Comparison of Two Methods of Volatile Analysis for Determining the Causes of Off-Odors in White Beet Sugars -- SPME and Headspace

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Abstract

White beet sugars periodically have off-odors, causing them to be rejected by customers. An understanding of the nature and source of the compounds responsible will help in eventually eradicating the problems that cause them. However, determining volatile substances in white sugar is challenging because the amounts present are very small, often in the parts-per-million or even parts-per-billion range. In this study, we describe a set of white beet sugar samples that were received from several locations. Each sugar was given an over-all sensory rating of: 1 = acceptable; 2 = borderline; or 3 = reject, by a sensory panel. The samples were analyzed by two methods of volatile analysis: Solid Phase Micro Extraction (SPME) and headspace analysis. Sample chromatograms were evaluated for compounds at mass to charge ratio (m/z) 60, where volatile fatty acids are found, with the exception of propionic acid. Representative chromatograms illustrating acceptable, borderline, and reject sugars are shown. Samples in the acceptable and borderline categories had lower levels of the volatile fatty acids than did the reject sugars. This was true for both SPME and Headspace. However, it was apparent that SPME was a better technique for volatile analysis.

Introduction

White beet sugars sometimes have off-odors, which not only affect the value of the sugar, but can also result in their rejection by customers, who do not wish to use a malodorous sugar in their product. Among the causes of off-odors are microbial infection, poor beet quality, beet deterioration in storage, improper beet washing, odorants in centrifugal wash water, mother liquor in crystals and acid or alkaline degradation of sugar in processing.
There has been longstanding interest in understanding and correcting the source of these odors, but only in recent years has technology become available that allows for the rapid analysis of trace volatiles in a matrix such as white sugar. Marsilli, et al., (1994) and Godshall, et al., (1995) identified some of the major components responsible for off-odors, using purge and trap procedures and direct thermal desorption. Although many compounds were identified that contributed to the odor of beet sugar, including pleasant odors and off-odors, the major off-odor compounds in crystalline beet sugar were found to be volatile fatty acids (VFA), in particular, butanoic and isovaleric acids. Pihlsgard, et al., (1998, 1999, 2000) combined sensory analysis and headspace analysis to identify volatiles from liquid beet sugars of different purities, and followed the progress of volatiles through processing. Pyrazines, furans and aldehydes were found.

Colonna, et al., (1996) examined factory processing streams, wash water and white sugars using purge and trap and various solid phase adsorbents with solvent desorption. Many volatiles responsible for off-odors were identified in the wash water and condensate, which could be a possible source of contamination. Recently, Batista, et al., (2002) reported on optimization of volatile analysis for semi-quantitative analysis of butanoic acid in cane and beet sugars using solid phase microextraction. It is of interest to note that Marsilli (1994) reported the presence of geosmin in white sugars but no other researchers have found it, although it has been found in wash water (Colonna et al., 1996) and beet peels (Godshall, unreported results) and early in the process but not later (Pihlsgard, et al., 2000). Geosmin is responsible for the earthy odor of sugarbeets.

In this study, two methods for determining the volatiles in white beet sugar were compared. The first method was SPME, an acronym for Solid Phase Micro Extraction. This is a recent technique that utilizes a fiber coated with an adsorbent material that is placed in the headspace of a sample for a specific period of time and temperature. During this time, the volatiles in the headspace are concentrated on the thin film of the fiber. The fiber is subsequently desorbed with heat into a gas chromatograph-mass spectrometer (GC-MS) for separation and identification of the volatiles.

Figure 1 shows a simplified diagram of a SPME apparatus. The second method studied was headspace analysis, which consists of removing a measured volume of the headspace from a sample of sugar and introducing it directly into the GC-MS for sample analysis and data collection. Both methods are simple, inexpensive, rapid, and do not use solvent extraction. Only small amounts of sugar are needed for either analysis and no sample preparation is required.

Figure 1. Diagram of SPME extraction apparatus.
EXPERIMENTAL

A set of white beet sugars was received from seven different locations, with four to seven sugars from each location. Each sample was sensory scored by a SPRI sensory panel, looking at a set of six odor categories. Each sugar was then given an over-all sensory rating as follows: 1 = acceptable; 2 = borderline; 3 = reject. The SPRI results were compared to those of a professional tester from a large confectionery company, and it was noted that SPRI testers tended to be stricter in some instances than the professional tester, rating more sugars as borderline.

