

This is the peer reviewed version of the following article:

Kupska M., Jeleń H., In-tube extraction for the determination of the main volatile compounds in *Physalis peruviana* L, JOURNAL OF SEPARATION SCIENCE, Vol. 40, iss. 2 (2017), pp. 532-541, which has been published in final form at <https://dx.doi.org/10.1002/jssc.201600797>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions. This article may not be enhanced, enriched or otherwise transformed into a derivative work, without express permission from Wiley or by statutory rights under applicable legislation. Copyright notices must not be removed, obscured or modified. The article must be linked to Wiley's version of record on Wiley Online Library and any embedding, framing or otherwise making available the article or pages thereof by third parties from platforms, services and websites other than Wiley Online Library must be prohibited.

## In tube extraction for determination of the main volatile compounds in

3 *Physalis peruviana* L.

4 Magdalena Kupska<sup>1</sup>, Henryk H. Jeleń\*<sup>2</sup>

5 <sup>1</sup> Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology,

6 11/12 Gabriela Narutowicza St., 80-233 Gdańsk, Poland

7 <sup>2</sup> Faculty of Food Science and Nutrition, Poznań University of Life Sciences, 31 Wojska

8 Polskiego St., 60-624 Poznań, Poland

9

10 \***Correspondence:** Professor Henryk Jeleń, Faculty of Food Science and Nutrition, Poznań

11 University of Life Sciences, 31 Wojska Polskiego St., 60-624 Poznań, Poland

12 Email: henrykj@up.poznan.pl

13 Fax: 004861-8487314

14 Tel: 004861-8487273

15

16 **Abbreviations:** CIM, conventional interpolative method; EI, electron impact; GC×GC, two  
17 dimensional gas chromatography, HS, headspace; ITEEX, in tube extraction;

18  
19

## Abstract

20 An analytical procedure based on in-tube extraction followed by gas chromatography mass  
21 spectrometry has been developed for the analysis of 24 main volatile components in cape  
22 gooseberry (*Physalis peruviana* L.) samples. According to their chemical structure, the  
23 compounds were organised in different groups: 1 hydrocarbon, 1 aldehyde, 4 alcohols, 4  
24 esters and 14 monoterpenes. By single-factor experiments, incubation temperature, incubation  
25 time, extraction volume, extraction strokes, extraction speed, desorption temperature and  
26 desorption speed were determined as 60 °C, 20 min, 1000 µL, 20, 50/50 µL/s, 280 °C, 100  
27 µL/s, respectively. Quantitative analysis using authentic standards and external calibration  
28 curves was performed. The limit of detection and limit of quantification for the analytical  
29 procedure were calculated. Results shown the benzaldehyde, ethyl butanoate, 2-methyl-1-  
30 butanol, 1-hexanol, 1-butanol,  $\alpha$ -terpineol, terpinen-4-ol were the most abundant volatile  
31 compounds in analysed fruits (68.6 - 585 µg/kg). The obtained data may contribute to qualify  
32 cape gooseberry to group of superfruits and therefore increase its popularity.

33

34

35

36

37

38

## Keywords

40 in-tube extraction; gas chromatography; cape gooseberry; fruit; terpenes

## 41 1. Introduction

42 The word “superfood” has been recently introduced to the nomenclature [1]. It comprises 14  
43 natural products among which can be found e.g. fruits, vegetables, corns and tea. These food  
44 ingredients introduced into human diet bring many health benefits and can easily enhance  
45 well-being. A large group of nutrient-rich fruits played an important role in folk medicine in  
46 Asia (China, Tibet) and Africa for thousands of years. Nowadays, the “superfruit” is treated  
47 more like a marketing term than a science and that is the reason why food and medicinal  
48 preparations based on these kind of fruits are more and more popular among consumers. The  
49 globalization of world markets enables the availability of even the most exotic fruits which  
50 can be used in order to enrich the diet with new flavours while providing many significant  
51 health natural ingredients [2]. The term superfruits is considered as a new marketing approach  
52 to promote the demand for rare fruits which can be consumed as foodstuffs or used as  
53 ingredients by manufacturers of functional foods, nutraceuticals, beverages. However, gaining  
54 the popularity of health-oriented, superfruits on market depend heavily on both research  
55 results and appropriate marketing. Fruits which contain powerful bioactive compounds such  
56 as polyphenols, anthocyanins or procyanidins, with high antioxidant capacity may be  
57 classified as a superfruits. Also very important is contents of terpenes, because they determine  
58 the flavour and taste of fruits and many of them have bioactive properties e.g.  $\alpha$ -phellandrene  
59 and  $\beta$ -myrcene has antioxidant properties [3], limonene has antimicrobial, antidiabetic,  
60 antifungal [4-7], *p*-cymene has antibacterial, antinociceptive and anti-inflammatory [8-10]  
61 properties. Considerable interest led to the increase of the number of research and  
62 publications focusing on health benefits of superfruits [11-14] and determination terpenes  
63 compounds in food products [15-17].

