



Effect of sugar reduction on flavour release and sensory perception in an orange juice soft drink model

Article

Accepted Version

Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0

Tsitlakidou, P., Van Loey, A., Methven, L. and Elmore, S. (2019) Effect of sugar reduction on flavour release and sensory perception in an orange juice soft drink model. *Food Chemistry*, 284. pp. 125-132. ISSN 0308-8146 doi: <https://doi.org/10.1016/j.foodchem.2019.01.070> Available at <http://centaur.reading.ac.uk/82059/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1016/j.foodchem.2019.01.070>

Publisher: Elsevier

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

1 Effect of sugar reduction on flavour release and sensory perception in
2 an orange juice soft drink model

3 Petroula Tsitlakidou¹, Ann Van Loey², Lisa Methven¹, J. Stephen Elmore^{1*}

4 ¹Department of Food and Nutritional Sciences, University of Reading, Whiteknights,
5 Reading RG6 6AP, UK

6 ²Laboratory of Food Technology (member of Leuven Food Science and Nutrition Research
7 Center, LFoRCe), Department of Microbial and Molecular Systems (M²S), KU Leuven,
8 Kasteelpark Arenberg 22 box 2457, 3001 Heverlee, Belgium

9 E-mail addresses:

10 Ms Petra Tsitlakidou: petratsitlak@hotmail.com

11 Prof. Ann Van Loey: Ann.VanLoey@kuleuven.be

12 Dr Lisa Methven; l.methven@reading.ac.uk

13 Dr Stephen Elmore j.s.elmore@reading.ac.uk (*corresponding author; tel.: +118-3787455)

14

15

16

17

18

19

20

21

22

23 ABSTRACT

24 To examine the effect of sugar reduction on the sensory perception of sweetened beverages,
25 an orange juice soft drink model flavoured with seven characteristic compounds (hexanal,
26 decanal, linalool, ethyl butanoate, α -pinene, β -myrcene and (*Z*)-3-hexen-1-ol) was developed.
27 Five samples were prepared with relevant sugar contents (5.2, 8.2, 9.7, 11.2 and 14.2 °Brix).
28 Using retronasal quantitative descriptive analysis (QDA), nine attributes were found to differ
29 significantly ($p < 0.05$) with sugar content. When the samples were evaluated orthonasally,
30 only the attribute “overripe orange” significantly decreased ($p < 0.05$) with reduction of sugar
31 content. Headspace solid-phase microextraction with gas chromatography–mass spectrometry
32 showed that as sugar concentration decreased, the headspace concentration of six of the
33 volatile compounds decreased, whilst ethyl butanoate remained constant. Principal component
34 analysis revealed that the total release of the flavour compounds was highly correlated with
35 the perceived intensity of the orthonasal attribute “overripe orange”.

36 **Keywords:** Orange flavour; sugar reduction; salting-out; sensory analysis; direct gas
37 chromatography-olfactometry; headspace solid phase microextraction with gas
38 chromatography-mass spectrometry; principal component analysis

39

40 **1. Introduction**

41 The interest in developing “light” or “diet” beverages is rising, driven largely by the
42 market potential for beverages that can maintain or promote the well-being of consumers.
43 Beverage manufacturers have shown strong interest in addressing the challenge of sugar
44 reduction in soft drinks, whilst maintaining the organoleptic characteristics, often using high
45 intensity sweeteners to replace sugars. Yet little is known about the interactions between
46 sugar and flavour in soft drinks and subsequent effects on sensory quality. To date, it has
47 been suggested that an increase in release of specific flavour compounds with increasing
48 sucrose concentration (from 20 to 60% *w/w*) is possibly due to a “salting-out” effect
49 (Hansson, Andersson, & Leufven, 2001). Nahon and co-workers (Nahon, Roozen, & de
50 Graaf, 1998) investigated the release of an orange aroma in various mixtures of sucrose and
51 sodium cyclamate. It was shown that there was a significant association between the retention
52 time of a volatile compound on a gas chromatography column and its release behaviour.
53 Specifically, the release rates of volatile compounds with short retention times intensified by
54 increasing sucrose content from 0 to 60% (*w/v*) whilst flavour compounds with higher
55 retention times were negatively influenced, if at all, by modifying sucrose concentration.
56 Similar results were obtained by Rabe and her co-workers (Rabe, Krings, & Berger, 2003),
57 who revealed that various flavour compounds showed an increased release with increasing
58 sugar content ranging from 0 to 500 g/L. Hence, it can be deduced from the aforementioned
59 studies that the release rate of the volatiles is selectively influenced by the sucrose content,
60 resulting in a significant shift of the flavour profile. However, when lower levels of sugars
61 (glucose 0–150 g/L and fructose 0–64 g/L) were applied in a model citrus-flavoured
62 beverage, it was suggested that flavour enhancement was not fully explained by
63 physicochemical interactions within the beverage matrix (Hewson, Hollowood, Chandra, &
64 Hort, 2008). Further investigation is required to clarify the effect on sensory perception of the

65 sugar content in these soft drink model systems (sugar level at approximately 10% *w/v*), since
66 a number of studies have shown conflicting results.

67 Solid-phase microextraction (SPME) is a widely applied technique for volatile analysis in
68 food/flavour chemistry, since it is a rapid, solvent-free and simple adsorption method for the
69 isolation of headspace flavour compounds (Zhang & Pawliszyn, 1993). To date, many studies
70 have conducted flavour analysis of different citrus species/varieties (González-Mas, Rambla,
71 Alamar, Gutiérrez, & Granell, 2011), of fresh (Moshonas & Shaw, 1994) or excessively
72 heated orange juice (Bazemore, Goodner, & Rouseff, 1999) and the presence of pulp in
73 orange juice (Rega, Fournier, Nicklaus, & Guichard, 2004), using headspace SPME followed
74 by gas chromatography–mass spectrometry (HS–SPME/GC–MS).

75 The optimisation of SPME sampling and gas chromatographic conditions for both
76 qualitative and quantitative analyses of volatile compounds in the headspace of orange juice
77 has been investigated in the past (Jia, Zhang, & Min, 1998). Several different types of fibres
78 have been used in citrus studies, with the divinylbenzene/CarboxenTM/polydimethylsiloxane
79 (DVB/CAR/PDMS) fibre predominantly used, due to its ability to extract from the orange
80 juice matrix a large number of flavour compounds with different polarities (Berlinet,
81 Guichard, Fournier, & Ducruet, 2007; González-Mas et al., 2011; Rega, Fournier, &
82 Guichard, 2003).

