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Article

GC-MS Investigation of Volatiles from Honeybee Worker Larvae and Larval Food at Different Instars

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Abstract: (*E*)-β-ocimene was the only found volatile chemical emitted by whole, live worker larvae of *Apis mellifera* L. by sampling in the vapor phase. While in addition to (*E*)-β-ocimene, there is evidence for the existence of other volatiles; but the changes of their composition and contents remain unknown during larval development, as are their differences from larvae to larval food. This is the main purpose of the study. We investigated volatile components of worker larvae and larval food using solid phase dynamic extraction (SPDE) coupled with gas chromatography-mass spectrometry (GC-MS). Nine compounds were identified with certainty and six tentatively, consisting of terpenoids, aldehydes, hydrocarbons, ester and ketone. The contents of volatiles of the second-instar worker larvae differ greatly from larvae of other stages mainly attributable to terpenoids, which made the second-instar worker larvae had significantly higher amounts of overall volatiles. Larval food contained significantly higher amounts of aldehydes and hydrocarbons than the corresponding larvae from the fourth to fifth-instar. We discovered volatiles in worker larvae and their food which were never reported before; we also mastered the change of these volatiles' contents during larval development.

Keywords: *Apis mellifera*; GC-MS; larva; terpenoids; volatiles

1. Introduction

Volatiles occur in honey bee colonies as a complex mixture, determined by pheromones produced by bees, and other chemicals emitted by beeswax, honey, pollen and larval food [1]. These volatiles have been identified as alcohols, aldehydes, benzenoid compounds, carboxylic acids, hydrocarbons, ketones and terpenoids [1].

Volatiles in honey bee colonies are easily dispersed and received by bees through antennal reception over long distance. Therefore, using volatiles are efficient means in moderating the bee social behaviors. With regard to the volatiles in maintaining social cohesion, most of them are found from adult worker bees, like alarm pheromone inducing defensive behavior of honeybees[2] Nasanov pheromone triggering aggregation[3], and other volatiles transmitting recognition cues; also a few of them are detected from queens specifically[4].

Very few efforts have been made to determine the volatiles emitted directly by intact and alive honey bee larvae. On the contrary, the non-volatile chemicals of honey bee larvae are widely studied based on the solvent extraction method [5-7]. (*E*)-β-ocimene is the only identified volatile chemical produced by alive worker larvae. There is evidence for the existence of other larvae volatiles, because different chromatogram peaks in addition to (*E*)-β-ocimene were

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discovered [8], but their compositions are not clear. There is also a lack of information about how these unknown larval volatiles change with larval development. However, an understanding of the composition and content variation of volatiles during larval development is necessary for interpreting their functions.

Food provided to honeybees could directly or indirectly affect honeybee volatiles production. There is evidence that food shortages might stimulate worker bee larvae into releasing more (*E*)-β-ocimene [9]; and feeding honeybee worker larvae essential oils via diet supplements may change their volatiles[10]. However, the volatiles extracted from worker larval food have not been reported before. Only volatile carboxylic acids were identified in drone larval food, and other unidentified non-acidic volatiles were noteworthy [11]. If the volatiles of the worker larval food were analyzed combined with the volatiles analysis of the worker larvae, it would provide a deep insight into the relationship between volatiles in larvae and their food during the same larval instar.

In this paper, we analyzed the volatiles from worker larvae and their food at different instars, using solid phase dynamic extraction (SPDE) combined with high resolution gas chromatography tandem mass spectrometry (GC-MS). Through this research, we discovered some volatiles in worker larvae and their food that have been overlooked before. We believe that these discoveries are a first step towards determining the underlying important functions of volatiles within honey bee nests.

2. Materials and Methods

Three standard Chinese commercial strain of western honeybee (Apis mellifera ligustica L.) were used in this experiment. Large double-deep colonies (30,000 to 50,000 adult bees) located at our apiary in the Institute of Apicultural Research in Beijing (39o N, 116.2 o E). Queens were caged within plastic controllers (interior dimensions: 457 × 50 × 245mm, only allowed passage of worker bees) to oviposit on empty new combs (without pollen and nectar stores) for 24 hours and were then removed to the outside of the controllers. Oviposition frames were reared in the controllers. At each caging interval, we obtained three combs with single-age cohorts of worker larvae for each replicate. Seven times caging were carried out, for a total of 21 combs.

After the combs were brought back to the laboratory, cells were randomly chosen to provide both the larval food and larvae samples for further analysis. Larvae and larval food was obtained with a small spatula. Larvae were inspected under the microscope, and only live and uninjured larvae were used for the study [9].

Treatments

The volatiles of grouped larvae at three development stages were monitored. Group sizes were 20 second-instar (2nd-instar) larvae, 10 fourth-instar (4th-instar) larvae, and 5 fifth- instar (5th-instar) larvae. The larval food volatiles were also detected at different stages. To monitor change in volatiles with ontogenetic development, the amount of volatiles released by individual larva was determined [8]. To compare volatiles content between larvae and food, the amount of volatiles released per unit weight of sample were calculated [12]. Larvae and food were carefully transferred into 20 mL glass vials. Before analysis, 2μL of 0.1 mg/L hexadecane hexane solution on a strip of filter paper was added as an internal standard for quantification. Clean empty vials were analyzed separately as controls to remove background interference.