64 *Physalis peruviana*, commonly known as goldenberry or cape gooseberry, is a solanaceous  
65 hairy plant native to tropical South America. Cape gooseberry is an herbaceous, semi-shrub,

66 upright and perennial growing in subtropical zones. Its general size is between 0.6 to 0.9 m  
67 but in some cases it can reach 1.8 m. The flower can be easily pollinated by insects, wind and  
68 also by auto-pollination. The fruit is a juicy berry with ovoid shape and a diameter between  
69 1.25 cm to 2.50 cm, 4 g and 10 g weight, containing inside around 100 to 200 small seeds  
70 [18]. The cape gooseberry is extensively used as medicinal herb for treating diseases such as  
71 cancer, malaria, asthma, hepatitis, dermatitis and rheumatism [19]. There are known  
72 additional attributed properties such as antispasmodic, diuretic, antiseptic, sedative, analgesic,  
73 helping to fortify the optic nerve, throat trouble relief, elimination of intestinal parasites and  
74 amoeba. There have also been reported antidiabetic properties, recommending the  
75 consumption of five fruits per day. There are studies indicating that eating the fruit of cape  
76 gooseberry reduces blood glucose after 90 min postprandial in young adults, causing a greater  
77 hypoglycaemic effect after this period [20]. So far, there are no studies that indicate possible  
78 adverse effects. Cape gooseberry is an attractive fruit for international markets due to its  
79 important nutritional as well as medicinal properties. Currently, there are different products  
80 made of this fruit such as jams, raisins and chocolate-covered candies. It can also be  
81 processed for juice, pomace and other products sweetened with sugar as a snack [21].  
82 However, it is still one of the less consumed raw materials of plant origin for human nutrition.  
83 In-tube extraction (ITEX) combines efficient sample extraction, with selective analytes  
84 concentration and rapid transfer to GC-MS system. A micro trap filled with adsorbent  
85 materials is placed between the HS syringe and a needle. This allows a rapid, simple and  
86 efficient extraction and concentration of volatile compounds. Analysis is carried out by  
87 multiple pumping of headspace fraction in the closed vial through adsorbent located in a  
88 special type of needle [22]. The main advantages of the in-tube extraction are: i) its  
89 effectiveness with highly volatile compounds, ii) the possibility of optimising its

90 concentration capability, depending on the analytes amount in the vapour phase by selecting a  
91 suitable number of pull/push cycles [23, 24].

92 The aim of the present work was to identify main volatile compounds from *Physalis*  
93 *peruviana* and optimize ITEX extraction method for their subsequent quantitation by GC-MS.

94 To our knowledge there are no reports on use of the ITEX for analysis of gooseberries volatile  
95 compounds. Previous publications on determination of volatile compounds in that fruit were  
96 related to liquid-liquid extraction [25, 26], dynamic headspace [27] and solid phase  
97 microextraction techniques [28-30]. Only in two publications information on quantitative data  
98 of few compounds [26, 29] were reported, as well as semiquantative using of relative percent  
99 area [27, 28].

## 100 **2. Materials and Methods**

### 101 **2.1. Materials**

102 All standard chemicals:  $\alpha$ -pinene  $\geq 99\%$ ;  $\beta$ -pinene  $\geq 99\%$ ; limonene  $\geq 99\%$ ; ocimene  $\geq 90\%$ ;  $\gamma$ -  
103 terpinene  $\geq 97\%$ ;  $\alpha$ -terpineol 97%;  $\beta$ -citronellol  $\geq 99\%$ ;  $\beta$ -myrcene  $\geq 90\%$ ; *p*-cymene 99%;  
104 eucalyptol  $\geq 99\%$ ;  $\alpha$ -terpinolene  $\geq 90\%$ ; terpinen-4-ol  $\geq 95\%$ ;  $\alpha$ -phellandrene  $\geq 95\%$ ; geraniol  
105  $\geq 99\%$ ; ethyl butanoate  $\geq 99.5\%$ ; butyl acetate  $\geq 99.7\%$ ; ethyl octanoate  $\geq 99\%$ ; ethyl decanoate  
106  $\geq 99\%$ ; 1-butanol  $\geq 99.9\%$ ; 1-hexanol  $\geq 99.9\%$ ; heptan-2-ol  $\geq 97\%$ ; *n*-pentanal  $\geq 97.5\%$ ; 2-  
107 methyl-1-butanol  $\geq 99\%$ ; benzaldehyde  $\geq 99\%$ ; were purchased from Sigma-Aldrich (Sigma-  
108 Aldrich, Poznań, Poland).

109 Samples of cape gooseberry (*Physalis Peruviana* L.) imported from Colombia and purchased  
110 at supermarket were analysed. Prior to analysis, fruit samples (calyx removed) were stored in  
111 the freezer at  $-35\text{ }^{\circ}\text{C}$ .

### 112 **2.2. Methods**

#### 113 **2.2.1. Sample preparation**

114 Before the extraction step, the fruits were pureed using a mortar and pestle. NaCl was added  
115 (10% *w/w*) during the blending stage in order to prevent possible enzymatic reactions that can  
116 lead to the conversion of some volatile compounds to their derivatives and to increase the  
117 concentration of analytes in the sample headspace [31]. The fruits reached the room  
118 temperature before proceeding with the ITEX extraction. Eight grams of sample were moved  
119 to 20 mL vial crimped with Teflon coated silicon rubber septa.