83 The number and nature of the extracted volatile compounds are strongly dependent on the
84 food type and matrix, as well as the sampling time of the fibre, heating temperature and time
85 (Yang & Peppard, 1994). In this sense, it has been suggested that a short time of sampling is
86 preferable, to better represent the original headspace of samples (Rega et al., 2003; Roberts,
87 Pollien, & Milo, 2000). Rega and co-workers (2003) developed an instrumental method to
88 evaluate odours from headspace extracts, in order to improve SPME performance, which they
89 termed direct gas chromatography–olfactometry (D–GC–O). In this method, headspace

90 extracts are injected into a deactivated fused silica capillary, which is attached to a GC-
91 sniffing port. Trained assessors sniff the extract coming out from the GC-sniffing port and
92 rate the similarity of the headspace extract to the original headspace of the sample. By this
93 method, various sampling conditions are validated, including type of fibre, extraction time,
94 heating time and temperature (Berlinet et al., 2007; Rega et al., 2003).

95 A plethora of volatile compounds and key odorants in orange juice has been identified and
96 their odour and flavour thresholds have been quantified (Plotto, Margaría, Goodner, &
97 Baldwin, 2008; Plotto, Margaría, Goodner, Goodrich, & Baldwin, 2004). There is no single
98 volatile in orange juice that can be considered as a character impact compound. Instead, the
99 perception of orange flavour is a result of a group of aroma-active compounds present in low
100 concentrations. In the current study, an orange flavour mixture was used, consisting of
101 decanal, hexanal, (Z)-3-hexen-1-ol, ethyl butanoate, linalool, β -myrcene and α -pinene, most
102 of which have been found at higher concentration levels in processed orange juices than
103 freshly-squeezed ones and are characterised as some of the key odorants in orange juice
104 flavour (Moshonas & Shaw, 1994). Decanal and hexanal are straight-chain aldehydes present
105 at low concentration in processed juice and considered as an important contributor to the
106 green, grassy note of orange juice flavour. Similarly, (Z)-3-hexen-1-ol contributes to the
107 green/woody top notes of freshly squeezed orange juice. Ethyl butanoate is the single most
108 important ester and most intense odorant in orange juice, imparting a fruity odour quality.
109 Linalool is a terpene alcohol which contributes floral, sweet and fruity aromas. β -Myrcene
110 and α -pinene are the most abundant terpenes after limonene, and their levels depend on the
111 peel oil content of the processed juice. These compounds are considered to possess a low
112 odour-active intensity, with β -myrcene imparting a mossy odour note and α -pinene a pine-
113 tree, resin odour quality (Perez-Cacho & Rouseff, 2008).

114 The objective of the current study was to examine how sugar reduction affects the release
115 of seven different flavour compounds (of known hydro-phobicity/philicity) and sensory
116 perception in an orange juice soft drink model. Initially, a sensorial approach was
117 implemented, conducting two independent quantitative descriptive analyses, one orthonasal
118 and one retronasal, in order to investigate how sugar reduction can influence the sensory
119 perception of orange juice soft drink samples. Then, D–GC–O was applied to determine the
120 optimum experimental conditions to obtain the most representative SPME extract.
121 Subsequently, samples were subjected to HS–SPME/GC–MS to examine whether the flavour
122 release of the volatile compounds was influenced by sugar content modification, and to relate
123 this to change in orthonasal perception.

124 2. Materials and Methods

125 2.1 *Materials and sample preparation*

126 The soft drink model system consisted of 7% (w/w) deodorised orange juice concentrate of
127 65 °Brix, a non-commercial orange flavour mixture, 0.25% (w/v) citric acid, and sucrose at
128 five different concentrations. Givaudan Ltd (Milton Keynes, UK) provided the flavour
129 mixture, which contained 7 compounds with known concentrations and different solubility
130 properties, all dissolved in triacetin. Hexanal, decanal and linalool were present at 5 g/L.
131 Ethyl butanoate was present at 50 g/L, α -pinene at 37.5 g/L, β -myrcene at 12.5 g/L and (Z)-3-
132 hexen-1-ol at 20 g/L. The flavour mixture was dosed at 300 mg/L in all samples; thus, it was
133 calculated that the dosage would deliver 1.5 mg/L for each of the compounds hexanal,
134 decanal and linalool, 15 mg/L of ethyl butanoate, 11.25 mg/L of α -pinene, 3.75 mg/L β -
135 myrcene and 6 mg/L of (Z)-3-hexen-1-ol in the soft drink model (Supplementary Table S1).
136 Five sucrose concentrations were chosen (2.0, 5.0, 6.6, 8.2 and 11.5% w/v) resulting in
137 samples of 5.2, 8.2, 9.7, 11.2 and 14.2 °Brix (the total sugar levels), determined by a hand-

138 held refractometer. All sample preparation, pasteurisation, aseptic bottling and measurement
139 of refractive index and acidity were performed by a commercial soft drinks manufacturer.
140 Subsequently, bottles of juice were shipped and stored at 4 °C at the Department of Food and
141 Nutritional Sciences, University of Reading, UK, until they were used for sensory and
142 volatile compound analysis.

143 2.2 *Sensory analysis by quantitative descriptive profiling*

144 Samples of juice (25 mL) were poured into tulip-shaped whisky glasses (200 mL volume;
145 Glencairn, East Kilbride, UK) and covered with a Petri dish for sensory evaluation. Samples
146 were coded with 3-digit random codes and prepared 2 hours prior to tasting, to allow
147 headspace equilibrium and ambient temperature to be reached.

148 A sensory panel ($n = 8$, $n = 9$) based at the Sensory Science Centre (Department of Food
149 and Nutritional Sciences, University of Reading) participated in two independent qualitative
150 descriptive analysis (QDA) tests, one orthonasal and one retronasal assessment. The assessors
151 were screened and trained, with a minimum of 6 months experience in sensory evaluation of
152 flavour. An experienced sensory panel was preferred, to dissociate possible interactions from
153 odour and taste modalities (Hewson, Hollowood, Chandra, & Hort, 2008).

154 The orthonasal assessment preceded the retronasal assessment. At the beginning of each
155 type of assessment, the panellists were asked to generate as many sensory terms as possible to
156 describe the characteristics of all samples. Subsequently, the panel leader initiated a
157 discussion to develop a consensus vocabulary, in which flavour characteristics of all samples
158 were described and defined. There followed four training sessions on separate days, where
159 various references were chosen to standardise the definitions of descriptors (Supplementary
160 Table S2). Next, panellists individually rated samples in duplicate on two separate days, in
161 individual booths under artificial daylight and at a room temperature of 23 °C. Samples were
162 presented monadically and in a balanced order. The intensity of each attribute was rated using

163 unstructured line scales (scaled 0–100), with anchors predetermined by the consensus panel;
164 data were captured using Compusense 5 software (Compusense, West Guelph, ON, Canada).

