.43 a (7.9) | 0.24 |
| β-(E)-ocimene | 97.56 a (1.2) | 85.12 a (0.9) | 137.94 a (1.3) | 95.42 a (1.0) | 65.71 a (0.7) | 0.35 |
| γ-terpinene | 94.41 a (1.2) | 214.61 a (2.3) | 145.11 a (1.3) | 114.40 a (1.3) | 102.55 a (1.1) | 0.46 |
| terpinolene | 529.08 b (6.5) | 251.54 a (2.7) | 1263.25 c (11.7) | 769.46 bc (8.3) | 620.50 bc (6.8) | 0.56 |
| 1,3,8-$p$-menthatriene | 2.83 ab (0.0) | 1.29 a (0.0) | 14.23 c (0.1) | 5.06 b (0.1) | 4.79 b (0.1) | 0.87 |
| $allo$-ocimene | 1127.08 a (13.6) | 1043.03 a (11.4) | 1445.55 a (13.4) | 1092.00 a (11.7) | 850.75 a (9.3) | 0.25 |
| myristicin | 695.22 b (8.4) | 29.70 a (0.3) | 304.86 b (2.9) | 35.49 a (0.4) | - | 1.35 |

| | | | | | | |
|---|---|---|---|---|---|---|
| dillapiol | 209.61 a (2.5) | 647.04 ab (7.0) | 1437.32 b (13.0) | 681.37 ab (7.0) | 466.60 ab (5.2) | 0.80 |
| δ-elemene | 212.13 b (2.6) | 106.95 a (1.2) | 308.11 b (2.9) | 262.85 b (2.9) | 182.78 b (2.0) | 0.39 |
| α-cubebene | 17.82 a (0.2) | 37.89 bc (0.4) | 26.88 b (0.3) | 27.05 b (0.3) | 42.46 c (0.5) | 0.34 |
| ylangene | 9.42 a (0.1) | 18.27 b (0.2) | 15.13 b (0.1) | 14.87 b (0.2) | 23.24 b (0.3) | 0.34 |
| α-copaene | 126.84 a (1.5) | 270.68 b (3.0) | 191.92 b (1.8) | 191.59 b (2.2) | 267.91 b (2.9) | 0.30 |
| β-bourbonene | 31.50 a (0.4) | 38.81 a (0.4) | 33.72 a (0.3) | 24.94 a (0.3) | 37.14 a (0.4) | 0.26 |
| β-cubebene | 65.69 a (0.8) | 125.79 a (1.4) | 97.80 a (0.9) | 82.21 a (0.9) | 128.60 a (1.4) | 0.36 |
| β-elemene | 44.61 a (0.5) | 40.34 a (0.4) | 36.59 a (0.3) | 37.62 a (0.4) | 55.69 a (0.6) | 0.38 |
| α-gurjunene | 5.19 b (0.1) | 3.17 a (0.0) | 7.16 c (0.1) | 6.81 c (0.1) | 5.53 bc (0.1) | 0.29 |
| β-caryophyllene | 397.24 a (4.8) | 847.67 b (9.3) | 513.18 ab (4.8) | 589.96 ab (6.7) | 907.06 b (9.9) | 0.37 |
| α-bergamotene | 26.55 a (0.3) | 98.95 bc (1.1) | 61.60 ab (0.6) | 272.64 cd (3.2) | 413.56 d (4.2) | 1.01 |
| γ-muurolene | 92.31 a (1.1) | 185.30 b (2.0) | 152.68 b (1.4) | 144.44 b (1.6) | 226.46 b (2.5) | 0.34 |
| α-caryophyllene | 126.70 a (1.5) | 282.00 b (3.1) | 221.88 b (2.1) | 203.10 b (2.3) | 311.64 b (3.4) | 0.33 |
| germacrene D | 906.92 a (11.0) | 1800.27 b (19.7) | 1502.69 b (14.0) | 1516.03 b (17.0) | 2063.93 b (22.5) | 0.28 |