The six odor categories were:
1. Sweet aromatic (like flowers or sweet spices)
2. Caramel, browned, chocolate, nutty
3. Barnyard, slightly ammoniacal, meaty
4. Mushroom-like, earthy, beety
5. Sour, fermented, volatile fatty acids, butanoic/isovaleric acids
6. Green

Experimental – SPME

For SPME analysis, approximately 0.75 g of each sugar was weighed into 2-ml vials. Sample vials were placed in a CTC SPME Autosampler (Leap Technologies, Carrboro, NC) and preheated at 65°C for 15 min. The headspace was extracted for 15 min using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS - 50/30 μm film) fiber (Supelco, Bellefonte, PA).

Experimental – Headspace Analysis

For headspace analysis, approximately 5 g of each sugar sample was weighed into 10-ml vials. The samples were placed in the autosampler, heated at 65°C and agitated for 20 min. The vials were then punctured and a measured volume of 2.5 ml of headspace was taken and introduced directly into the injection port of the GC-MS.

GC-MS Conditions

Sample volatiles were desorbed from the SPME fiber for 2 min at 270°C into the injection port of an Agilent 6890 GC equipped with a 5973 MS system (Agilent Technologies, Palo Alto, CA). Helium was utilized as the carrier gas under a constant flow of 36 cm/s through a 30 m, 0.25 μm, DB-5 capillary column (J & W Scientific, Folsom, CA). An initial GC temperature of 50°C was held for 1 minute. The temperature was then increased at 5°C/min to 100°C, then at 15°C/min to 270°C and held 5.67 min.

The mass spectrometer was operated in scan mode from m/z 45 to m/z 350 employing 70 eV electron ionization. Compounds were identified using the Wiley mass spectral library (7th Edition).

The total ion chromatogram (TIC) for each sample was run for both techniques, but this study focused on those compounds with a mass to charge ratio of 60, characteristic of volatile fatty acids, with the exception of propionic acid. Each sample was run in duplicate.
RESULTS AND DISCUSSION

Good profiles of the volatile fatty acids (VFA) were obtained using m/z 60 detection. The total ion chromatogram showed lower levels of other compounds, and many of the compounds previously identified in beet sugar were not picked up. This was felt to be due to the small sample size of 0.75 g for SPME. The normal range of white beet sugar VFA (m/z 60) was observed in most of the sugars. These included acetic, butanoic, isovaleric (3-methyl butanoic acid), pentanoic, hexanoic, heptanoic, octanoic and nonanoic acids. Reject sugars did not appear to contain very much of the higher molecular weight volatile fatty acids (heptanoic, octanoic, nonanoic). This was probably due to the very high levels of acetic, butanoic, and isovaleric acids in those samples which had a saturating effect on the fiber used. The odors associated with the VFA identified in the sugars are shown in Table 1.

Table 1. Volatile fatty acids identified in beet sugar and type of odor. (From Fenaroli’s Handbook (Furia and Bellanca, 1975), Sigma-Aldrich (2003), and personal observation.)

<table>
<thead>
<tr>
<th>Volatile fatty acid (Common name)</th>
<th>Type of odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>Vinegar, sour, acetic</td>
</tr>
<tr>
<td>Butanoic acid (Butyric acid)</td>
<td>Rancid, sour, cheesy</td>
</tr>
<tr>
<td>Isovaleric acid (3-Methylbutyric acid)</td>
<td>Rancid, cheesy, sweaty</td>
</tr>
<tr>
<td>Pentanoic acid (Valeric acid)</td>
<td>Sweaty, rancid</td>
</tr>
<tr>
<td>Hexanoic acid (n-Caproic acid)</td>
<td>Sweaty, rancid, sour, cheesy, fatty</td>
</tr>
<tr>
<td>Heptanoic acid</td>
<td>Disagreeable, rancid, tallow-like</td>
</tr>
<tr>
<td>Octanoic acid (Caprylic acid)</td>
<td>Slightly unpleasant rancid, fruity-acid, oily</td>
</tr>
<tr>
<td>Nonanoic acid (Pelargonic acid)</td>
<td>Fatty, cheesy, waxy</td>
</tr>
</tbody>
</table>