SPDE System

Volatile extraction was performed using SPDE equipment installed in a CTC-Combi-PAL auto sampler (CTC Analytics, Zwingen, Switzerland), as described by Castro [13]. The SPDE needle (SPDE-01/AC-50-56, 50 μm × 56 mm), coated with 90% polydimethylsiloxane (PDMS) and 10% active charcoal (AC), and was preconditioned before use. Equilibration between sample and headspace lasted for 30 min at 35 °C in an incubation pool. After equilibration, the extraction procedure was operated as follows: extraction volume – 1 mL, agitator temperature – 35 °C,

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94 headspace syringe temperature – 35 °C, number of strokes – 30, filling/eject speed – 25 μL/s.
95 During the equilibration and extraction procedure, the larvae were alive and isolated from food
96 for more than 45 min [9]. The needle was then withdrawn and introduced into the injection port
97 of the gas chromatograph, and pumped with 1 mL nitrogen at 100 μL/s for desorption, at 250°C
98 for 2 min in splitless mode.
99 GC-MS analysis was performed using a Shimadzu gas chromatograph-2010 equipped with
100 a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA, USA)
101 coupled to a Shimadzu quadrupole-2010 mass spectrometer (Shimadzu, Kyoto, Japan). The oven
102 program was as follows: 35 °C for 2 min, 35–200 °C at 5 °C/min, 200 °C for 2 min, 200–250 °C at 10
103 °C/min, then 250 °C for 3 min. Injector temperature was maintained at 250 °C, transfer line
104 temperature was 250 °C, and ion source temperature was 200 °C. Helium was used as the carrier
105 gas, at a flow rate of 1.7 mL/min.
106 Qualitative and quantitative analysis
107 The identification of the compound with authentic standards was performed by comparing
108 the mass spectra (Wiley6 and NIST05) and retention times to those of authentic standards.
109 Compounds without standards were identified by comparing the mass spectrum peaks with
110 data system libraries (Wiley6 and NIST05) and other published spectra (Mass Spectrometry Data
111 Centre 1974). Additionally, the linear retention indices (LRI) of the compounds were calculated
112 by injecting a series of n-alkanes (C10–C25) (o2si Smart Solutions) into the GC-MS on two
113 columns of different polarities under identical conditions. Authentic standards (listed in
114 supplementary data) purchased from Alfa Aesar (Karlsruhe, Germany) were serially diluted
115 with hexane to make standard solutions. The peak areas on the total ion chromatogram were
116 used for quantification. The calibration curve derived from a step-series of standard compounds
117 for individual target compounds was built by plotting the area ratio of target compounds to the
118 internal standard against the concentration ratio. The concentrations of volatile compounds were
119 calculated based on the corresponding calibration curves.
120 Statistical analysis
121 Principal component analysis (PCA) using a correlation matrix was applied to the data to
122 establish relationships between the different samples and their volatile compounds. The SPSS
123 software package Version 21 for Windows (SPSS, Chicago, IL, USA) was used for statistical
124 analysis. A one-way ANOVA ($p < 0.05$) was used to test for significant differences in volatile
125 compound concentrations among treatments. The SigmaPlot software package Version 12 for
126 Windows (Systat Software Inc., California USA) was used to create the artwork.

127 **3. Results**

128 We detected fifteen compounds from the developing larvae and their corresponding food,
129 which could be sorted into seven groups: three aldehydes, one ester, three hydrocarbons, one
130 ketone, and seven terpenoids (Figure 1, Table 1).

131

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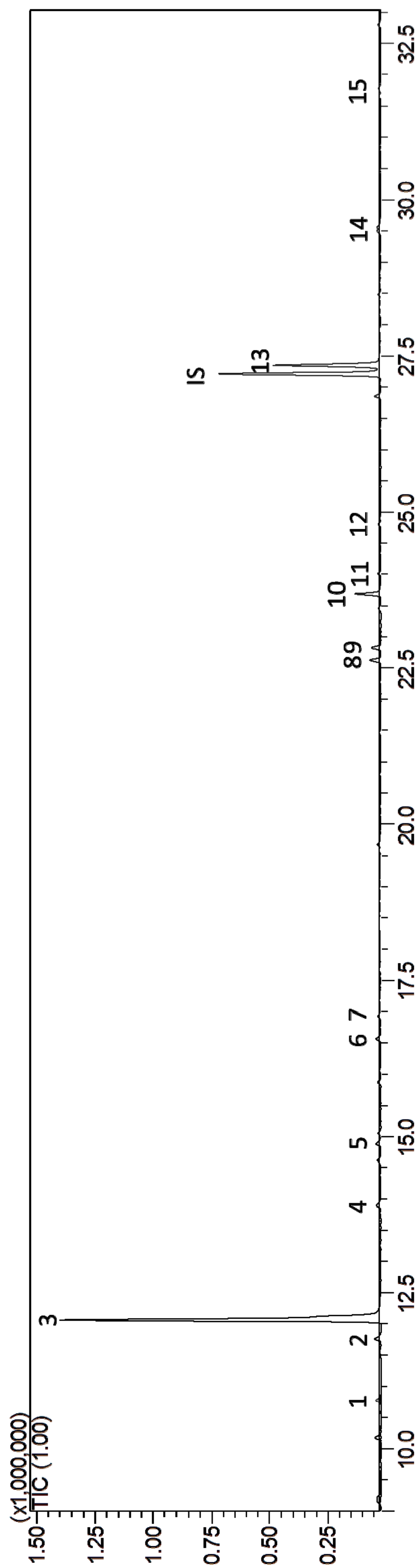

Figure 1 Gas chromatogram of all identified volatiles reflected in the 2nd instar larvae (IS: internal standard)

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133 **Table 1** Volatile compounds tentatively identified from larvae and larval food at different larval instars.