| | | | | | | |
|---|---|---|---|---|---|---|
| γ-cadinene | 60.87 a (0.7) | 267.10 b (2.9) | 276.45 b (2.6) | 129.64 b (1.4) | 143.26 b (1.6) | 0.60 |
| δ-cadinene | - | - | - | - | 395.55 a (4.3) | 2.09 |
| cadala-(1,10)-3,8-triene | 8.89 a (0.1) | 17.41 b (0.2) | 13.87 b (0.1) | 14.18 b (0.2) | 20.96 b (0.2) | 0.33 |
| cadalene | 2.20 a (0.0) | 3.25 a (0.0) | 2.70 a (0.0) | 2.94 a (0.0) | 4.28 a (0.0) | 0.31 |
| caryophyllene oxide | 6.20 a (0.1) | 6.89 a (0.1) | 4.34 a (0.0) | 5.68 a (0.1) | 7.12 a (0.1) | 0.34 |
| 1,2-benzothiazole | 0.74 a (0.0) | - | 0.78 a (0.0) | 1.04 a (0.0) | 0.91 a (0.0) | 0.58 |
| | | | | | | |
| *Total monoterpenes* | 5050 ab (61.3) | 4187 ab (45.8) | 5566 b (51.4) | 4689 ab (51.0) | 3573 a (39.1) | 0.2 |
| *Total sesquiterpenes* | 2134 a (25.8) | 4144 bc (45.3) | 3462 b (32.3) | 3521 b (39.7) | 4954 c (53.9) | 0.29 |
| *Total phenylpropanoids* | 904 a (10.9) | 667 a (7.2) | 1641 b (15.0) | 705 a (7.3) | 467 (5.2) | 0.6 |
| *Total terpenoid derived compounds* | 153 a (1.9) | 134 a (1.5) | 128 a (1.2) | 125 a (1.4) | 143 a (1.6) | 0.19 |
| *Total others* | 16 a (0.2) | 19 a (0.2) | 13 a (0.1) | 52 b (0.6) | 18 a (0.2) | 0.64 |

| *Total* | 8259 a | 9151 a | 10810 a | 9091 a | 9156 a | 0.13 |

556 Different letters within rows indicate significant differences at $P < 0.05$, according to the Student-Newman-Keuls test

557 [a] tr indicates compound detected as traces

558 **Table 3.** Mean values of the individual VOCs targeted in water celery (WaCel, n=5), carrot leaves (CtL, n=3), carrot roots (CtR, n=3), celery

559 (two samples, Cel1, n=3, and Cel2, n=3) and parsley (two samples, Par1, n=3, and Par2, n=3), expressed as GC peak area (x10$^6$). The relative

560 abundance is also given, in parentheses, as percentage of each compound against the total identified.

