Optimization of Strawberry Volatile Sampling by Direct Gas Chromatography Olfactometry

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The aim of this work was to determine the most suitable sampling headspace technique for the study of strawberry (Fragaria xananassa Duch.) fruit aroma. The aromatic qualities of different headspace extracts of strawberry (‘Festival’) puree were evaluated using direct gas chromatography-olfactometry (D-GC-O), a technique that allows assessing global odor from solvent-free extracts without chromatographic separation. Two solid phase microextraction (SPME) extracts, with different types of fibers, polydimethylsiloxane/divinylbenzene (PDMS/DVB) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), and two static headspace extracts, with different phase ratios (ratio of gas to sample phase volumes), were evaluated using D-GC-O. A similarity test with three panelists allowed comparison of the odor of these extracts to that of strawberry puree. The results indicated that SPME using a DVB/CAR/PDMS fiber generated the most representative odor, a “green” impression and a strong “fruity” note. A fruity note was also obtained with the 10-mL static headspace extracts, which exhibited a volatile concentration that was higher than that in the 20-mL headspace. When comparing the aroma profile of each extract, the DVB/CAR/PDMS extract showed the highest concentration of methyl butanoate and ethyl butanoate, two key compounds in strawberry aroma.

Strawberry aroma is unique, highly desirable, and depends on many factors such as genetic variability within the species, climatic conditions and postharvest environment. Among the hundreds of volatiles identified in strawberry, esters are known to be important contributors to typical fruity notes (Gomes da Silva and Chaves das Neves, 1999; Pérez et al., 1992; Schieberle and Hofmann, 1997). Furanones are also contributors to aroma, providing sweet caramel-like notes (Larsen and Poll, 1992; Pérez et al., 1996; Sanz et al., 1994). Additionally, aldehydes and alcohols such as hexanal, (E)-2-hexenal are responsible for green and pungent notes (Schieberle and Hofmann, 1997; Schulbach et al., 2004).

Headspace and solvent extraction methods are mostly used to characterize strawberry aroma by sampling and analyzing volatile compounds. Among headspace methods, purge-and-trap technique allowed extraction and quantification of volatile compounds from the whole fruit (Pérez et al., 1992) or fresh strawberry slices (Gomes da Silva and Chaves das Neves, 1999; Shamaila et al., 1992). More recently, solid phase microextraction (SPME), an inexpensive and rapid method, has been widely employed to trap volatiles from whole strawberries (Song et al., 1998) and strawberry puree (de Boishebert et al., 2004; Schulbach et al., 2004; Song et al., 1998; Urruty et al., 2002). Generally, these headspace methods trap most volatiles except compounds with a low vapor pressure. For example, furanones, weakly detected by headspace methods (Pérez et al., 1992), are usually collected by solvent extraction and analyzed by high-pressure liquid chromatography (HPLC) (Pérez et al., 1996; Sanz et al., 1994). This latter technique is also used for thermally sensitive compounds. Nevertheless, headspace sampling remains the best compromise to extract most other volatiles.

All the above-described headspace techniques use a trapping step, which has the potential of creating artifacts or extracting volatiles in a selective manner. By using direct gas chromatography-olfactometry (D-GC-O), a technique developed to assess global odor from solvent-free extracts, Lecanu et al. (2002) found that cheese extracted with a carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber under conditions that had been optimized to obtain a large amount of chromatographic peaks had an overwhelming “garlic” aroma, unlike the odor of the cheese sample. Therefore, these researchers changed the extraction conditions to obtain a headspace extract representative of the cheese sample (Lecanu et al., 2002). Following the same approach, Rega et al. (2003) described in detail orange juice headspace sampling using different SPME fibers and extraction conditions. From these two studies, it appears that the D-GC-O technique is a useful tool to optimize sample extracts for flavor analysis.