RT[a]	Compound	Code[b]	Peak	LRI Calc[c]		LRI Lit[d]	Identify[e]	Quantifier ions
				DB5	BPX5			
	Terpenoids							
11.75	(*Z*)-β-Ocimene	t1	2	1040	1229	976	S. N. L	93, 41, 79
12.06	(*E*)-β-Ocimene	t2	3	1050	1250	976	S. N. L	93, 79, 105
14.61	(*E, Z*)-Alloocimene	t3	5	1134	1371	1088	N. L	119, 91, 134
16.56	α-Terpineol	t4	6	1199		1143	S. N. L	59, 93, 121
22.62	α-Cedrene	t5	8	1421	1556	1403	N. L	119, 93, 105
22.82	β-Cedrene	t6	9	1429	1560	1403	N. L	161, 69, 204
27.34	Cedrol	t7	13	1615	2112	1543	N. L	
	Aldehydes							
10.73	Octanal	a1	1	1008		1005	S. N. L	43, 56, 84
13.88	Nonanal	a2	4	1109		1104	S. N. L	57, 41, 70
16.91	Decanal	a3	7	1212		1204	S. N. L	43, 57, 70
	Hydrocarbons							
24.80	Pentadecane	h1	12	1508	1498	1512	S. N. L	57, 43, 71, 85
29.48	Heptadecane	h2	14	1709	1699	1711	S. N. L	57, 43, 71, 86
31.77	Octadecane	h5	15	1816	1799	1852	S. N. L	57, 71, 85, 43
	Ester							
24.03	Ethyl 2(*E*)-decenoate	e1	11	1489	1758	1389	N. L	43, 55, 73
	Ketone							
23.65	(*E*)-Geranylacetone	k1	10	1454		1420	N. L	43, 41, 69

134 [a] RT: Retention time (min).
135 [b] Code: abbreviation of the compound.
136 [c] LRI Calc: Linear retention index calculated through n-alkanes.
137 [d] LRI Nis: Linear retention index reported in the NIST Chemistry Web Book 2005.

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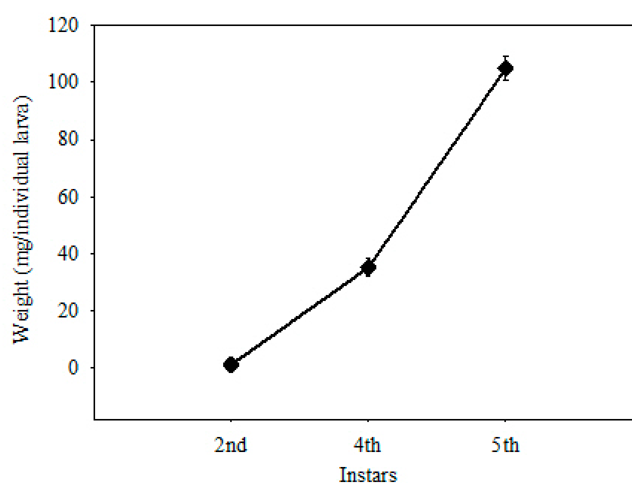
138 [e] The reliability of the identification or structural proposal is indicated by the following: (S) mass spectrum
139 and retention time consistent with those of an authentic standard; (N) structural proposals given on the
140 basis of mass spectral data (NIST98); (L) mass spectrum consistent with spectra found in literature.

141 *3.1. Volatiles*

142 3.1.1. Terpenoids

143 Terpenoids were the largest group of larvae except at 5th instar, accounting for 83.3%, and
144 73.7% of total larval volatiles at 2nd and 4th instar separately, calculated as ng/mg. The major
145 terpenoids of food volatiles were α-cedrene (t5) and cedrol (t7), accounting for 65.5–89.2% of
146 total terpenoids, while the major terpenoids of larval was (*E*)-β-ocimene (t2), and accounting for
147 60.9–93.8%.
148 When terpenoid amount was calculated as ng/ individual larva while larval weight
149 increased (Figure 2), the amount of all terpenoids (Figure 3, column St), (*E*)-β-ocimene (Figure 3,
150 column t2) and α-terpineol (Figure 3, column t4) continuously decreased. The amount of
151 (*Z*)-β-ocimene (Figure 3, colum t1) and (*E*, *Z*)-alloocimene (Figure 3, column t3) disappeared
152 at 5th instar. The amount of α-cedrene (Figure 3, column t5) and β-cedrene (Figure 3, column t6)
153 just fluctuated without significant difference by instar. The amount of cedrol (Figure 3, column
154 t7) increased sharply from 2nd to 4th instar, and remained stable from 4th to 5th instar.