### 120 **2.2.2. Optimized extraction procedure**

121 The extraction process was carried out with a commercial version of ITEX installed in  
122 autosampler (Alpha M.O.S. HS100) with PAL1 Cycle Composer software (version 1.5.4). A  
123 2.5 mL headspace ITEX syringe (Hamilton Bonaduz AG, CTC Analytics, Switzerland) was  
124 used with the ITEX trap (Tenax TA 80/100 mesh). The ITEX extraction parameters were a  
125 subject of study, the optimal parameters have been provided in Table 1.

### 126 **2.2.3. Instrumentation**

127 The analysis was carried out on an Agilent 7890A gas chromatograph with single quadrupole  
128 mass detector (Agilent Technologies, 5975C VL MSD, (TAD)). The injector was a standard  
129 split/splitless. The injection was carried out in a split mode (1:10). The carrier gas was He at a  
130 constant linear velocity of 32.4 cm/sec (pressure 15.7 psi, flow of 0.8 mL/min) during the run.  
131 The column was DB-5 (Agilent Technologies, 30 m × 0.2 mm I.D., 0.2 μm film thickness).  
132 The chromatographic oven was held at 40 °C for 1 min, then raised to 200 °C at 10 °C/min,  
133 then to 280 °C at 20 °C/min and finally the temperature was held at 280 °C for 1 min.  
134 Analyses were performed in electron impact (EI) mode. The ion source temperature was 230  
135 °C GC/MS interface was kept at 280 °C. Detection was in a scan mode with *m/z* 33 to 333  
136 range. The ITEX/GC-MS process was carried out according to optimized conditions.

### 137 **2.2.4. Data analysis**

138 Tentative identification was accomplished through MS library search using the NIST (version  
139 2.0) mass spectral library. Positive identification of 24 analytes ( $\alpha$ -pinene,  $\beta$ -pinene,  
140 limonene, ocimene,  $\gamma$ -terpinene,  $\alpha$ -terpineol,  $\beta$ -citronellol,  $\beta$ -myrcene, *p*-cymene, eucalyptol,  
141  $\alpha$ -terpinolene, terpinen-4-ol,  $\alpha$ -phellandrene, geraniol, ethyl butanoate, butyl acetate, ethyl  
142 octanoate, ethyl decanoate, 1-butanol, 1-hexanol, heptan-2-ol, *n*-pentanal, 2-methyl-1-butanol,  
143 benzaldehyde) was confirmed by the comparison of retention times with authentic standards.  
144 Moreover, an ITEX blank run was done every one analysis of fruit samples as well as  
145 standards to consider the influence of column or Tenax degradation. The analysis of fruits  
146 sample was performed in five repetition. The calculation were performed using Excel 2010,  
147 Microsoft Office 2010. In order to define significance of differences the statistical tests were  
148 used (the Fisher-Snedecor test, the Student's t-test, the c-Cochran and Cox test).

### 149 **3. Results and discussion**

#### 150 **3.1. Optimization of extraction conditions**

151 To provide the highest peak responses and best resolution of analysed compounds the  
152 following parameters were optimized for the ITEX extraction: incubation temperature,  
153 incubation time, extraction volume, extraction strokes, extraction speed, desorption  
154 temperature and desorption time. For the extraction method optimization, a mixture of 12  
155 compounds detected in cape gooseberry was used and peak areas were compared in these  
156 experiments. The following compounds were chosen to represent main classes of volatiles  
157 present in gooseberries: alcohols (2-methyl-1-butanol and 1-hexanol), esters (butyl acetate  
158 and ethyl octanoate), monoterpene hydrocarbons ( $\beta$ -myrcene and  $\alpha$ -terpinolene), monoterpene  
159 alcohols (terpinen-4-ol and  $\alpha$ -terpineol), monoterpene aromatic hydrocarbon (*p*-cymene),  
160 monoterpene cyclic ether (eucalyptol), aromatic and aliphatic aldehydes (benzaldehyde and  
161 pentanal). The average dry matter of cape gooseberry is 20,7 % (*w/w*). The vast majority of  
162 the fruit consists of water, therefore the water standards solutions was used to select optimal

163 parameters of the extraction process. The repeatability of the extraction under tested  
164 conditions were calculated as relative standard deviation of absolute peak areas for the  
165 triplicate analyses of model samples. Table 1 presents summarized optimization parameters  
166 and tested values.

### 167 **3.1.1. Effect of incubation temperature and incubation time**

168 In the sample analysis via ITEX, analytes are extracted from the sample headspace.  
169 Therefore, the temperature and the time at which equilibrium is reached between the  
170 concentration of analytes in the sample and the sample headspace are crucial parameters.  
171 Figure 1A and Figure 1B show the results of analyses performed in order to optimize the  
172 temperature and time of extraction steps.

173 All the extraction temperatures were tested at the same extraction time of 10 minutes. The  
174 highest extraction efficiency for all compounds was noted at 60 °C. The extraction efficiency  
175 increased along with an increase of the extraction (incubation) temperature. It is known that in  
176 the higher temperatures thermal degradation of compounds can take place. Therefore, the  
177 extraction temperature was established at 60 °C and it was used for subsequent analyses.