| | WaCel | CtL | CtR | Cel1 | Cel2 | Par1 | Par2 |
|---|---|---|---|---|---|---|---|
| *p*-cymen-8-ol | 1.89 a (0.0) | - | 0.89 a (0.0) | - | 0.20 a (0.0) | - | - |
| (Z)-carveol | 0.28 a (0.0) | - | - | 1.54 b | 0.43 b (0.0) | - | - |
| germacrene D-4-ol | 5.70 b (0.1) | - | - | - | - | 0.95 a (0.0) | 1.06 a (0.0) |
| spathulenol | 5.46 a (0.1) | - | - | - | - | - | - |
| α-cadinol | 3.92 a (0.0) | - | - | - | - | 2.98 a (0.0) | 1.74 a (0.0) |
| α-bisabolol | 25.25 c (0.3) | 3.23 b (0.1) | 0.66 a (0.0) | - | - | - | - |
| phytol | 87.06 e (0.9) | 29.58 bc (1.0) | - | 71.50 de (1.5) | 41.03 cd (1.6) | 16.38 a (0.2) | 22.83 ab (0.4) |
| (Z)-3-hexenyl acetate | - | 181.37 a (6.4) | - | - | - | - | 261.92 a (5.1) |
| bornyl acetate | - | - | 60.11 b (2.7) | - | - | - | 0.77 a (0.0) |
| carvyl acetate | - | - | - | 5.34 b (0.1) | 1.10 a (0.0) | - | - |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| α-terpinyl acetate | - | - | 0.77 a (0.0) | - | - | - | - |
| 4,7-dimethyl benzofuran | - | - | - | - | - | 1.83 a (0.0) | 1.47 a (0.0) |
| 3-butylidenephtalide | - | - | - | 4.75 a (0.1) | - | - | - |
| 1-Methyl-4-sec-butylbenzene | - | - | - | - | - | 1.50 a (0.0) | 1.34 a (0.0) |
| β-methylnaphthalene | 7.27 ab (0.1) | 0.44 a (0.0) | tr[a] | 20.30 b (0.4) | 8.26 ab (0.3) | 1.04 ab (0.0) | tr |
| $p$-methylacetophenone | - | - | - | - | - | 9.23 a (0.1) | 6.86 a (0.1) |
| carvone | 1.39 b (0.0) | - | - | 0.55 a (0.0) | 0.29 a (0.0) | - | - |
| 4-hydroxy-3-methyl acetophenone | 15.65 a (0.2) | - | - | - | - | - | - |
| α-thujene | - | - | - | - | - | tr | tr |
| α-pinene | 33.03 c (0.3) | 258.01 e (9.0) | 152.61 d (6.9) | 8.74 a (0.2) | 16.89 b (0.6) | 173.80 d (2.3) | 167.93 d (3.3) |
| camphene | tr | tr | tr | tr | 2.82a (0.1) | tr | tr |
| β-pinene | 404.29 d (4.3) | 110.47 c (3.9) | 75.36 bc (3.4) | 19.55 a (0.4) | 42.93 b (1.6) | 61.69 bc (0.8) | 109.21 c (2.1) |
| β-myrcene | 121.62 c (1.3) | 750.64 f (26.3) | 30.67 a (1.4) | 195.28 d (4.2) | 86.72 b (3.3) | 569.54 e (7.4) | 466.73 e (9.1) |
| α-phellandrene | 29.22 b (0.3) | tr | 13.19 a (0.6) | tr | tr | 223.73 c (2.9) | 36.54 b (0.7) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| α-terpinene | 2.28 a (0.0) | tr | 1.40 a (0.1) | tr | 2.42 a (0.1) | 14.64 b (0.2) | tr |
| *p*-cymene | 42.41 b (0.5) | 21.77 a (0.8) | 22.08 a (1.0) | 23.74 a (0.5) | 33.06 ab (1.3) | 171.21 d (2.2) | 89.12 c (1.7) |
| limonene | 1036.81 d (11.1) | 91.19 b (3.2) | 52.08 a (2.3) | 1478.66 e (31.9) | 786.04 d (30.2) | 141.67 c (1.8) | 81.70 b (1.6) |
| β-phellandrene | - | - | - | - | - | 1084.18 a (14.1) | 826.50 a (16.2) |
| β-(*Z*)-ocimene | 911.32 e (9.7) | 6.83 a (0.2) | tr | 178.84 c (3.9) | 257.28 d (9.9) | 10.39 ab (0.1) | 18.60 b (0.4) |
| β-(*E*)-ocimene | 96.35 c (1.0) | 133.20 c (4.7) | 14.98 a (0.7) | 26.11 b (0.6) | 12.65 a (0.5) | 68.28 c (0.9) | 113.72 c (2.2) |
| γ-terpinene | 134.22 d (1.4) | 27.02 ab (0.9) | 51.66 c (2.3) | 40.27 bc (0.9) | 66.46 c (2.6) | 22.82 ab (0.3) | 20.60 a (0.4) |
| terpinolene | 686.77 c (7.3) | 167.98 ab (5.9) | 687.45 c (31.0) | 122.60 a (2.6) | 247.06 b (9.5) | 1063.08 c (13.8) | 684.95 c (13.4) |
| 1,3,8-*p*-menthatriene | 5.64 a (0.1) | - | 2.15 a (0.1) | 4.39 a (0.1) | 2.33 a (0.1) | 2164.54 b (28.2) | 1010.39 b (19.8) |
| *allo*-ocimene | 1111.68 d (11.9) | 14.82 b (0.5) | 0.55 a (0.0) | 291.12 c (6.3) | 321.99 c (12.4) | 12.59 b (0.2) | 20.74 b (0.4) |
| myristicin | 213.05 a (2.3) | tr | 253.71 a (11.4) | - | - | 526.86 a (6.9) | 385.92 a (7.6) |