165 2.3 *Measuring representativeness of extraction by HS–SPME/D–GC–O*

166 The D–GC–O method was performed to determine the SPME extract most representative
167 of orange juice aroma. The trained panel ($n = 7$), who had previously undertaken the sensory
168 profiling of the two sets and hence were familiar with the samples and the descriptors,
169 participated in the D–GC–O analysis. A similarity test was carried out in duplicate on five
170 SPME extracts obtained using different extraction conditions from the headspace of reference
171 sample 11.2 °Brix. Sample 11.2 °Brix was chosen since it was the sample most assessed
172 during QDA training of the panellists, thus it was the most familiar to them. The five
173 different sets of experimental conditions for aroma extraction are shown in **Error! Reference**
174 **source not found.** Initially longer fibre exposure periods were tested (15–30 minutes).
175 However, this led to fibre overload and poor chromatography (data not shown) All samples
176 were subjected to agitation during equilibration and extraction. SPME extracts were
177 presented in balanced order and labelled with numbers from 1 to 5. Assessors were firstly
178 asked to read the list of odour descriptors, then to smell the reference sample (3.75 mL)
179 contained in an amber bottle (30 mL). Subsequently, they evaluated the different SPME
180 extracts using D–GC–O in one session, rating the similarity to the reference using a 10-cm
181 line scale, ranging from 0 (far from reference) to 10 (close to reference). At the end of the
182 evaluation, the panellists were asked to describe the differences between the odour of the
183 SPME extract and that of the reference. Between sample evaluations, panellists had to smell
184 the reference again. Panellists individually rated samples in duplicate on two separate days.

185 The SPME extracts were evaluated by D–GC–O, using a Hewlett-Packard 5890 gas
186 chromatograph equipped with a sniffing port (ODO II; SGE, Ringwood, Australia). A short
187 capillary made of untreated fused silica (80 cm × 0.32 mm i.d.; Supelco, Bellefonte, PA)

188 connected the injection port to the sniffing port. The carrier gas was helium with a flow rate
189 of 10 mL/min. The SPME extracts were injected in splitless mode (injector temperature at
190 240 °C) and the oven temperature was kept at 50 °C. Since the short capillary contained no
191 stationary phase, there was no chromatographic separation of compounds in the SPME
192 extract and the extracts were assessed as “global” odour (Rega et al., 2003).

193 2.4 *Gas chromatography–mass spectrometry (GC–MS)*

194 Sample aliquots (2.5 mL) were added to 20-mL screw-cap glass vials and headspace
195 SPME was performed using an Agilent GC Sampler 120 PAL autosampler (Agilent
196 Technologies, Santa Clara, CA). A Stableflex 50/30 µm DVB/CAR/PDMS SPME fibre was
197 used for extraction (Supelco, Bellefonte, PA) and samples were agitated during equilibration
198 and extraction. Analyses were conducted using a 7890A gas chromatography system (Agilent
199 Technologies) attached to a 5975C inert MSD triple-axis detector (Agilent Technologies).
200 The injection port was kept at 240 °C and the fibre was desorbed in the injection port in
201 pulsed splitless mode for 45 s, with a pressure pulse of 25 psi. Helium was used as carrier gas
202 and flow through the column was constant at 1.2 mL/min (8.5 psi at 30 °C). A ZB-5MSi (30
203 m, 0.25 mm i.d., 1 µm film thickness; Phenomenex, Torrance, CA) capillary column was
204 used for volatile compound separation. The initial oven temperature was held at 30 °C for 4
205 min. It was then raised at 4 °C/min to 200 °C, and finally at 8 °C/min to 300 °C, where the
206 temperature remained constant for 1 min.

207 The mass spectrometer operated in electron impact mode with an electron energy of 70 eV
208 and scanned from m/z 29 to m/z 400. The interface was at 280 °C, the ion source at 230 °C and
209 the quadrupole at 150 °C. Peaks were identified by comparing retention times and mass spectra
210 with those of reference compounds. The GC peak area was measured for each compound, in
211 order to determine the release of volatiles from the sample, and all data were obtained in
212 triplicate.

213 2.5 *Statistical analysis*

214 The QDA results were statistically analysed by two-way analysis of variance (ANOVA)
215 with sample and assessors fitted as fixed and random effects, respectively, and main effects
216 tested against the assessor by sample interaction. Tukey's post hoc test was performed to
217 identify significant differences between sample pairs ($p < 0.05$) (SENPAQ software; Qi
218 Statistics, Ruscombe, UK). The GC peak areas of the flavour compounds recovered by GC-
219 MS were statistically analysed by one-way ANOVA and Tukey's post hoc test was applied to
220 determine differences between samples. Principal component analysis, using the mean
221 volatile data as the variables and with the mean sensory ratings (that were significantly
222 different between samples) regressed onto the space as supplementary variables, was carried
223 out using XLSTAT software Version 2014.6.01 (Addinsoft, Paris, France).

224 **3. Results and Discussion**

225 *3.1 Sensory analysis*

226 Figures 1 and 2 show the mean intensity scores for the sensory attributes, when samples
227 were assessed orthonasally and retronasally, respectively. When analysed retronasally, 9 out
228 of the 16 described attributes (listed in Supplementary Table S1) differed significantly
229 between samples. However, when assessed orthonasally, only one attribute, "overripe
230 orange", was found to vary significantly with sugar concentration. As sugar was not replaced
231 with sweeteners in this study, then the taste attributes rated were expected to significantly
232 differ between samples when assessed retronasally. Indeed, as can be seen in Figure 2, sweet
233 taste increased significantly with sugar content and the sample with the least sugar (5.2 °Brix)
234 had a significantly higher bitter taste, as might be expected because sweetness suppresses
235 bitterness (Green, Lim, Osterhoff, Blacher, & Nachtigal, 2010). As expected, "syrupy"
236 mouthfeel significantly increased with sugar content, especially at 11.2 and 14.2 °Brix. In

237 addition, the tactile sensation “astringency” (drying in nasal cavity) and “mouth-drying” were
238 found to significantly decrease with sugar content, as supported by previous literature
239 (Lyman & Green, 1990). Considering the ratings of the retronasal flavour attributes “fresh
240 ripe orange”, “cooked orange”, “artificial orange”, “citrus/non-orange” and “overall orange
241 strength”, these were found to vary significantly with the sucrose content, as shown in Figure
242 2. Apart from “citrus/non-orange”, the perceived intensities of the remaining attributes
243 showed an increase with increasing sugar content. The effect of sugar concentration on the
244 “overall orange strength” perception was the most pronounced. This overall odour intensity
245 significantly increased as sugar content increased. Likewise, the intensity of the attributes
246 “cooked orange” and “artificial orange” increased, from the low-sugar samples to the high-
247 sugar samples. However, this effect was not consistent in the case of “fresh ripe orange”,
248 where the scores increased gradually from samples 5.2 °Brix until 11.2 °Brix, but a
249 significant reduction occurred in the score of the 14.2 °Brix sample. As this evaluation was
250 retronasal, significant differences in flavour perception with sugar content may be attributed
251 to either differences in flavour release or to cross-modal taste enhancement.