155

156 **Figure 2.** The change of larva weight during larval development

157 When terpenoid amount (St, t1, t2, t3, t4, t5, t6, t7) released by larva was calculated as
158 ng/mg (Figure 3), there was a decreasing trend during larval development. The 2nd instar larvae
159 always had significantly higher contents than larvae at other instars. When terpenoid amount in
160 larval food was calculated as ng/mg, the detectable terpenoid amount changed insignificantly
161 among instars.
162 When comparing the content in larvae and food at the same stage as ng/mg, the larvae had
163 significantly higher terpenoid content than food at the 2nd instar, except α-cedrene, β-cedrene
164 and cedrol. At the 2nd instar, the contents of α-cedrene and β-cedrene were insignificant
165 between larva and food; the content of cedrol was significantly lower in larvae than in food. At
166 the 4th instar, the larvae still had significantly higher content of (*E*)-β-ocimene than food; the
167 content of (*Z*)-β-ocimene was insignificant between larvae and food. As for other terpenoid
168 compounds, larvae had substantially lower contents than food after the 2nd instar.

169 3.1.1. Aldehydes

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170 Aldehydes had higher concentrations in food (25.4–59.6%) than in larvae (7.9–22.3%) when
171 calculated as ng/mg. Nonanal (a2) was the most abundant aldehyde in each sample, accounting
172 for 65.3–93.3% of total aldehydes, followed by decanal (a3) and octanal (a1).
173 Octanal (Figure 3, column a1) was only detected in larvae at the 2nd instar, but was present
174 in food at every stage. Nonanal (Figure 3, colum a2) and decanal (Figure 3, column a3) was
175 detected in both larvae and food at every stage. When calculated by ng/individual larva, the
176 content of each aldehyde and the total content of all aldehydes (Figure 3, column Sa) only
177 changed slightly and non-significantly ($p > 0.05$) with the sharp increase in larval body weight
178 (Figure 2). However, when calculated as ng/mg, both measures were significantly higher in
179 larvae at the 2nd instar than at the other stages. When comparing both measures in larvae and
180 food at the same stage as ng/mg, there was no significant difference at the 2nd instar, while at
181 other stages food contained higher contents than larvae ($p < 0.01$). Trends in aldehyde content in
182 food were similar. There were fluctuations during larval development, but no significant
183 differences occurred.

184 3.1.1. Hydrocarbons

185 The amounts of hydrocarbons to total volatiles content in food were the third highest after
186 terpenoids and aldehydes, when calculated as ng/mg. Pentadecane (Figure 4, column h1) and
187 heptadecane (Figure 4, column h2) were the hydrocarbons that accounted for the major amount
188 (69.7–88.1%).

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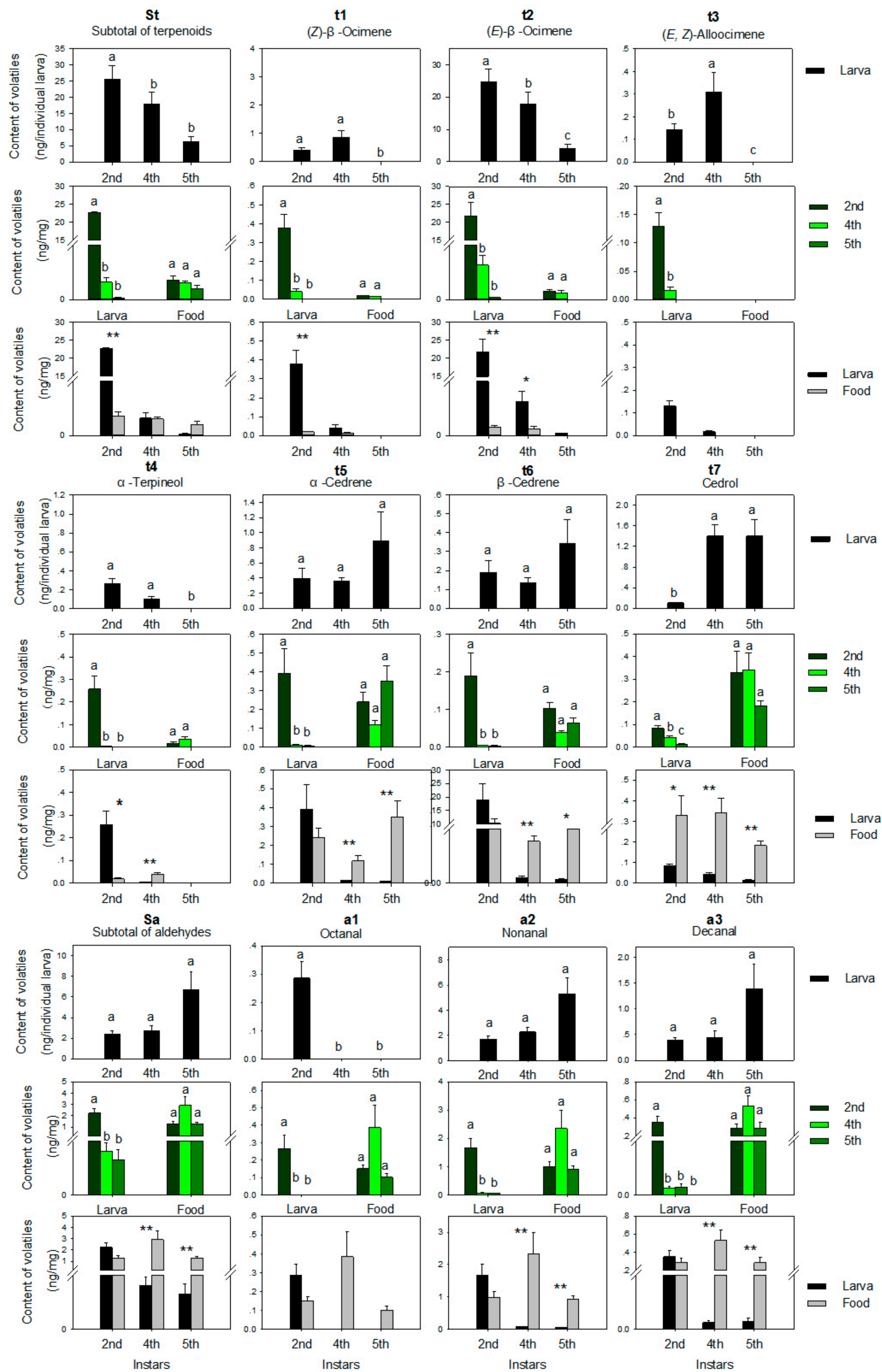