| dillapiol | 688.39 a (7.3) | - | - | - | - | - | - |
| apiol | - | - | - | - | - | 682.78 a (8.9) | 465.83 a (9.1) |
| δ-elemene | 214.56 e (2.3) | 44.58 d (1.6) | 15.75 c (0.7) | - | - | 4.55 b (0.1) | 2.06 a (0.0) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| α-cubebene | 30.42 d (0.3) | 16.73 c (0.6) | tr | - | - | 8.71 b (0.1) | 5.33 a (0.1) |
| ylangene | 16.19 d (0.2) | 4.77 c (0.2) | tr | - | - | 2.22 b (0.0) | 1.04 a (0.0) |
| α-copaene | 209.79 g (2.2) | 38.21 d (1.3) | 1.60 b (0.1) | 2.73 c (0.1) | 0.97 a (0.0) | 112.94 f (1.5) | 57.26 e (1.1) |
| β-bourbonene | 33.22 c (0.4) | 8.62 b (0.3) | - | - | - | 11.04 b (0.1) | 3.06 a (0.1) |
| β-cubebene | 100.02 e (1.1) | 17.21 c (0.6) | 1.56 a (0.1) | 5.94 b (0.1) | 1.73 a (0.1) | 46.93 d (0.6) | 24.16 c (0.5) |
| β-elemene | 42.97 d (0.5) | 8.80 c (0.3) | 2.65 b (0.1) | 4.96 c (0.1) | 1.35 a (0.0) | 8.57 c (0.1) | 2.96 b (0.1) |
| α-gurjunene | 5.57 a (0.1) | - | - | - | - | - | - |
| α-cedrene | - | - | - | 13.34 d (0.3) | 5.97 c (0.2) | 1.92 b (0.0) | 0.95 a (0.0) |
| β-caryophyllene | 651.02 d (6.9) | 278.24 c (9.7) | 525.64 d (23.7) | 1088.77 e (23.5) | 338.32 c (13.0) | 145.41 b (1.9) | 89.20 a (1.7) |
| α-bergamotene | 174.66 c (1.9) | 34.86 b (1.2) | 49.74 b (2.2) | 2.07 a (0.0) | 2.36 a (0.1) | 7.54 a (0.1) | 6.97 a (0.1) |
| β-sesquiphellandrene | - | 9.25 b (0.3) | 2.24 a (0.1) | - | - | - | - |
| γ-muurolene | 160.24 d (1.7) | 37.48 c (1.3) | - | - | - | 16.86 b (0.2) | 5.69 a (0.1) |
| α-caryophyllene | 229.06 c (2.4) | 90.82 b (3.2) | 84.33 b (3.8) | 138.79 b (3.0) | 33.21 a (1.3) | 39.00 a (0.5) | 24.77 a (0.5) |
| (Z)-β-farnesene | - | 29.63 b (1.0) | 7.07 a (0.3) | - | - | - | - |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| α-curcumene | - | - | - | 14.35 b (0.3) | 6.75 a (0.3) | - | - |
| germacrene D | 1557.97 e (16.6) | 376.43 d (13.2) | 21.50 a (1.0) | - | - | 211.18 c (2.7) | 68.46 b (1.3) |
| β-selinene | - | - | - | 693.87 b (15.0) | 233.83 a (9.0) | - | - |
| α-zingiberene | - | - | 18.13 a (0.8) | - | - | - | - |
| α-selinene | - | - | - | 167.18 b (3.6) | 43.72 a (1.7) | - | - |
| β-bisabolene | - | 9.94 a (0.3) | 61.18 d (2.8) | - | - | 28.14 c (0.4) | 17.80 b (0.3) |
| γ-cadinene | 175.46 c (1.9) | 11.80 b (0.4) | - | - | - | 8.67 b (0.1) | 2.59 a (0.0) |
| δ-cadinene | 79.11 a (0.8) | 32.12 a (1.1) | - | tr | 1.50 a (0.1) | - | - |
| cadala-(1,10)-3,8-triene | 15.07 e (0.2) | 2.29 d (0.1) | 0.34 a (0.0) | - | - | 1.05 c (0.0) | 0.54 b (0.0) |
| cadalene | 3.07 b (0.0) | 0.71 a (0.0) | - | - | - | - | - |
| caryophyllene oxide | 6.05 d (0.1) | 3.28 c (0.1) | 5.77 d (0.3) | 5.78 d (0.1) | 1.49 b (0.1) | 0.61 a (0.0) | 1.30 b (0.0) |
| 1,2-benzothiazole | 0.73 a (0.0) | - | - | - | - | - | - |
| *Total* | 4615 e | 1582 b | 1104 a | 2389 c | 1879 b | 5782 f | 3647 d |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *monoterpenes* | (49.2) | (55.5) | (49.8) | (51.6) | (72.2) | (75.3) | (71.4) |
| *Total sesquiterpenes* | 3698 e (39.4) | 1052 c (36.9) | 792 bc (35.7) | 2132 d (46.0) | 670 b (25.7) | 655 b (8.5) | 313 a (6.1) |
| *Total phenylpropanoids* | 901 a (9.6) | tr | 254 a (11.4) | - | - | 1210 a (15.8) | 852 a (16.7) |
| *Total terpenoid derived compounds* | 137 e (1.6) | 36 bc (1.3) | 68 d (3.1) | 85 d (1.8) | 44 c (1.7) | 21 a (0.3) | 28 ab (0.5) |
| *Total others* | 24 a (0.2) | 181 ab (6.4) | - | 25 ab (0.5) | 8 a (0.3) | 14 a (0.2) | 272 b (5.3) |
| *Total* | 9376 e | 2852 b | 2218 a | 4631 c | 2601 ab | 7681 d | 5111 c |