178 The next step involved optimization of the extraction time. In case of five compounds ( $\beta$ -  
179 myrcene,  $\alpha$ -terpinolene, p-cymene, eucalyptol, benzaldehyde) the highest extraction  
180 efficiency was obtained at 5 min. For esters and monoterpene alcohols the most convenient  
181 time was 20 min. The lowest repeatability of the analysis was observed for 10 min of  
182 incubation. In case of alcohols, the difference in the extraction efficiency in different  
183 incubation time was not significant. Considering the above the optimal incubation time was  
184 established at 20 min.

### 185 **3.1.2. Effect of extraction volume**



186 The following volumes of extraction were tested: 300, 500, 1000, 2000  $\mu\text{L}$ . For the 300  $\mu\text{L}$   
187 the significant problem with repeatability of the peaks area in subsequent analyses was  
188 observed. For almost all compounds the highest efficiency of extraction in the volume of 2000  
189  $\mu\text{L}$  were obtained (only for butyl acetate in 1000  $\mu\text{L}$  peaks were the most abundant). In case  
190 of 500  $\mu\text{L}$  for esters the lowest repeatability was observed. The results are presented in Figure  
191 1C.

192 At first 2000  $\mu\text{L}$  was chosen as the optimal extraction volume. However, in further analyses  
193 the leaks in syringe was observed (twice). Therefore, it was decided to choose 1000  $\mu\text{L}$  as  
194 optimal volume (the leaks at syringe was not observed).

### 195 **3.1.3. Effect of extraction strokes**

196 The relationship between the number of extraction cycles and signal of analytes is presented  
197 in Figure 1D. For nine of the compounds with increasing number of strokes extraction  
198 efficiency increased. Only for monoterpene hydrocarbons and monoterpene aromatic  
199 hydrocarbon the maximum of extraction efficiency was obtained at 20 and 30 strokes,  
200 respectively. However, it should be noticed that increasing numbers of strokes increases the  
201 extraction time (in case of 40 strokes extraction time is 4 times longer than for 10 strokes, it  
202 gives 5 min and 20 min respectively, the test were carried out in 50  $\mu\text{L}/\text{s}$  of aspirate speed and  
203 100  $\mu\text{L}/\text{s}$  of dispense speed). Moreover, the greater is the number of stokes the higher is the  
204 risk of the syringe leaks in subsequent analyses. Therefore, the optimum value as 20 strokes  
205 was chosen.

### 206 **3.1.4. Effect of extraction speed**

207 Extraction speed consists of aspirate and dispense speed. The first is related to the speed of  
208 rising the syringe plunger, the second with the speed of lowering the syringe plunger during  
209 extraction process. Figure 2A presents the relationship between the number of extraction



210 cycles and signal from analytes. An increase of the extraction (aspiration and dispense) speed  
211 lead to decrease of the extraction time. For the tested parameters the extraction times were as  
212 follows: 13.3 min (50/50), 10 min (50/100), 6.6 min (100/100), 4.4 min (100/300) and 2.2 min  
213 (300/300). However, the higher is the dispense speed the higher is pressure in syringe and the  
214 risk of the syringe leaks increases [32]. In case of all analysed monoterpene hydrocarbons, the  
215 extraction speed is decreasing with growing extraction speed. For the rest compounds the  
216 minimum is reach in 100/100  $\mu\text{L/s}$ . Taking into account the above, the optimal extraction  
217 speed established 50/50  $\mu\text{L/s}$ .

### 218 **3.1.5. Effect of desorption temperature and desorption speed**

219 In order to ensure quantitative transfer of the analysed compounds adsorbed on the ITEX trap  
220 to the chromatography system, an adequate trap temperature during desorption and speed of  
221 the desorption process is required. Both conditions should not promote the formation of  
222 artefacts and thermal degradation of the stationary phase of the ITEX sorbent, whereas the  
223 analytes should be completely desorbed from the Tenax TA.

224 The results of analyses carried out using various desorption temperature of analytes by the  
225 ITEX trap are present in Figure 2B. For esters, monoterpene hydrocarbons and monoterpene  
226 aromatic hydrocarbon, the most intense peaks were observed in desorption temperature of 240  
227  $^{\circ}\text{C}$ , while the most reproducible results were obtained at 280  $^{\circ}\text{C}$ . For the remaining  
228 compounds (especially alcohols and monoterpene alcohols) no significant difference was  
229 observed.

230 The carry over effect was checked for all standards and was tested in different desorption  
231 temperatures. It was found that this effect occurs for four compounds ( $\beta$ -myrcene, p-cymene,  
232 eucalyptol,  $\alpha$ -terpinolene). The results of analyzes carried out using various desorption  
233 temperatures from the Tenax TA are present in Table 2. In the desorption temperature of 240

234 °C carry over is more than 0.1% after the first analysis, moreover the highest is for β-  
235 myrcene. The lowest carry over was achieved at 280 °C. The highest decline in the size of the  
236 peaks area as the temperature increases was observed for β-myrcene (240 °C - 0.258%, 260  
237 °C - 0.158%, 280°C - 0.099%). Furthermore, the highest repeatability of the analyses was  
238 achieved in the temperature of 280 °C. Therefore, the 280 °C of desorption temperature was  
239 chosen as optimal for further analysis.