189

190 **Figure 3.** Terpenoids and aldehydes extracted from larvae and larval food at different larval 191 instars. N> 10 for each group; error bars represent standard errors, different letters and '*' on top 192 of bars denote significant difference at the level of 0.05, '**' at the level of 0.01 under Fisher's 193 PLSD test, after ANOVA showed a significant effect. Bars sharing a superscript letter are not 194 significantly different.

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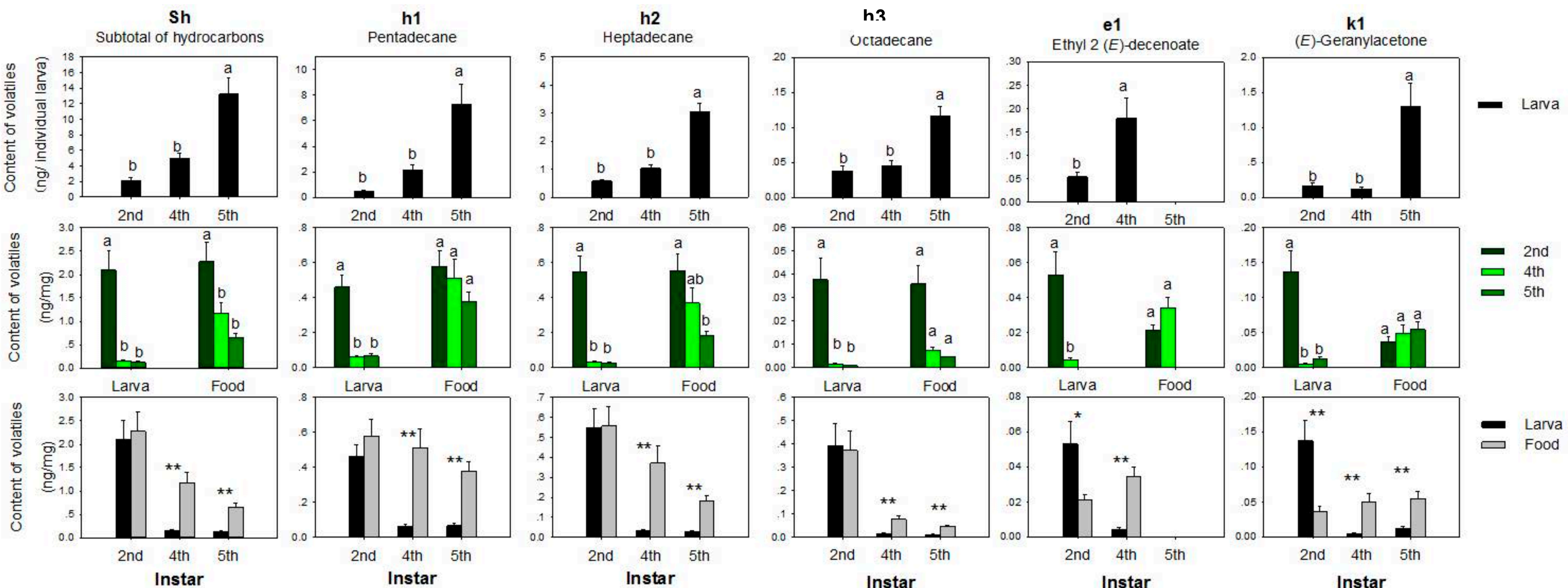

Figure 4. Hydrocarbons and other minor volatiles extracted from larvae and larval food at different larval instars. N> 10 for each group; error bars represent standard errors, different letters and '*' on top of bars denote significant difference at the level of 0.05, '**' at the level of 0.01 under Fisher's PLSD test, after ANOVA showed a significant effect. Bars sharing a superscript letter are not significantly different.

195

3.1.1. Hydrocarbons

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196 The amounts of hydrocarbons to total volatiles content in food were the third highest after terpenoids and aldehydes, when calculated as ng/mg. Pentadecane (Figure 4, column h1) and heptadecane (Figure 4, column h2) were the hydrocarbons that accounted for the major amount (69.7–88.1%).