561 Different letters within rows indicate significant differences at $P < 0.05$, according to the Student-Newman-Keuls test
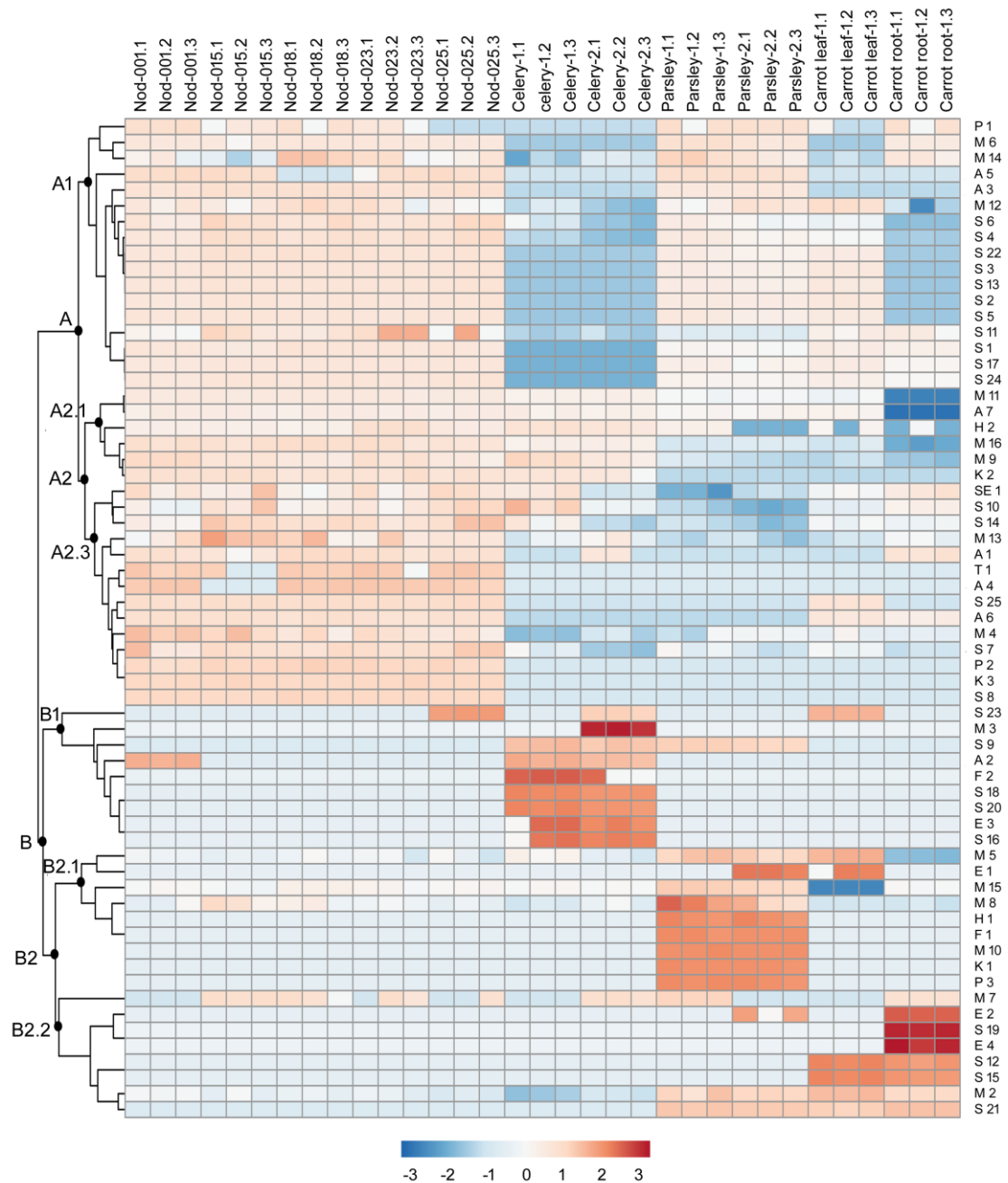
562 [a]tr indicates compound detected as traces