The aim of the present work was to choose a suitable headspace technique to study the aroma of strawberry fruit. Two SPME fibers were compared to each other and to the direct headspace technique at different phase ratios. The extraction conditions were set as already published. Different SPME and static headspace extracts from puree were evaluated using D-GC-O to determine the extract giving the “truest” odor of the strawberry puree. The method used to obtain this extract can then be used in future studies involving strawberry aroma.
**Materials and Methods**

**Fruit preparation.** About 10 fruits of ‘Festival’ strawberry cut in halves (~80g) were homogenized for 20 s in a Waring blender. A volume of 2.5 mL of this homogenate was introduced into a 20-mL vial with 3-hexanone as an internal standard (1 µL·L⁻¹) (Sigma-Aldrich, St. Louis, MO). Saturated aqueous CaCl₂ (2.5 mL) was added after 1 min to inhibit enzymatic activity (Buttery, 1993). Therefore, in each 20-mL vial, the phase ratio (ratio of gas to sample volumes) was 3. The same procedure was performed in 10-mL vials with 1.5 mL puree and 1.5 mL CaCl₂ in order to obtain a smaller phase ratio (2.33). The vials were sealed with magnetic crimp caps (Gerstel, Inc., Baltimore, MD) and agitated using a Genie 2 vortex (Scientific Industries, Inc., Bohemia, NY) for several seconds. When all the strawberries were processed, the vials were stored in a freezer at −80 °C until analysis.

**Extraction of volatile compounds.** Only 20-mL vials were used for SPME extracts and two types of fiber were tested: a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (1 cm) and a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (2 cm) (Supelco, Bellefonte, PA). After a sample equilibration time of 10 min at 40 °C in a water-bath, the fiber was exposed to the headspace for 30 min at 40°C (de Boishebert et al., 2004; Schulbach et al., 2004; Urruty et al., 2002). The fiber was then introduced into the injector of the GC-FID for 3 min at 240 °C for desorption of volatiles.

For static headspace extracts, 10-mL and 20-mL vials were used and experiments were performed with an autosampler (MPS2, Gerstel). After an equilibration time of 40 min at 40 °C (same total equilibration time and temperature as with SPME), a gas-syringe removed automatically 2.5 mL of the headspace. The experimental conditions for both techniques (SPME and static headspace) are summarized in Table 1.

**Odor comparison.** A 15-cm deactivated silica column (0.32-mm internal diameter) was installed on a gas chromatograph (GC) (Perkin Elmer, Autosystem XL, Waltham, MA) equipped with a sniffing port (Gerstel) and a FID. This configuration allows for the evaluation of global odor of headspace extracts without first having to perform chromatographic separation. The flow rate of the carrier gas (He) was 18 mL·min⁻¹, and the oven temperature was kept at 70 °C. A similarity test was performed in triplicate for the different extracts with three panelists. The panelist smelled the odor of the extract from the GC sniff port, a task that lasted less than 1 min. Then, the vial was opened and the panelist smelled the sample. The panelist rated the similarity between the two odors using a 10-cm scale ranging from 0 (far from the sample) to 10 (close to the sample).

Once the D-CG-O experiment was completed, a DB5ms capillary column (30 m × 520 µm × 1.00 µm) (Agilent Technologies, Santa Clara, CA) was installed on the GC to record the FID peak areas of the main compounds in the same extracts as sniffed. Oven program was from 40 °C for 5 min to 250 °C for 10 min at 7 °C·min⁻¹. Helium was used as carrier gas at a constant flow (2 mL·min⁻¹).

**Identification of volatile compounds using GC-MS.** Identification of volatile compounds was carried out using an Agilent 6890 GC coupled with a 5973N MS detector (Agilent Technologies). Mass units were monitored from 40 to 250 m/z and ionized at 70 eV. The separation was performed with a DB5ms capillary column (60 m × 250 µm × 1.00 µm) (Agilent Technologies) at a constant flow (2 mL·min⁻¹ He). The oven temperature was held at 40 °C for 5 min followed by a 7 °C·min⁻¹ increase to a final temperature of 250 °C that was held for 10 min. Compounds were first identified by matching mass spectra with library values (NIST/EPA/NIH Mass Spectral Library, Version 2.0d; National Institute of Standards and Technology, Gaithersburg, MD). The identification of volatile compounds was confirmed using retention indices (RI) calculated using retention time data from a series of alkane standards (C₇⁻C₁₇) run under the same chromatographic conditions.