200 When calculated by ng/ individual larva, the contents of hydrocarbons generally increased during larval development. The 2nd instar larva had the lowest content of each hydrocarbon and the lowest total content of hydrocarbons. However, the situation was reversed when calculating as ng/mg. The 2nd instar larva had significantly higher content than other instars larva, and there is insignificant among other instars. Food at the 2nd instar also had higher content than food at other instars, h2 significantly, h1 and h3 insignificantly. When comparing the content in larvae and food at the same stage by ng/mg, the differences were always significant, except at the 2nd instar. Larvae had substantially lower content than food.

208 3.1.1. Ester

209 Ethyl 2(*E*)-decenoate (Figure 4, column e1) was the only detected ester present before the 5th instar. This ester was significantly higher in 4th than 2nd instar larvae when calculated as ng/ individual larva ($p = 0.02$). The trend was reversed when it was calculated as ng/mg. There were few changes in food of different instars. At the 2nd instar, the larvae had significantly higher content than food, but significantly lower content at the 4th instar.

214 3.1.1. Ketone

215 (*E*)-Geranylacetone (Figure 4, column k1) was the only ketone detected in the materials of this experiment. When calculated as ng/individual larva, the 5th instar larvae had significantly higher content than larvae at other instars, which had comparable levels. When calculated as ng/mg, the 2nd instar larvae had significantly higher content than larvae at other instars. Food of various instars contained comparable levels of (*E*)-geranylacetone. When comparing the content in larvae and food at the same stage as ng/mg, larvae had substantially higher contents than food at the 2nd instar, while the opposite was true at the other stages.

222 *3.2. Principal component analysis*

223 PCA was performed to reveal relationships between the samples (scores) and their volatile compounds (loadings) (Figure 5). A total of 96 samples and 15 compounds were used (Kaiser-Meyer-Olkin Measure of Sampling Adequacy: 0.731; Bartlett's Test of Sphericity: $P < 0.01$). The majority of information is contained in the first two PCs and accounts for 62.2 % of the explained variation. A scatter plot of the PCA scores (Figure 5A) shows the distribution of samples, and the corresponding loading plot (Figure 5B) shows the volatiles in larvae and food at different development stages. Combining these results allows interpreting the relationships between the samples and their compounds.

231 The analyses show clear differences between the volatile profiles of larvae and the volatile profile of food. The two kinds of samples were separated by PC2. The majority of food samples had positive PC2 scores, attributable to the contribution of all aldehydes (a1, a2, a3), hydrocarbons (h1, h2, h3), two of the terpenoids (t5, t7). Meanwhile, all the larvae samples had negative PC2 scores, driven by most of the terpenoids (t1, t2, t3, t4, t6), one ester (e1), one ketone (k1).

237 Samples of the same type were distinguished by PC1 according to development stages. Both larvae and food samples generally shifted from positive to negative along the PC1 axis, from the 2nd to 5th instar. Samples of 2nd larvae located at the fourth quadrant were essentially characterized by the compounds distributed in that quadrant, especially terpenoids. Larvae at other instars were located in the third quadrant, implying that the volatiles of these stages were highly conservative and had relatively low levels. Part t of the 2nd and 4th instars food located at the first quadrant was mainly characterized by the compounds distributed in that quadrant,

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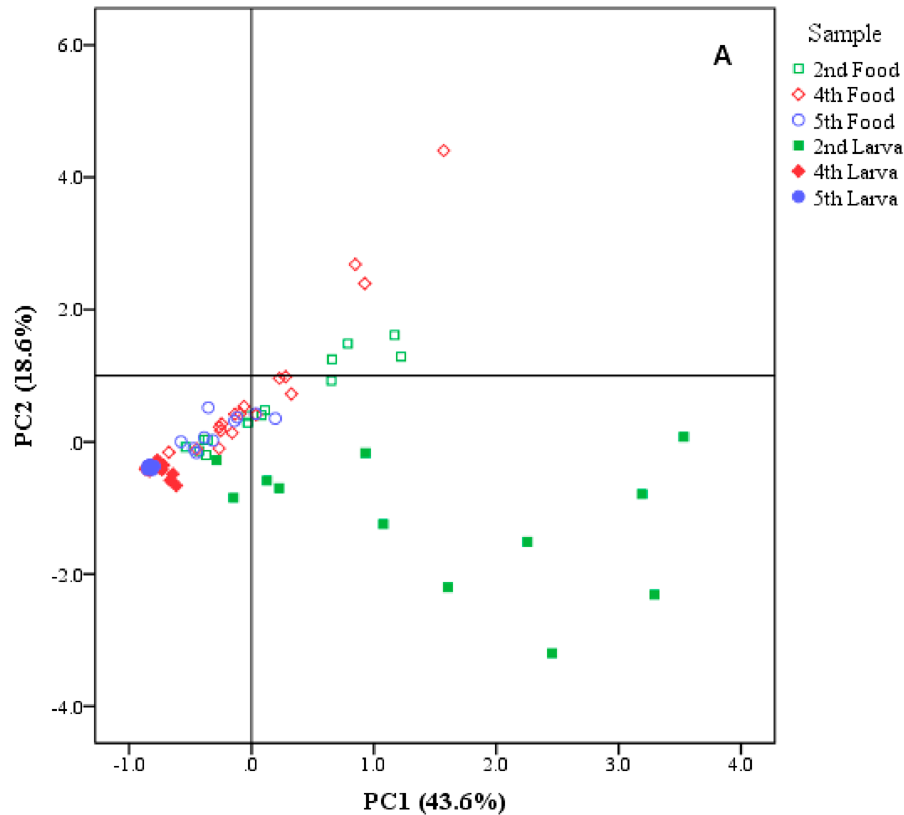
244 especially aldehydes and hydrocarbons. The rest of the food samples were located in the third
245 quadrant, with low PC scores.