240 The results of analyses carried out using various desorption speed of analytes from the Tenax  
241 TA are presented in Figure 2C. For all compounds the most intense peak areas at 100 µL/s  
242 were observed, while for esters the smallest repeatability of the analyses. Also in the lowest  
243 desorption speed the best peaks shapes (narrows and fully separated) was achieved.

244 Based on the literature data, for the volatile compounds determination (including terpenes) in  
245 fruits samples the injector temperature of 250 °C and 3 min was found as optimum [33-35].

#### 246 **3.1.6. Exhaustion extraction of analytes from the sample using in-tube extraction**

247 In order to check the number of analysis that can be performed on one sample, provides the  
248 result reliable, it was carried out a exhaustion test of the sample. For the first two analyses no  
249 significant decrease in total peak areas was observed (statistically significant). For subsequent  
250 analyses decline in the total peak area with the number of repetitions was evident. The results  
251 of analyses are presented in Figure 3.

#### 252 **3.2. Performance of the analytical procedure**

253 The performance of the optimized analytical procedure for the analysis of 24 the most  
254 concentrated volatile compounds in cape gooseberry fruit by ITEX/GC-MS was evaluated by  
255 applying the extraction procedure as described in Table 1.

256 For the creation of standard curves, 8g of cape gooseberry (after isolation step using Dering  
257 apparatus) with salt addition were spiked with mix of standards in the range of concentrations.  
258 The analysis was performed by GC-MS using an external calibration curve method (CIM -  
259 Conventional Interpolative Method). Using this method several standard solutions of various,  
260 known concentrations of the analytes were prepared (in range 5 - 500 µg/kg). Making  
261 measurements for mix of standard solution and for sample, the calculations was done in the  
262 interpolative way (in the linearity range of calibration curves). The stock solutions of  
263 standards were prepared in methanol. For each standard the following concentrations were  
264 analysed: 5, 10, 25, 50, 100, 250, 500, µg/kg. Each of standard solution concentrations was  
265 run three times.

266 The LOD and LOQ for the analytical procedure were calculated on the basis on the standard  
267 deviation of a set of signals and the angle of inclination of the calibration curve. The  
268 equations of calibration curve in the range of linearity, linearity range, coefficient of  
269 determination, detection and quantification limits in order of retention time of analytes were  
270 shown in Table 3.

### 271 **3.3. Analysis of compounds in cape gooseberry sample**

272 The volatile compounds determined in cape gooseberry are presented in Table 4. Compounds  
273 with the highest peak areas (24) after first test analysis with use of ITEX/GC-MS were  
274 chosen. This group consisted of 1 hydrocarbon, 1 aldehyde, 4 alcohols, 4 esters and 14  
275 monoterpenes.

276 19 of 24 compounds were previously identified by using LLE/GC-MS [25], this include 1  
277 hydrocarbon, 1 aldehyde, 4 alcohols, 4 esters and 9 monoterpenes. 21 of 24 compounds were  
278 previously identified by using HS-SPME/GC-MS, this include 1 aldehyde, 4 alcohols, 4 esters  
279 and 12 monoterpenes [28] and additional 112 compounds were determined.



280 Moreover, the profile of volatile terpenes in cape gooseberry was determined using GC×GC-  
281 ToFMS. The 62 terpenes were identified [30], and it confirms 14 chosen monoterpenes in this  
282 work. The cape gooseberry is known for its high percentage content of compounds from the  
283 group of terpenes, compared to other fruits [29]. For this reason, it is assumed that cape  
284 gooseberry is starting to be known as superfruit.

285 The 1-butanol, 2-methyl-1-butanol, heptan-2-ol, 1-hexanol and  $\alpha$ -terpineol were also reported  
286 by Mayorga et al. [36], with additional 39 compounds as a glycosidically bound flavour  
287 compounds. Also  $\alpha$ -pinene, ethyl octanoate and eucalyptol (as 1,8-cineole) were previously  
288 determined by Ramadan et al. [27] with additional 31 compounds.

289 Previously reported quantitative results by Ymaztekin [26] for some of analytes are different  
290 comparing to data obtained in this paper (benzaldehyde 110.4  $\mu\text{g}/\text{kg}$ , 1-butanol 514.3  $\mu\text{g}/\text{kg}$ ,  
291 heptan-2-ol 10.07  $\mu\text{g}/\text{kg}$ , 1-hexanol 292.9  $\mu\text{g}/\text{kg}$ , 2-methyl-1-butanol 470.4  $\mu\text{g}/\text{kg}$ , butyl  
292 acetate 19.4  $\mu\text{g}/\text{kg}$ , ethyl decanoate 130.2  $\mu\text{g}/\text{kg}$ , ethyl octanoate 28.8  $\mu\text{g}/\text{kg}$ ,  $\beta$ -citronellol 26.0  
293  $\mu\text{g}/\text{kg}$ , terpinen-4-ol 128.5  $\mu\text{g}/\text{kg}$ ,  $\alpha$ -terpineol 160.7  $\mu\text{g}/\text{kg}$ ,  $\beta$ -myrcene 7.9  $\mu\text{g}/\text{kg}$ , ocimene 2.8  
294  $\mu\text{g}/\text{kg}$ ,  $\alpha$ -terpinolene 13.2  $\mu\text{g}/\text{kg}$ ). For determination of volatile compounds the liquid-liquid  
295 extraction with combination of GC-FID and GC-MS was used. However, for quantification  
296 only 4-nonanol,  $\gamma$ -valerolactone and cyclohexyl butanoate were used. Whereas Dymerski et  
297 al. [29] determined terpinen-4-ol (50  $\mu\text{g}/\text{kg}$ ),  $\gamma$ -terpinene (95  $\mu\text{g}/\text{kg}$ ) and  $\alpha$ -terpinolene (180  
298  $\mu\text{g}/\text{kg}$ ) using HS-SPME/GC×GC-ToFMS. The difference in the obtained results may be due  
299 to the biological sample, different origin, agronomic and climatic conditions, as also store the  
300 fruits during transportation.