A representative sample of the SPME and headspace chromatograms of an acceptable sugar is shown in Figure 2. The abundance measurements shown on the y-axis are relative measurements of peak intensity and not quantitative measurements. The SPME chromatogram (upper left) showed very good integration for the fatty acid peaks and high abundance readings ranging from under 20,000 to about 140,000. In contrast, the same sugar run by headspace analysis (upper right) had much lower abundance ranges from under 2,000 to a maximum of only about 10,000. There is also a lot of noise along the baseline, which is common when dealing with lower concentrations of compounds. Note also that acetic acid was not present on the headspace chromatograms. MS data was not acquired during the time that acetic acid eluted from the column because it coincided with the elution of the large, interfering air peak obtained from the headspace. The bottom chromatogram in Figure 2 shows an overlay of the SPME and headspace chromatograms for acceptable sample A1; the difference is dramatic.
A representative sample of the SPME and headspace chromatograms of a reject sugar (E1) is shown in Figure 3. The upper left chromatogram shows the SPME analysis. Compared to acceptable sugar A1 (Figure 2), there are much higher abundance levels of acetic, butanoic, and isovaleric acids, ranging as high as 260,000 counts for acetic acid. Reject sugars appeared to have lower concentrations of the higher volatile acids, such as hexanoic and octanoic, but this was attributed to the fact that the fiber was probably saturated by the high levels of acetic, butanoic and isovaleric acids present. Headspace analysis is shown in the upper right of the figure. Although headspace analysis clearly reflected the higher levels of butanoic and isovaleric acids in this sample, the maximum abundance measurement was only about 22,000. The bottom chromatogram in Figure 3 shows the overlay of SPME and headspace, again demonstrating the large difference in sensitivity for the two methods.
Figure 3. Chromatograms of SPME (upper left) and headspace (upper right) of a reject sugar (E2). Bottom - overlay of SPME and headspace chromatograms.

Figure 4 shows a SPME comparison for two acceptable sugars A1, A2), two borderline sugars (B3, D4), and two reject sugars (E1, E2). The reject sugars have much higher levels of acetic, butanoic, and isovaleric acids. Borderline sugar D4 had a high level of acetic acid, but low levels of butanoic and isovaleric acids. In this case, acetic acid could be suspected of helping to move this sugar into the borderline category.

Figure 5 shows a headspace analysis comparison for the same samples as shown in Figure 4. It is evident that headspace analysis tracks a similar trend as SPME, except that the abundance measurements are only about one-tenth that of the SPME analysis. Headspace clearly shows the higher levels of the most objectionable volatile fatty acids, butanoic and isovaleric acids, in the reject sugars.

Figures 6, 7, and 8 compare SPME and headspace analysis of butanoic acid, isovaleric acid and hexanoic acid in the sugars referred to in Figures 4 and 5. In all cases, not only does SPME show much higher area counts for the VFA than does headspace, but SPME also differentiates the sugars much better than headspace does. Butanoic and isovaleric acid are shown to be much higher in the rejected sugars compared to the acceptable and borderline sugars. Hexanoic acid, by contrast, is higher in the acceptable sugars, although the hexanoic acid maximum abundance for all the sugars (10,000 - 40,000) is quite a lot lower than butanoic and isovaleric in most of the sugars.
Figure 4. Comparative abundance of VFA by SPME in five sugars. A1 and A2 = acceptable; B3 and D4 = borderline; E1 and E2 = reject.

Figure 5. Comparative abundance of VFA by headspace in five sugars. A1 and A2 = acceptable; B3 and D4 = borderline; E1 and E2 = reject.
Figure 6. Comparison of butanoic peak area counts by SPME and by headspace in five sugars. A1 and A2 = acceptable; B3 and D4 = borderline; E1 and E2 = reject.

Figure 7. Comparison of butanoic peak area counts by SPME and by headspace in five sugars. A1 and A2 = acceptable; B3 and D4 = borderline; E1 and E2 = reject.
CONCLUSIONS

In conclusion, reject sugars had higher levels of the objectionable volatile fatty acids, butanoic and isovaleric, than did the acceptable or borderline sugars. This was shown to be true for both SPME and headspace. Acetic acid was also much higher in reject sugars, and may serve as a marker for assessing sugars. While headspace analysis showed similar trends to SPME, it was not able to analyze acetic acid because of interference by the air peak, and it was only about one-tenth as sensitive as SPME. Acetic acid is not necessarily always a cause off-odor, but its presence at a high concentration is a good indicator or marker that the other acids will also be present. If, however, there is a sufficiently high concentration of acetic acid, it can produce a strong, sour odor in the sugar. Butanoic and isovaleric acids are responsible for much of the off-odors in white beet sugar, and a rapid method to assess them would be useful to confirm a reject sugar. While quantitation of these acids is desirable, it would not be necessary in a quality control laboratory, as relative abundance could serve as a good indicator. Both SPME and headspace analysis are rapid, environmentally friendly methods. The SPME apparatus is very inexpensive, and given its high level of sensitivity for VFA in beet sugars, would be recommended for quality assessment of beet sugars instead of headspace analysis.
REFERENCES


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