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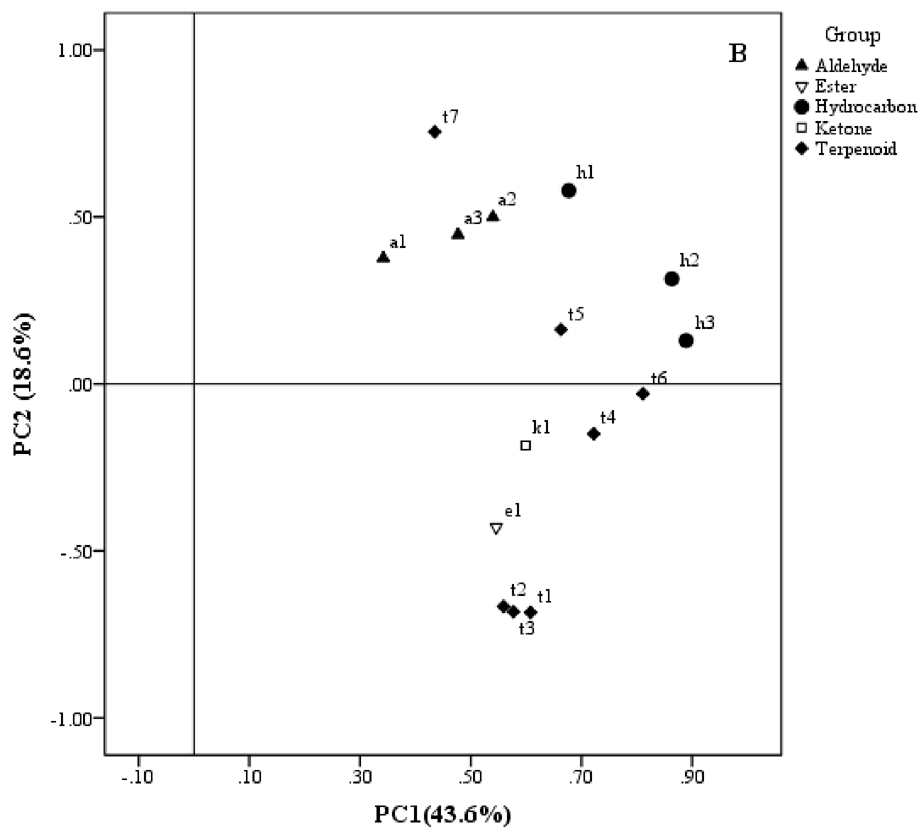

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248 **Figure 5.** Positions of PC scores of samples including larvae and larval food at different larval
249 instars (2nd: 2nd instar, 4th: 4th instar, and 5th: 5th instar). (A): Scores scatter plot of PCA. (B):
250 loadings plot of PCA. The codes in (B) correspond to the compound codes listed in Table 1.