301 The benzaldehyde, ethyl butanoate, 1-hexanol, 2-methyl-1-butanol, 1-butanol,  $\alpha$ -terpineol was  
302 found in greatest concentrations (more than 70  $\mu\text{g}/\text{kg}$ ). 1-butanol and 2-methyl-1-butanol have  
303 a sweet, floral and fruity notes [26]. This alcohols are reported in many exotic fruits as



304 acerola, jackfruit, *Annona cherimolia* or *Spondias mombin* [37].  $\alpha$ -Terpineol is known in  
305 antimicrobial effects [38].

306 From the state of the art it is known the Tenax is releasing aldehydes (e.g. benzaldehyde) and  
307 ketones during thermal desorption, which can obscure the determination of these compounds  
308 [32, 39]. However, in consideration of linearity and reproducibility of the content of  
309 benzaldehyde, it can be assumed that the impact of benzaldehyde derived from sorbent is not  
310 significant. It is certain that the benzaldehyde is present in the fruit, as indicated in the  
311 literature [25, 29].

312 As was observed during optimization of the ITEX/GC-MS method the concentration level  
313 was different for single compounds (very abundant peak of one compound, in fact, did not  
314 indicate a high concentration of this compound). It depends of the LOD and LOQ, the  
315 linearity of calibration curve, selectivity and sensitivity of GC system. This is related to all  
316 relative quantitative methods - they are not so accurate and reliable as quantification using  
317 authentic standards and calibration curves.

#### 318 **4. Concluding remarks**

319 The method development for in-tube extraction and gas chromatography was successfully  
320 applied to the analysis of the volatile fractions from cape gooseberry fruit. The results indicate  
321 that the ITEX/GC-MS technique is a good alternative for the determination of volatile and  
322 semi-volatile compounds, in particular terpenes, compared with other concentration  
323 techniques and separation methods for volatile analytes. Also it limits the use of chemical  
324 reagents in the sample preparation step. The principal components analysis indicated that the  
325 *Physalis peruviana* L. is mainly composed of compounds from the branched esters, alcohols  
326 and monoterpene groups. Literature data about flavour compounds of cape gooseberry are  
327 rare. It is assumed the obtained data will contribute to qualify these fruits to group of



328 superfruits and also increase their popularity. The results of this research may encourage both  
329 the food and pharmaceutical industry to utilize these fruits as raw material or additives for  
330 new, health-oriented food products (such as fruity juices, wines and liqueurs) and  
331 nutraceuticals including dietary supplements. As a result, the human diet will be  
332 supplemented with additional healthy and valuable products.

### 333 **Acknowledgments**

334 This work was financially supported by the National Science Centre under research project  
335 no. DEC-2012/07/N/ST4/00629.

### 336 **Conflict of interest**

337 The authors have declared no conflict of interest.

### 338 **References**

339 [1] Steven, G., Pratt, M. D., Matthews, K., SuperFoods Rx: Fourteen foods that will change  
340 your life, HarperCollins Publishers, Australia 2004.

341 [2] Felzenszwalb, I., da Costa Marques, M. R., Mazzei, J. L., Aiub, C. a F., Toxicological  
342 evaluation of Euterpe edulis: a potential superfruit to be considered. Food Chem. Toxicol.  
343 2013, 58, 536–544.

344 [3] Ciftci, O., Ozdemir, I., Tanyildizi, S., Yildiz, S., Oguzturk, H., Antioxidative effects of  
345 curcumin,  $\beta$ -myrcene and 1,8-cineole against 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced  
346 oxidative stress in rats liver. Toxicol. Ind. Health 2011, 27, 447–453.

347 [4] Vuuren, S. F. Van, Viljoen, A. M., Antimicrobial activity of limonene enantiomers and 1 ,  
348 8-cineole alone and in combination, Flavour Frag. J. 2007, 22, 540–544.