252 Considering flavour release, it is reported that odour perception is directly related to the
253 aroma compounds released in the vapour phase of a model system (Rega et al., 2004). The
254 perceived intensity of an aroma is a function of the initial concentration in the model system
255 as well as the physical parameters that determine molecular transfer into the headspace.
256 Subsequently, any perceived alterations detected by the trained panel between samples could
257 be explained by differences in release of the various volatiles when sugar concentration is
258 modified. Therefore, it is hypothesised that as sucrose concentration increased, the perceived
259 intensity of these attributes may have increased, due to a salting-out effect. As literature
260 suggests, the “fresh” quality could be associated with terpenic compounds, whereas hexanal
261 and decanal could be responsible for the “fruity/fresh” intensity. Finally, the “artificial” and

262 “overall” intensity could be related to ethyl butanoate, which is characterised by a strong
263 “sweet orange” quality. Linalool has been found to impart “cooked” quality (Rega et al.,
264 2004). However, from the retronasal evaluation we cannot rule out cross-modal enhancement
265 of the “cooked orange”, “artificial orange”, “citrus/non-orange” notes as well as “overall
266 orange strength” by the sweet taste, as sweetness is known to enhance the perception of
267 sweet-congruent flavours (Lim, Fujimaru, & Linscott, 2014).

268 When the assessment was orthonasal any differences with sucrose content should be due
269 to flavour release as they cannot be due to perceptual cross-modal effects. Therefore, we
270 propose that the trend of “overripe orange ” orthonasal aroma to increase with sugar content
271 (Figure 1), which was significant between the 8.2 and 14.2 °Brix samples, is due to a salting-
272 out effect.

273 *3.2 Aroma quality analysis by HS-SPME/D-GC-O*

274 Five SPME extraction procedures were examined, varying three sampling conditions
275 (Table 1), with the sensory panel comparing each extract to the complete reference juice at
276 11.2 °Brix. Generally, a short time of fibre exposure was preferred, since many studies
277 suggested that a shorter time of sampling shows better sensitivity and less likelihood of fibre
278 overloading from compounds with high affinity to the coated material of the fibre (Rega et
279 al., 2003; Roberts et al., 2000). The mean similarity ratings (scored out of 10) obtained from
280 the trained sensory panel for each of the extraction conditions varied from 4.8 to 6.3,
281 although these differences were not significant ($p = 0.068$). Overall, this showed that the
282 odour of the SPME extracts did not perfectly match that of the reference sample. Although
283 the differences were not significant, it is interesting to note that the aroma of the extract with
284 the highest fibre sampling time (5 min) was rated to be the least like the reference sample,
285 which agreed with previous studies (Rega et al., 2003; Roberts et al., 2000). The equilibration
286 temperature of 40 °C tended to give greater similarity than 30 °C, while the combination of

287 40 °C for 30 min with agitation has been previously reported as one of the most suitable
288 sampling conditions for the headspace analysis of orange flavour compounds (Jia et al.,
289 1998). The experimental conditions that provided the most representative extract (40 °C
290 equilibration for 30 min followed by 1 min fibre exposure) were used for all subsequent HS–
291 SPME/GC–MS analyses.

292 3.3 Gas chromatography-mass spectrometry (GC–MS)

293 The experimental data of flavour release from the orange juice model system at varying
294 sucrose concentrations are presented in Figure 3. Although limonene was absent from the
295 flavouring, it was present as the compound with the largest peak area in the GC–MS trace.
296 The limonene is a component of the orange pulp, which was not fully removed by the
297 deodorisation process. Because of its importance in citrus, it was decided to examine how this
298 compound varied as a result of sucrose reduction.

299 Overall, the results show a significant decrease in the concentration of hexanal, α -pinene,
300 β -myrcene, limonene, linalool and decanal by decreasing sugar content ($p < 0.05$). These
301 findings might be attributed to the salting-out of flavour volatiles into the headspace above
302 the samples when sucrose interacts with water, resulting in increased concentration of the
303 flavour compounds in the remaining “unbound water” (Friel, Linforth, & Taylor, 2000;
304 Hansson et al., 2001; Rabe et al., 2003).

305 The decreasing sugar did not lead to a consistent decrease in the release of ethyl butanoate
306 and although the release of hexanal was significantly different between samples, the extent of
307 difference was small and the relationship between sugar concentration and hexanal release
308 was not consistent. There was an observable trend that the release of (*Z*)-3-hexen-1-ol slightly
309 varied with sugar content (at a significance value, $p < 0.05$), whereas linalool release
310 significantly decreased with decreasing sugar concentration. The release of these flavour
311 compounds has been reported to increase at elevated sucrose levels in soft drink model

312 systems (Hansson et al., 2001; Rabe et al., 2003). It is an indication that changes in the sugar
313 levels used in commercial soft drinks slightly modify the amount of “free water”, which
314 subsequently has a minor effect on salting-out of some aroma compounds.

315 Moreover, one robust finding to have emerged from the current HS–SPME analysis is the
316 clear and significant pattern observed in the concentrations of α -pinene, β -myrcene, limonene
317 and decanal, aroma compounds with relatively high molecular weights and relatively low
318 polarities. Release of these volatile compounds showed a significant increase from 5.2 °Brix
319 to 14.2 °Brix while release at the intermediate sucrose levels of 8.2 °Brix, 9.7 °Brix and 11.2
320 °Brix did not statistically differ. Regardless of the aforementioned salting-out effect, the up to
321 4-fold increased release of these compounds could be additionally explained by the strong
322 polar environment of the model system, the hydrophobic nature of these flavour compounds
323 and the low pulp content which such hydrocarbons are more associated with; a positive
324 correlation between reduction of pulp content and decreasing release of hydrophobic
325 compounds has been found (Berlinet et al., 2007). A previous study reported that limonene
326 did not show any significant changes in its release into the headspace above a soft drink
327 model system across different sugar concentrations ranging from 20 to 60% w/v, due to the
328 non-polar character of this compound (Hansson et al., 2001).