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251 **4. Discussion**

252 Terpenoids have previously been reported as characteristic products of the Nasanov gland
253 of worker bees, and included nerol, geraniol, (*E*) and (*Z*)-citral, nerolic acid, geranic acid and (*E*,
254 *E*)-farnesol [14]. In the present study, we found that additional terpenoids are the major
255 constituents of worker bee larvae. Larva and food at the 2nd instar was demonstrated to have
256 greater similarity of dry substance weight per unit volume, compared to other stages (data not
257 shown). Assuming that larvae and food at the 2nd instar had similar densities, larvae would
258 have released significantly higher amount of terpenoids than food, thus, these terpenoids, such
259 as (*Z*)-β-ocimene (t1), (*E*)-β-ocimene (t2), (*E*, *Z*)-alloocimene (t3) and α-terpineol (t4), were likely
260 produced by the larvae. Some terpene synthases have been identified for larvae [9]. Terpenoid
261 concentration could be diluted in old larvae because body fluid accounts for most of their
262 weight. Under these conditions, terpenoid contents in larvae were still significantly greater than
263 in food, suggesting that compounds such as t1, t2 and t3 could be identified as larval volatiles.
264 t1, t2 and t3 were once reported as the components of Tagetes minuta essential oil, which is
265 highly lethal to *V. destructor* [15]. T4 was demonstrated to have a repellent effect on the mite in a
266 laboratory assay, and may therefore repress its entry into the brood cells of hives [16]. These
267 terpenoids might be noteworthy in *V. destructor* control. Cedrol is a sesquiterpene with tertiary
268 alcohol functionality. This alcohol could easily eliminate to give mixtures of α- and β-cedrene.
269 These sesquiterpene alcohols might be gathered by bees from gum and pollen of *Cedrus deodara*.
270 All aldehydes detected in this study have been previously reported as the volatiles of hives.
271 The aldehydes are emitted by adult worker bees [17] and virgin queens [18], present in enclosed
272 brood combs containing active larvae and attending workers [1]. Our results found that
273 aldehyde contents per unit weight were higher in larval food than in larvae. This supports the
274 assumption that aldehydes are secreted by worker bees, because larval food is a material that is
275 manipulated by worker bees, and is consistent with the findings of Torto et al.[19] who reported
276 these aldehydes in pollen. The result also explains why aldehyde contents in food did not show
277 significant fluctuations during larval development. Aldehyde contents in larvae also remained
278 steady, regardless of larval growth, except that a1 disappeared after the 2nd instar.
279 Hydrocarbons have transpiration-reducing functions in arthropods, including linear and
280 branched, saturated and unsaturated hydrocarbons, with different numbers of carbon atoms
281 ranging from 15 to 35[20]. Some of these compounds function in nestmate recognition and social
282 acceptance [21]. Pentadecane has been reported as a larval volatile [22] and was also detected in
283 nurse bees, coupled with heptadecane [23]. Octadecane is found in comb wax, with worker bees
284 treated with this compound becoming less acceptable to their untreated nestmates [24]. If we
285 assume cuticular hydrocarbons have an even and constant distribution on the surface of larvae,
286 hydrocarbon content should increase with larval volume. A previous report has indicated that
287 the level of tricosane and pentacosane would increase with drone larval development [25]. In
288 the present study, hydrocarbon content increased at different degrees in individual larva during
289 larval development. To some extent, this provides another support for the hypothesis. In food,
290 hydrocarbon content showed different degrees of decrease in per unit weight. This suggests that
291 the production of hydrocarbons in food is independent from that in larvae. In other words,
292 hydrocarbons in food are affected by the food provider, while hydrocarbons in larvae are
293 affected by the larvae themselves.
294 Ethyl 2(*E*)-decenoate is tentatively identified in honeybees for the first time in the present
295 study. Aliphatic ester is another group released by honeybee which is ubiquitous in colonies.
296 Decyl decanoate is secreted by virgin queens from the tergal gland; ethyl oleate is produced by
297 forager bees, suppressing the onset of foraging among younger bees; and ethyl and methyl
298 esters of palmitic, linoleic, linolenic, stearic, and oleic acids are brood pheromones mediating the

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299 communication between brood and worker bees [14]. Ethyl 2(*E*)-decenoate might be a product
300 of incomplete beta oxidation of longer fatty acids.
301 (*E*)-Geranylacetone has not been previously identified as a honeybee volatile.
302 Concentrations of this compound in larvae did not show obvious changes with the growth of
303 individual larva, and only displayed a dramatic increase during the capping stage (the 5th
304 instar). Neither did the significant differences of the contents were found in food at different
305 stages. This may suggest that the compound is released by larvae for a specific role in this
306 particular period.
307 When tracing the roots of the volatiles of worker larvae and larval food, the factor of the
308 adult worker bee also needs to be considered. Additionally, constancy of volatile components in
309 samples from different localities has not yet been established. Finally, only a single extraction
310 method was applied in this study, and use of more advanced extraction or detection methods
311 for worker larvae volatiles might yield additional compounds. The present study should
312 provide some basis for further research into the molecular mechanism of the volatiles along
313 these lines, and for verification of the role of the identified components

314 **5. Conclusions**

315 Our results show that more volatiles could be identified from honeybee worker larvae and
316 their food, in addition to (*E*)-β-ocimene. We provide evidence that these volatiles change and
317 have a set of rules during larvae development.

318 **Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Figure S1: title,
319 Table S1: Linearity of response for standards. Calibration fitting: y=kx+m[a].

Compound	Linear range (ng)	Slope (k, mean±SD[b], n=3)	Intercept (m, mean±SD[b], n=3)	Regression (r^2, n=6)	LOD[c] (ng)	LOQ[d] (ng)	Codes[e] of the compounds calculated by this curve
Aldehyde							
Octanal	0.004-40	0.047±0.0019	0.01±0.0008	0.99982	0.0003	0.0011	a1, a2
Decanal	0.0041-41	0.096±0.0027	0.007±0.0002	0.9974	0.0004	0.0014	a3
Ester							
Methyl tridecanoate	0.0044-44	0.168±0.0047	0.019±0.0041	0.9994	0.00048	0.0016	e1
Hydrocarbon							
Tetradecane	0.0017-17	0.097±0.0061	0.0063±0.00059	0.9997	0.00039	0.0013	h1, h2, h3
Ketone							
6-Methyl-5-hepten-2-one	0.0038-38	0.277±0.0005	0.08±0.0015	0.9992	0.00036	0.0012	k1
Terpenoid							
(E)-β-Ocimene	0.005-50	0.282±0.005	0.080±0.008	0.9993	0.00102	0.0034	t1, t2, t3, t4, t5, t6, t7

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[a] In the regression equation y=kx+m, y refers to the peak area ratio of target compound to internal standard , x is the concentration ratio of target compound to internal standard, r2 is the correlation coefficient of the equation.
[b] Standard deviation is abbreviated as SD.
[c]Limit of detection, S/N= 3
[d] Limit of quantitation, S/N= 10
[e] The codes correspond to the volatile codes listed in Table 1.

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