- 349 [5] Roberto, D., Micucci, P., Sebastian, T., Graciela, F., Anesini, C., Antioxidant activity of  
350 limonene on normal murine lymphocytes: relation to H<sub>2</sub>O<sub>2</sub> modulation and cell proliferation.  
351 *Basic Clin. Pharmacol. Toxicol.* 2010, 106, 38–44.
- 352 [6] Ibrahim, N., El-Sakhawy, F., Mohammed, M., Farid, M., Abdel-Wahed, N., Deabes, D.,  
353 Chemical composition, antimicrobial and antifungal activities of essential oils of the leaves of  
354 *Aegle marmelos* (L.) Correa growing in Egypt. *J. App. Pharm. Sci.* 2015, 5, 001–005.
- 355 [7] Murali, R., Saravanan, R., Antidiabetic effect of d-limonene, a monoterpene in  
356 streptozotocin-induced diabetic rats. *Biomed. Prev. Nutr.* 2012, 2, 269–275.
- 357 [8] Quintans, J. S. S., Menezes, P. P., Santos, M. R. V., Bonjardim, L. R., Almeida, J. R. G.  
358 S., Gelain, D. P., Araújo, A.A.S., Quintans-Júnior, L. J., Improvement of p-cymene  
359 antinociceptive and anti-inflammatory effects by inclusion in  $\beta$ -cyclodextrin. *Phytomedicine*  
360 2013, 20, 436–440.
- 361 [9] Sonboli, A., Salehi, P., Kanani, M. R., Ebrahimi, S. N., Antibacterial and antioxidant  
362 activity and essential oil composition of *Grammosciadium scabridum* Boiss. from Iran.  
363 *Zeitschrift Für Naturforschung - Section C, J. Biosci.* 2005, 60, 534–538.
- 364 [10] Wang, C. Y., Wang, S. Y., Chen, C., Increasing antioxidant activity and reducing decay  
365 of blueberries by essential oils. *J. Agric. Food Chem.* 2008, 56, 3587–3592.
- 366 [11] Jamin, E., Superfruits: are they authentic? *Fruit Processing* 2009, 19, 170–175.
- 367 [12] Medina, M. B., Determination of the total phenolics in juices and superfruits by a novel  
368 chemical method. *J. Funct. Foods* 2011, 3, 79–87.



- 369 [13] Crozier, S. J., Preston, A. G., Hurst, J. W., Payne, M. J., Mann, J., Hainly, L., Miller, D.  
370 L., Cacao seeds are a “Super Fruit”: A comparative analysis of various fruit powders and  
371 products. *Chem. Cent. J.* 2011, 5, 5.
- 372 [14] Gross, P. M., Superfruits: (Top 20 Fruits Packed with Nutrients and Phytochemicals,  
373 Best Ways to Eat Fruits for Maximum Nutrition, and 75 Simple and Delicious Recipes for  
374 Overall Wellness), 2009.
- 375 [15] Arceusz, A., Occhipinti, A., Capuzzo, A., Maffei, M. E., Comparison of different  
376 extraction methods for the determination of  $\alpha$ - and  $\beta$ -thujone in sage (*Salvia officinalis* L.)  
377 herbal tea. *J. Sep. Sci.* 2013, 36, 3130–3134.
- 378 [16] Herrera, C., Castro, R., García-Barroso, C., Durán-Guerrero, E., Development of a stir  
379 bar sorptive extraction method for the determination of volatile compounds in orange juices.  
380 *J. Sep. Sci.* In press DOI:10.1002/jssc.201600590
- 381 [17] Bajer, T., Ligor, M., Ligor, T., Buszewski, B., Design of the extraction process for  
382 terpenes and other volatiles from allspice by solid-phase microextraction and  
383 hydrodistillation. *J. Sep. Sci.* 2016, 39,769–775.
- 384 [18] Puente, L. A., Pinto-Muñoz, C. A., Castro, E. S., Cortés, M., *Physalis peruviana*  
385 Linnaeus, the multiple properties of a highly functional fruit: A review. *Food Res. Int.* 2011,  
386 44, 1733-1740.
- 387 [19] Wu, S.J., Ng, L.T., Lin, D.L., Wang, S.S., Lin, C.C., *Physalis peruviana* extract induces  
388 apoptosis in human Hep G2 cells through CD95/CD95L system and the mitochondrial  
389 signaling transduction pathway. *Cancer Lett* 2004, 215, 199–208.



- 390 [20] Rodríguez, S., Rodríguez, E., Efecto de la ingesta de *Physalis peruviana* (aguaymanto)  
391 sobre la glicemia postprandial en adultos jóvenes. *Revista Médica Vallejana* 2007, 4, 43–52.
- 392 [21] Ramadan, M. F., Moersel, J. T., Impact of enzymatic treatment on chemical composition,  
393 physicochemical properties and radical scavenging activity of goldenberry (*Physalis*  
394 *peruviana* L.) juice. *J. Sci. Food Agr.* 2007, 87, 452–460.
- 395 [22] Salanță, L.-C., Tofană, M., Socaci, S. A., Lazar (Pop), C., Michiu, D., Fărcas, A.,  
396 Determination of the volatile compounds from hop and hop products using ITEX/GC-MS  
397 technique. *J. Agroaliment. Proc. Technol.* 2012, 18, 110-115.
- 398 [23] Jochmann, M.A., Yuan, X., Schilling, B. and Schmidt, T.C., In-tube extraction for  
399 enrichment of volatile organic hydrocarbons from aqueous samples. *J. Chromatogr. A* 2008,  
400 1179, 96-105.
- 401 [24] Laaks, J., Jochmann, M.A., Schilling, B., Molt, K. and Schmidt, T.C., In-tube extraction-  
402 GC-MS as a high-capacity enrichment technique for the analysis of alcoholic beverages. *J.*  
403 *Agric. Food Chem.* 2014, 62, 3081-3091.
- 404 [25] Berger, R. G., Drawert, F., Kollmannsberger, H., The flavour of cape gooseberry  
405 (*Physalis peruviana* L.). *Z. Lebensm. Unters. Forsch.* 1989, 188, 122–126.
- 406 [26] Yilmaztekin, M., Characterization of potent aroma compounds of cape gooseberry  
407 (*Physalis Peruviana* L.) fruits grown in Antalya through the determination of odor activity  
408 values. *Int. J. Food Prop.* 2014, 17, 469-80.
- 409 [27] Ramadan, M. M., El-Ghorab, A. H., Ghanem, K. Z., Volatile compounds, antioxidants,  
410 and anticancer activities of Cape gooseberry fruit (*Physalis peruviana* L.): an in-vitro study. *J.*  
411 *Arab Soc. Med.Res.* 2015, 10, 56–64 .