329 Low variation in the quantification of flavour compounds between replicates was observed
330 using the SPME/GC–MS conditions optimised in this study. This indicates both that
331 equilibrium had been reached in sampling and that the ratio between the added volume of the
332 orange juice and the total volume of the vial (1:8) was optimal. Likewise, the sugar levels
333 used in the current model system are low, resulting in limited complexity of the hydration
334 processes and low variability of the experimental data, as has been suggested in a previous
335 study (Rabe et al., 2003).

336 *3.4 Comparison of sensory and analytical data*

337 Principal component analysis (PCA) was performed using the relative values of volatile
338 compounds determined in the HS–SPME extracts of the samples, with the sensory data for
339 the same samples fitted onto the PCA plot as supplementary variables (Figure 4), assuming
340 that the sensory perception of the orange juice soft drinks was affected by their volatile
341 flavour profile. Specifically, one orthonasal (O) and five retronasal attributes (R) were used
342 for this analysis; the attributes that differed significantly between samples. Similarly, among
343 the volatile compounds of the model flavour mixture, only ethyl butanoate was excluded
344 from the principal component analysis, since it did not vary with sugar concentration. The
345 first two principal components accounted for 94% of the variance in the data; principal
346 component 1 (PC1) explained 73.5% of the variance, discriminating samples in terms of
347 “sugar content”, and principal component 2 (PC2) explained a further 20.5% variance. PC1
348 placed the 5.2 °Brix soft drink on the left-hand side and the 14.2 °Brix on the right-hand side.
349 8.2 °Brix, 9.7 °Brix and 11.2 °Brix soft drink samples were located close to the origin.

350 It is well established that differences in the release of volatile compounds into the
351 headspace of juices and soft drinks are directly associated with the orthonasal perception of
352 them. Therefore, the perceived differences in the orthonasal attribute “overripe orange”
353 detected by the sensory panel with increasing sugar content could be partly attributed to
354 changes found in the volatiles released into the headspace. Specifically, the correlation matrix
355 showed that the orthonasal “overripe orange” attribute was strongly correlated with all the
356 flavour volatiles apart from the aldehyde hexanal (no correlation was found with this volatile
357 molecule). This indicates that the release of these flavour compounds, as a total, gave a major
358 sensorial impact on the perceived intensity of the sensory orthonasal quality of “overripe
359 orange”; as reflected by PC1 (Figure 4). Finally, this finding confirms our hypothesis that the
360 detected differences in the orthonasal perception by the sensory panel could be attributed to
361 the “salting-out” of the volatiles into the headspace above samples.

362 The retronasal perception is more complex and could be affected by many factors (not
363 only the physical parameters of the soft drink) and interactions between different sensory
364 modalities. Therefore, differences in the release of the flavour volatiles into the headspace
365 cannot directly correspond to differences detected during the retronasal evaluation. The
366 volatile compounds (*Z*)-3-hexen-1-ol, α -pinene, β -myrcene, limonene, linalool and decanal
367 were very well correlated with all the sensory variables apart from the retronasal “fresh ripe
368 orange”. Under closer inspection, this finding has a two-fold importance for the current study.
369 On the one hand, the flavour volatiles appeared to impart a substantial contribution to the
370 perceived intensity of these sensory attributes as a group and not as individual compounds; on
371 the other hand, the retronasal attribute “fresh ripe orange” showed weak correlations with all
372 volatiles, indicating that differences in the release of these volatiles did not reflect changes in
373 the intensity of this sensory attribute. In contrast, the “citrus/non-orange” attribute was found
374 to be negatively associated with all other dependent variables. Moreover, only the sample at
375 5.2 °Brix was positively correlated with the retronasal “citrus/non-orange” attribute. This
376 finding indicates that the retronasal quality of “citrus/non-orange” could be a result of low
377 release concentrations of the flavour volatiles in the soft drink containing 5.2 °Brix sugar
378 content and not due to a single flavour compound. Also, the increased intensity of
379 “citrus/non-orange” found in 5.2° Brix sample could be attributed to the increased perceived
380 bitterness, as a similar pattern in ratings was observed for this odour and taste descriptor. The
381 sample with 14.2 °Brix sugar content was found to be highly correlated with all the volatile
382 and sensory data used in the principal component analysis. In fact, the aforementioned
383 extreme sensory findings between samples with 5.2 and 14.2 °Brix sugar content suggest that,
384 apart from the observed physical effects (salting-out of volatiles), the perceived differences in
385 the retronasal evaluation might be attributed to bitterness and sweetness enhancement,

386 respectively (cognitive cross-modal enhancement) (Hornung & Enns, 1986; Stampanoni,
387 1993).

388 In the case of hexanal, negligible correlations with all the remaining variables were found.
389 This finding suggests that hexanal had very little, if any, contribution to the flavour
390 perception of the samples. This is not supported by the literature, which suggests that fresh
391 flavour notes are imparted by aldehydes (Rega et al., 2004). However, it could be explained
392 by the low concentration of hexanal in the flavour mixture and subsequently in its lower
393 release compared to the other volatiles in the current model system. This is also supported by
394 the very low intensity rating of green/grassy odour given by the sensory panel. At 5.2 °Brix
395 the relatively high headspace concentrations of linalool, ethyl acetate, (*Z*)-3-hexen-1-ol and
396 hexanal, relative to those of decanal and the monoterpenes, may also be associated with the
397 increase in the citrus/non-orange attribute. For example, at 5.2 °Brix, the ratio between the
398 headspace peak areas for β -myrcene and ethyl butanoate is about 1.5, while at 14.2 °Brix, the
399 ratio is about 6. This change could affect the balance of the flavouring at reduced sugar
400 content, resulting in the observed sensory differences.

401 Although the viscosities of the model orange soft drinks were not measured in this work,
402 other workers (Hewson, Hollowood, Chandra, & Hort, 2008), who examined sugar levels
403 close to the range used in this study, suggested that the small viscosity differences they
404 observed (0.4 mPa s) may not affect assessor perception. Kappes, Schmidt, and Lee (2006)
405 plotted sucrose solution concentration against viscosity. Their results suggested that the
406 difference in viscosity between the 5.2 °Brix and 14.2 °Brix model solutions was
407 approximately 0.5 mPa s. These authors suggested that a viscosity difference of 0.527 mPa s
408 could cause a perceived difference in mouthfeel. However, they were focusing on the effect
409 of sweetener removal on mouthfeel and they were comparing diet and regular cola
410 carbonated beverages, with an added sweetness suppressant. Based on these observations, it

411 seems unlikely that viscosity differences in the five orange juice model solutions would affect
412 flavour release.