**Statistical analysis.** Sensory and instrumental data were analyzed by analysis of variance (ANOVA) with the XLSTAT software (Addinsoft, Paris, France). Separation of means was performed with the Fisher’s least significant difference (LSD) test with α = 0.05.

**Results and Discussion**

The two-way ANOVA on similarity rates showed a significant “panelist” and “method” effect on the variance but there was no significant panelists x methods interaction (Table 2). This indicates panelists could discriminate between extracts and agreed as to which extract was most similar to the sample, but they tended to use different parts of the scale to rate similarity. Similarity rates for the different extracts ranged from 6.72 to 8.89 and indicated that, overall, the global odor of these extracts was similar to the reference (strawberry homogenate). However, the DVB/CAR/PDMS extracts, which had a strong “fruity” note together with a “green” impression, generated an odor that was most representative of the sample. A fruity note was also obtained from the 10-mL vial headspace extracts, but the overall odor was somehow weaker than with the DVB/CAR/PDMS fiber. The 20-mL vial headspace extracts generated a weak odor and the PDMS/DVB extracts exhibited a strong “green” note, lacking of the “fruity” odor and not representative of the sample.

The DVB/CAR/PDMS extract contained the highest amount of volatiles, followed by the PDMS/DVB, 10-mL vial headspace and 20-mL vial headspace extracts (Fig. 1). The aroma profile of DVB/CAR/PDMS extract was dominated by esters (methyl and ethyl butanoate, methyl hexanoate), which could explain the strong fruity note perceived by the panelists (Table 3). On the contrary, the aroma profile of PDMS/DVB extract was dominated...
by terpenes and γ-decalactone and exhibited the lowest concentration in methyl and ethyl butanoate among the four types of extracts. The imbalance of this volatile profile, with low ester content, explains the low score attributed to this extract. The better ester recovery by the DVB/CAR/PDMS fiber, compared to the PDMS/DVB fiber, could be attributed to two factors: the additional absorbent (carboxen) and a higher absorbing surface due to the length of this fiber (twice as long as the PDMS/DVB fiber). By adding an adsorbent specific to low molecular weight volatiles (carboxen; Supelco, personal communication), and with additional fiber length and absorption surface from the 2-cm fiber, it is very likely that there was less competition on the polar DVB phase, leaving more sorption sites for esters. Song et al. (1998) showed compound partition coefficients varied greatly with different fiber phases, and concluded that the PDMS/DVB fiber was the best compromise for studying the aroma of fruit such as tomato or strawberry. However, when this study was conducted, the DVB/CAR/PDMS fiber had not been developed yet (Supelco, personal communication). When the DVB/CAR/PDMS fiber became available, researchers found it gave a better performance than PDMS/DVB (Rega et al., 2003) or other commercial fibers (Urruty et al., 2002). Our results confirm this observation, not only because the amount of volatiles extracted from the headspace was higher, but also because the volatile profile was the closest to the odor of the strawberry sample.

In the direct headspace technique (i.e., removing an aliquot of the gas phase over the sample) the extract is representative of the headspace composition above the product and is expected to have an odor similar to that of the original product. That is not always the case for SPME methods, which may selectively trap volatile compounds having a strong affinity for the fiber coating (Song et al., 1998). However, the SPME method is very sensitive while the static headspace technique tends to collect only highly volatile compounds. In this study, to make up for the low sensitivity of the headspace technique, two vial volumes were tested in order to evaluate the influence of two different phase ratios, 3 and 2.33 respectively, on the volatile amount in the headspace. The 20-mL headspace extract (the highest phase ratio) exhibited a lower volatile concentration than the 10-mL headspace extract (the lowest phase ratio) (Fig. 1). This result could explain the weak odor perceived by the panelists for the 20-mL headspace extract (Table 3). The odor emerging from 10-mL headspace extract was close to that of strawberry homogenate according to the panelists. The aroma profile of this extract revealed a relatively high ester concentration, thus explaining the sensory result and highlighting the positive effect of a lower phase ratio on volatile amount in the headspace. However, the concentration of terpenes, (E)-2-hexenal and γ-decalactone remained low in this extract. The comparison with the SPME extract (Fig. 2) showed that several compounds were missing, especially less volatile compounds such as hexanoic acid and ethyl hexanoate, both reported as important volatiles in strawberry aroma (Larsen and Poll, 1992; Ulrich et al., 1997). Although a lower phase ratio allowed for an improvement of the method, with an acceptable