- 412 [28] Yilmaztekin, M., Analysis of volatile components of cape gooseberry (*Physalis*  
413 *peruviana* L.) grown in Turkey by HS-SPME and GC-MS. *Scientific World J.* 2014, 2014, 1-  
414 8.
- 415 [29] Dymerski, T., Namieśnik, J., Vearasilp, K., Arancibia-Avila, P., Toledo, F., Weisz, M.,  
416 Katrich, E., Gorinstein, S., Comprehensive two-dimensional gas chromatography and three-  
417 dimensional fluorometry for detection of volatile and bioactive substances in some berries.  
418 *Talanta*, 2015, 134, 460-467.
- 419 [30] Kupska, M., Wasilewski, T., Jędrkiewicz, R., Gromadzka, J., Namieśnik, J.,  
420 Determination of terpene profiles in potential superfruits. *Int. J. Food Prop.* In press DOI:  
421 10.1080/10942912.2016.1144066
- 422 [31] Klesk, K., Qian, M., Aroma extract dilution analysis of Cv. Marion (*Rubus spp. hyb*) and  
423 Cv. Evergreen (*R. laciniatus* L.) blackberries. *J. Agric. Food Chem.* 2003, 51, 3436-3441.
- 424 [32] Laaks, J., Jochmann, A. M., Schilling, B., Schmidt, T. C., Optimization strategies of in-  
425 tube extraction (ITEX) methods. *Anal. Bioanal. Chem.* 2015, 407, 6827–6838.
- 426 [33] Rocha, S. M., Coelho, E., Zrostlíková, J., Delgadillo, I., Coimbra, M. A., Comprehensive  
427 two-dimensional gas chromatography with time-of-flight mass spectrometry of  
428 monoterpenoids as a powerful tool for grape origin traceability. *J. Chromatogr. A* 2007, 1161,  
429 292–299.
- 430 [34] Kupska, M., Chmiel, T., Jędrkiewicz, R., Wardencki, W., Namieśnik, J., Comprehensive  
431 two-dimensional gas chromatography for determination of the terpenes profile of blue  
432 honeysuckle berries. *Food Chem.* 2014, 152, 88–93.



- 433 [35] Socaci, S. A., Socaciu, C., Tofană, M., Rațiș, I. V., Pinteș, A., In-tube extraction and  
434 GC–MS analysis of volatile components from wild and cultivated sea buckthorn (*Hippophae*  
435 *rhamnoides* L. ssp. *Carpatica*) berry varieties and juice. *Phytochem. Analysis* 2013, 24, 319-  
436 328.
- 437 [36] Mayorga, H., Knapp, H., Winterhalter, P., Duque, C., Glycosidically bound flavour  
438 compounds of cape gooseberry (*Physalis peruviana* L.). *J. Agr. Food Chem.* 2001, 49, 1904-  
439 1908
- 440 [37] Bicas, J. L., Molina, G., Dionísio, A. P., Barros, F. F. C., Wagner, R., Maróstica, Jr. M.  
441 R., Pastore, G. M., Volatile constituents of exotic fruits from Brazil. *Food Res. Int.* 2011, 44,  
442 1843–1855.
- 443 [38] Friedman, M., Henika, P. R., Mandrell, R. E., Bactericidal Activities of Plant Essential  
444 Oils and Some of Their Isolated Constituents against *Campylobacter jejuni*, *Escherichia coli*,  
445 *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Protect.* 2002, 65, 1545-1560.
- 446 [39] Dettmer, K., Engewald, W., Adsorbent materials commonly used in air analysis for  
447 adsorptive enrichment and thermal desorption of volatile organic compounds. *Anal. Bioanal.*  
448 *Chem.* 2002, 373, 490–500.
- 449

450

451 **Figure captions**

452 **Figure 1.** Optimization of the extraction parameters: (A) incubation temperature, (B)  
453 incubation time, (C) extraction volume, and (D) extraction strokes by single factor-  
454 experiments. The error bars based on triplicate analyses are included.

455 **Figure 2.** Optimization of the extraction parameters: (A) extraction speed, (B) desorption  
456 temperature, and (C) desorption speed by single factor-experiments. The error bars showing  
457 standard deviation based on triplicate analyses are included.

458 **Figure 3.** Exhaustive extraction for gooseberry sample performed from a single vial.