413

414 *4 Conclusions*

415 The results obtained from sensory and flavour release analysis concluded that a “salting-
416 out” effect of volatiles into the headspace could be observed within sugar levels normally
417 used in commercial soft drinks. It was observed that the ratings of some orthonasal and
418 retronasal attributes significantly ($p < 0.05$) varied by sugar level reduction.

419 The D–GC–O method, which was conducted, in order to assess the odour quality of the
420 SPME extracts by applying different sampling conditions, proved to be a valuable research
421 tool, capable of giving to the researcher confidence about the quality and the
422 representativeness of an SPME extract.

423 The HS–SPME/GC–MS analysis successfully determined an association between the
424 release behaviour of volatile compounds (selected because of their odour quality) and their
425 importance in orange juice flavour. Principal component analysis was able to explain about
426 95% of the data variability and strongly correlated the perceived intensity of the orthonasal
427 attribute “overripe orange” with the release of the flavour compounds, as a total, supporting
428 the “salting out” hypothesis. However, it remains questionable whether the perceived
429 differences in the retronasal evaluation might be attributed to sweetness enhancement rather
430 than flavour release.

431

432 **5 Acknowledgements**

433 The authors would like to gratefully acknowledge the financial support by the Seventh
434 Framework programme (FP7) of the European Union under the Marie Curie Initial Training
435 Network ‘HST FoodTrain’ (Grant agreement 264470).

437 **6 References**

438 Bazemore, R., Goodner, K., & Rouseff, R. (1999). Volatiles from unpasteurized and
439 excessively heated orange juice analyzed with solid phase microextraction and GC-
440 olfactometry. *Journal of Food Science*, 64 (5), 800–803.

441 Berlinet, C., Guichard, E., Fournier, N., & Ducruet, V. (2007). Effect of pulp reduction and
442 pasteurization on the release of aroma compounds in industrial orange juice. *Journal of Food*
443 *Science*, 72 (8), S535–S543.

444 Friel, E. N., Linforth, R. S. T., & Taylor, A. J. (2000). An empirical model to predict the
445 headspace concentration of volatile compounds above solutions containing sucrose. *Food*
446 *Chemistry*, 71(3), 309–317.

447 González-Mas, M. C., Rambla, J. L., Alamar, M. C., Gutiérrez, A., & Granell, A. (2011).
448 Comparative analysis of the volatile fraction of fruit juice from different citrus species. *PLoS*
449 *ONE*, 6 (7), e22016.

450 Green, B. G., Lim, J., Osterhoff, F., Blacher, K., & Nachtigal, D. (2010). Taste mixture
451 interactions: Suppression, additivity, and the predominance of sweetness. *Physiology &*
452 *Behavior*, 101 (5), 731–737.

453 Hansson, A., Andersson, J., & Leufven, A. (2001). The effect of sugars and pectin on flavour
454 release from a soft drink-related model system. *Food Chemistry*, 72 (3), 363–368.

455 Hewson, L., Hollowood, T., Chandra, S., & Hort, J. (2008). Taste-aroma interactions in a
456 citrus flavoured model beverage system: Similarities and differences between acid and sugar
457 type. *Food Quality and Preference*, 19, 323–334.

458 Hornung, D. E., & Enns, M. P. (1986). The contributions of smell and taste to overall
459 intensity: A model. *Perception and Psychophysics*, *39*, 385–391.

460 Jia, M., Zhang, Q. H., & Min, D. B. (1998). Optimization of solid-phase microextraction
461 analysis for headspace flavor compounds of orange juice. *Journal of Agricultural and Food*
462 *Chemistry*, *46* (7), 2744–2747.

463 Kappes, S. M., Schmidt, S. J., & Lee, S. Y. (2006). Mouthfeel detection threshold and
464 instrumental viscosity of sucrose and high fructose corn syrup solutions. *Journal of Food*
465 *Science*, *71*, S597–S602.

466 Lim, J., Fujimaru, T., & Linscott, T. D. (2014). The role of congruency in taste-odor
467 interactions. *Food Quality and Preference*, *34*, 5–13.

468 Lyman, B. J., & Green, B. G. (1990). Oral astringency - Effects of repeated exposure and
469 interactions with sweeteners. *Chemical Senses*, *15* (2), 151–164.

470 Moshonas, M. G., & Shaw, P. E. (1994). Quantitative determination of 46 volatile
471 constituents in fresh, unpasteurized orange juices using dynamic headspace gas
472 chromatography. *Journal of Agricultural and Food Chemistry*, *42* (7), 1525–1528.

473 Nahon, D. F., Roozen, J. P., & de Graaf, C. (1998). Sensory evaluation of mixtures of sodium
474 cyclamate, sucrose, and an orange aroma. *Journal of Agricultural and Food Chemistry*, *46*
475 (9), 3426–3430.

476 Perez-Cacho, P. R., & Rouseff, R. L. (2008). Fresh squeezed orange juice odor: a review.
477 *Critical Reviews in Food Science and Nutrition*, *48* (7), 681–695.

478 Plotto, A., Margaría, C. A., Goodner, K. L., & Baldwin, E. A. (2008). Odour and flavour
479 thresholds for key aroma components in an orange juice matrix: esters and miscellaneous
480 compounds. *Flavour and Fragrance Journal*, 23 (6), 398–406.

481 Plotto, A., Margaría, C. A., Goodner, K. L., Goodrich, R., & Baldwin, E. A. (2004). Odour
482 and flavour thresholds for key aroma components in an orange juice matrix: terpenes and
483 aldehydes. *Flavour and Fragrance Journal*, 19 (6), 491–498.

484 Rabe, S., Krings, U., & Berger, R. G. (2003). Dynamic flavor release from sucrose solutions.
485 *Journal of Agricultural and Food Chemistry*, 51 (17), 5058–5066.

486 Rega, B., Fournier, N., & Guichard, E. (2003). Solid phase microextraction (SPME) of
487 orange juice flavor: odor representativeness by direct gas chromatography olfactometry (D-
488 GC-O). *Journal of Agricultural and Food Chemistry*, 51 (24), 7092–7099.

489 Rega, B., Fournier, N., Nicklaus, S., & Guichard, E. (2004). Role of pulp in flavor release
490 and sensory perception in orange juice. *Journal of Agricultural and Food Chemistry*, 52 (13),
491 4204–4212.