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**Table 2. Two-way ANOVA of the similarity test on different extraction methods.**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panelist (P)</td>
<td>2</td>
<td>14.43</td>
<td>7.22</td>
<td>5.90</td>
<td>0.008</td>
</tr>
<tr>
<td>Method (M)</td>
<td>3</td>
<td>26.19</td>
<td>8.73</td>
<td>7.14</td>
<td>0.001</td>
</tr>
<tr>
<td>P × M</td>
<td>6</td>
<td>9.29</td>
<td>1.55</td>
<td>1.27</td>
<td>0.309</td>
</tr>
</tbody>
</table>

DF: degrees of freedom; F: mean squares effect/mean squares error.

**Table 3. Similarity rates obtained from the different sampling methods by a sensory panel of 3 assessors.**

<table>
<thead>
<tr>
<th>Sampling methods</th>
<th>Means</th>
<th>Odor impressions</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVB/CAR/PDMS fiber</td>
<td>8.89 a</td>
<td>green/strong fruity</td>
</tr>
<tr>
<td>10 mL vial - headspace</td>
<td>7.44 ab</td>
<td>green/fruity</td>
</tr>
<tr>
<td>20 mL vial - headspace</td>
<td>6.89 b</td>
<td>weak odor</td>
</tr>
<tr>
<td>PDMS/DVB fiber</td>
<td>6.72 b</td>
<td>strong green/fruity</td>
</tr>
</tbody>
</table>

Means followed by the same letter were not significantly different by the Fisher’s least significant difference test at α = 0.05.

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Fig. 1. Peak area (average of three replicates) of the main volatile compounds identified in ‘Festival’ puree using GC-MS, for each extract. Vertical bars represent standard deviations.
level of esters, the 10-mL direct headspace extract did not provide a complete aroma profile of ‘Festival’ due to the low sensitivity of the method. On the contrary, SPME yielded a more complete mixture of volatile compounds and seemed more appropriate for the study of strawberry aroma. Nevertheless, the choice of the fiber coating remains a critical point because, as shown in Fig. 1, the fiber with PDMS/DVB generated an imbalanced strawberry aroma profile in comparison with the DVB/CAR/PDMS fiber, and that can lead to a misinterpretation of the chemical data. The conditions that were used to extract the samples (equilibration time, extraction time and temperature) were those that had been used by other researchers (de Boishebert et al., 2004; Schulbach et al., 2004; Urruty et al., 2002) and were assumed to have been already optimized.

D-GC-O is a simple technique that can help a flavor researcher optimize sample preparation and extraction by choosing the method with the closest aroma profile to the sample. In this study, D-GC-O allowed us discriminate between four types of headspace extracts and showed the ability of the 2-cm SPME fiber with DVB/CAR/PDMS to produce an odor most representative of the sample. This extraction method will be used in further studies with strawberry aroma analysis.

**Figure 2.** Comparison of aroma profile in SPME extract (DVB/CAR/PDMS) and in headspace extract from 10-mL vials. 1: methyl butanoate; 2: internal standard (3-hexanone); 3: ethyl butanoate; 4: butyl acetate; 5: (E)-2-hexenal; 6: methyl hexanoate; 7: hexanoic acid; 8: ethyl hexanoate; 9: linalool; 10: α-terpineol; 11: γ-decalactone.

**Literature Cited**