563

**Figure 1.** A1. Population of water celery growing in a ditch. A2. Material representing

the edible part of this wild vegetable.

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**Figure 2.** Hierarchical cluster analysis of the VOCs targeted in the materials studied:
water celery (Nod), celery, parsley, and carrot leaf and root, including the three
biological replicates of each material. VOCs are clustered in two clusters, A and B, and
the correspondent subclusters. Codes of the VOCs correspond to codes indicated in
Table 1.
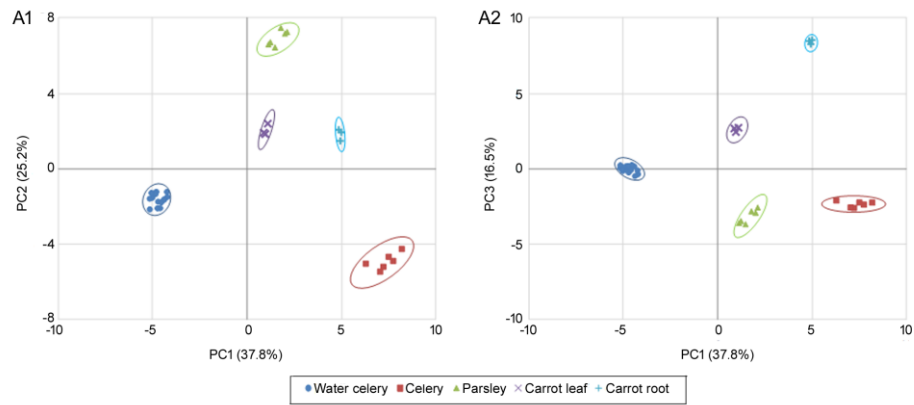
573

574 **Figure 3.** Scores plot from the Principal Component Analysis of the materials studied:

575 water celery, celery, parsley, and carrot leaf and root, including the three biological

576 replicates. A1. Analysis of the first and second principal components, A2. Analysis of

577 the first and third principal components.