492 Roberts, D. D., Pollien, P., & Milo, C. (2000). Solid-phase microextraction method
493 development for headspace analysis of volatile flavor compounds. *Journal of Agricultural*
494 *and Food Chemistry*, 48 (6), 2430–2437.

495 Stampanoni, C. R. (1993). Influence of acid and sugar content on sweetness, sourness and the
496 flavour profile of beverages and sherbets. *Food Quality and Preference*, 4(3), 169–176.

497 Yang, X., & Peppard, T. (1994). Solid-phase microextraction for flavor analysis. *Journal of*
498 *Agricultural and Food Chemistry*, 42 (9), 1925–1930.

499 Zhang, Z., & Pawliszyn, J. (1993). Headspace solid-phase microextraction. *Analytical*
500 *Chemistry*, 65 (14), 1843–1852.

501

502 **Figure legends**

503 **Fig. 1.** Orthonasal evaluation of samples: mean perceived intensity (0–100) of all sensory
504 attributes (different letters above the bars represent significant differences at $p < 0.05$ from
505 Tukey's HSD test)

506 **Fig. 2.** Retronasal evaluation of samples: mean perceived intensity (0–100) of all sensory
507 attributes (flavour, taste and mouthfeel) (different letters above the bars represent significant
508 differences at $p < 0.05$ from Tukey's HSD test)

509 **Fig.3.**Effect of sugar concentration on relative amounts of aroma compounds in the
510 headspace SPME extract of a model orange juice soft drink. Effect significant at:* $p < 0.05$;
511 ** $p < 0.01$; *** $p < 0.001$; ns: not significant (error bars indicate standard deviation between
512 replicates ($n = 3$)).

513 **Fig. 4.** Principal component analysis biplot where the volatile compound data (■) that were
514 significantly different between samples (◇) formed the multidimensional space and the
515 sensory attributes (◆) were regressed onto the space as supplementary variables; orthonasal
516 and retronasal evaluations have "O" and "R" letters, respectively.

517

518 **Table 1**

519 Examined experimental conditions of SPME extractions using a Stableflex 50/30 μm
520 DVB/CAR/PDMS fibre

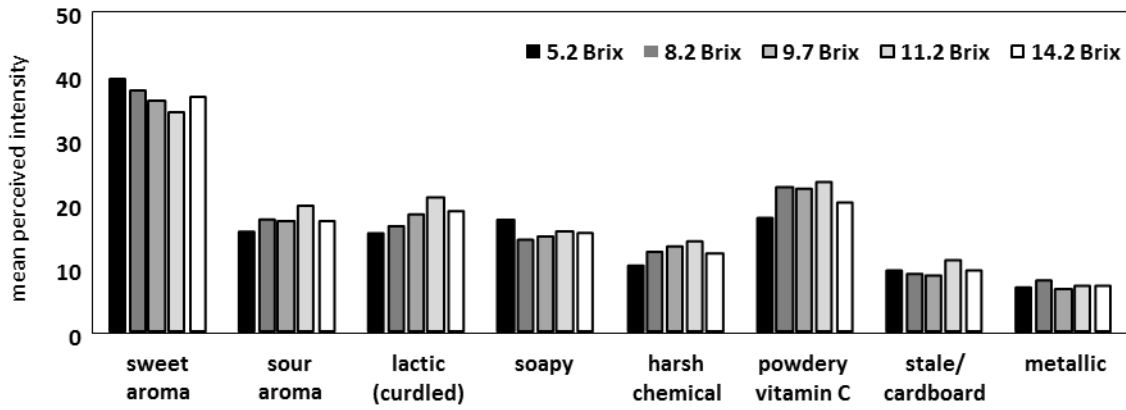
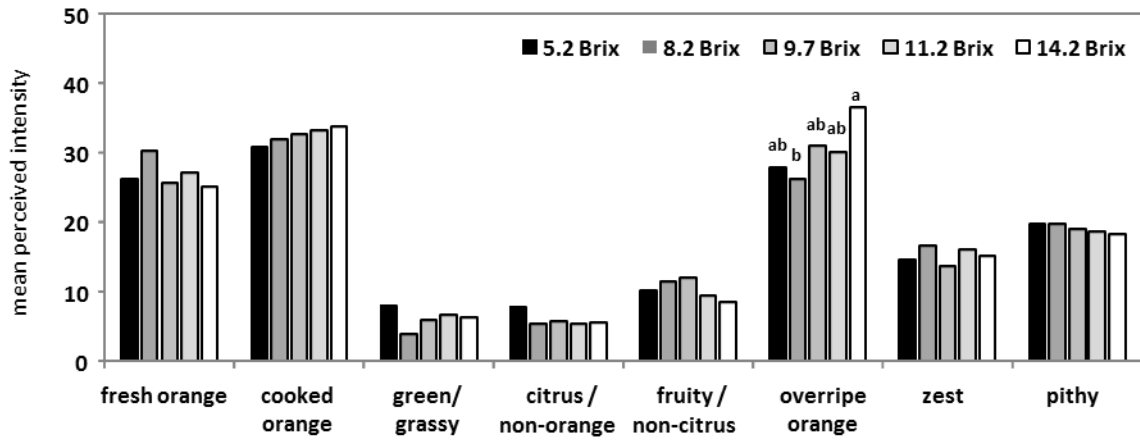
| equilibrium temperature (°C) | equilibrium time (min) | fibre exposure time (min) |
|---|-----------------------------------|--|
| 30 | 30 | 1 |
| 30 | 30 | 5 |
| 40 | 15 | 1 |
| 40 | 30 | 0.5 |
| 40 | 30 | 1 |

521

522

523 Figure 1:

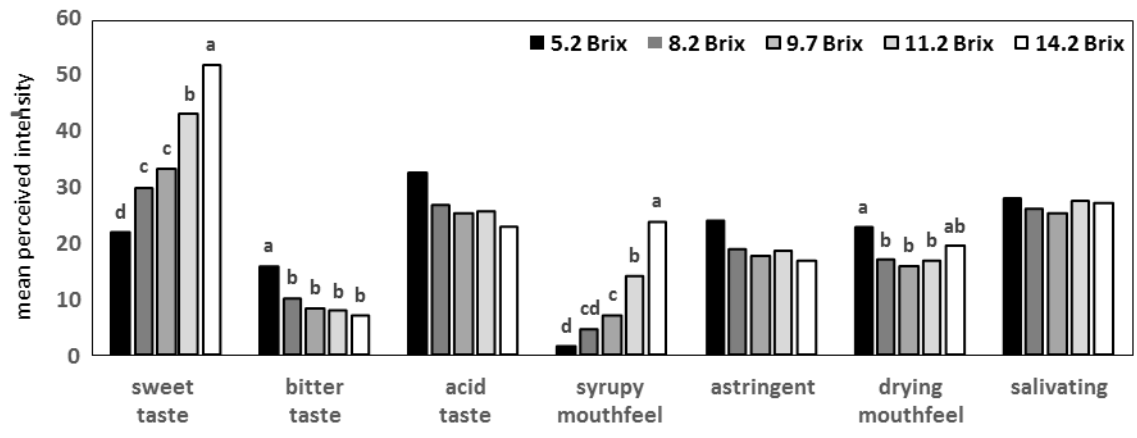
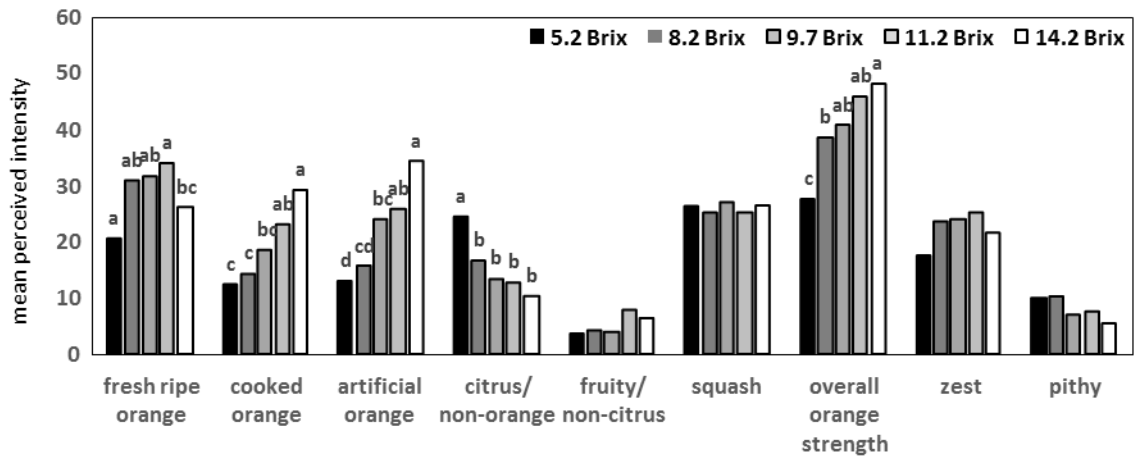
524



525

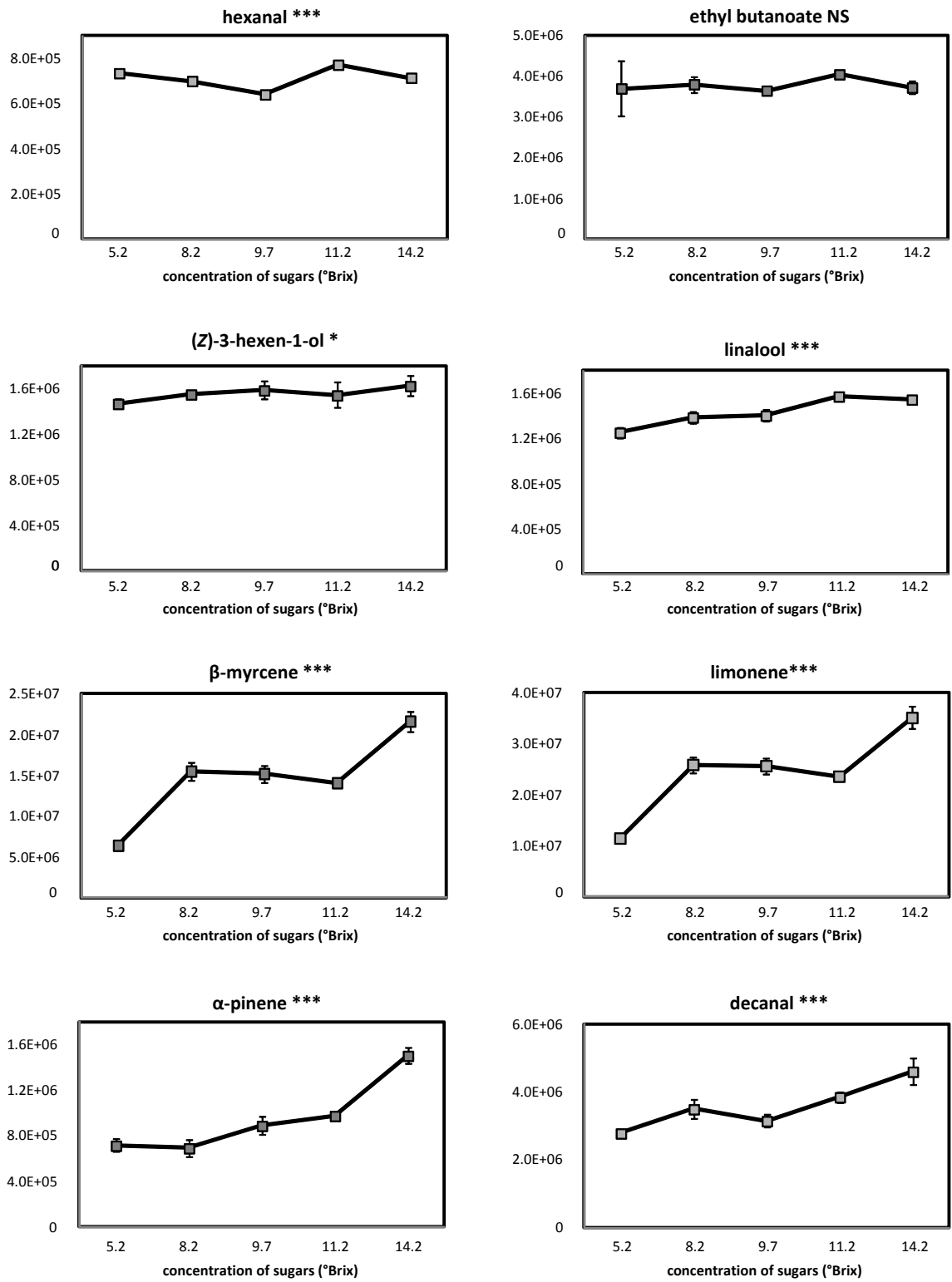
526 Figure 2:

527



528

Figure 3:



532

533 **Figure 4:**

534

535

536

537

538

539

540

541

542

543

