How Low pH Can Intensify β-Damascenone and Dimethyl Trisulfide Production through Beer Aging

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INTRODUCTION

Flavor quality is of major importance to the consumer, but the flavor characteristics of beer appear to deteriorate greatly with time, at a rate depending on the composition of the beer and its storage conditions (notably pH). Prior to identifying the influence of pH on the development of the most intense staling flavors found in aged lager beers, the corresponding key flavor compounds were determined by aroma extract dilution analysis. In addition to trans-2-nonenal, β-damascenone seems at least as important in the flavor of aged beer. Ethyl butyrate, dimethyl trisulfide, 2-acetylpyrazine, 3-(methylthio)-propionaldehyde, 2-methoxypyrazine, maltol, γ-nonalactone, and ethyl cinnamate are also relevant to the sensory profile of aged beer. Upon aging, a beer having a higher pH produces less β-damascenone, because acid-catalyzed glycoside hydrolysis is decreased. On the other hand, it produces more 3-(methylthio)propionaldehyde, owing to Strecker degradation of methionine. Raising the beer pH additionally causes the release of 3-(methylthio)propionaldehyde from sulfitic adducts. These adducts, more stable at a lower pH, protect the aldehyde against premature oxidation to 3-(methylthio)propionic acid, thus making it available for dimethyl trisulfide formation during aging.

KEYWORDS: Dimethyl trisulfide; beer accelerated aging; methional; β-damascenone; GC–olfactometry

EXPERIMENTAL PROCEDURES

Accelerated Aging of Bottled Beer at Various pH Values. Bottles of a commercial lager beer were opened and struck to produce foam. When foam reached the top of the bottle, the bottle was sealed with a silicone top (Vel no. 5). Beer pH was adjusted to 3, 4.2, 5, 6, or 7 by injection of HCl or NaOH with a glass syringe into bottles through the silicone top. The bottles were then crown-sealed and the beers aged at 40 °C for 5 days in a dark room. As far as beer off-flavors are concerned, our experiment indicates that this accelerated aging mimics very well a natural 20 °C storage.

Extraction of Most Aroma Compounds [for GC–Olfactometry and GC-MS Identifications, 3-(Methylthio)propionaldehyde Determination, and Ethyl Butyrate, γ-Nonalactone, and Ethyl Cin...
The ether extract was dried with anhydrous sodium sulfate and then eliminated sugars and other water-soluble substances. Aroma compounds were first rinsed with 100 mL of ultrapure water in order to recover tenoid derivatives. Instead of ether as described above, dichloromethane (25 ppm) was added before transfer of the extract to a chromatographic vial.

Dimethyl Trisulfide Quantification by Dynamic Headspace and GC—Sulfur Chemiluminescence Detection (SCD). Two hundred and fifty milliliters of beer sample was poured into a 500-mL flat-bottom flask fitted with a sintered Drechsel head. The flask was placed in a thermostatic bath maintained at 30 °C. A conditioned Tenax cartridge (90 mg, 25–30 mesh) was fitted to the gas vent branch of the Drechsel head and another attached to the purge unit. Volatiles were purged to the Tenax phase for 10 min with a 30 mL/min nitrogen flow. The Tenax cartridge was then dried using a reversed 15 mL/min nitrogen current for 3 min and transferred to the Chrompack TCT/PTI 4001 GC unit for adsorption. The aroma compounds adsorbed on the Tenax were desorbed, condensed onto a cold trap, and again desorbed from this trap to be injected onto the capillary column. Desorption/injection was carried out in four steps: (1) precolling of the trap [CP-Sil8 CB capillary column, 0.53 mm i.d.; film thickness, 5 μm; the trap was cooled (−95 °C) for 4 min in a stream of liquid nitrogen]; (2) first desorption, Tenax cartridge heated to 230 °C, remaining at this temperature for 10 min with a helium gas flow of 10 mL/min; (3) second desorption, cooling of the cold trap stopped and the surrounding metal capillary immediately heated to 200 °C; (4) Tenax cartridge heated to 275 °C for 45 min, with a 10 mL/min reversed helium flow for reconditioning. GC analyses were carried out on a 50 m × 0.32 mm, WCOT CP-Sil5 CB (Chrompack, Antwerpen, Belgium) capillary column (film thickness, 1.2 μm). The oven temperature, initially kept at 40 °C for 4 min, was programmed to rise from 40 to 132 °C at 2 °C/min and then to 200 °C at 10 °C/min, remaining at the maximum temperature for 15 min thereafter. Helium carrier gas was used at a flow rate of 1.0 mL/min. In the 800 °C combustion chamber of a sulfur chemiluminescence detector (Sievers, model 355 SCD), the air and hydrogen flows were maintained at 40 and 100 mL/min, respectively. A 6 psi air flow was applied in the oxygen generator under vacuum (150–275 Torr obtained with an Edwards oil-sealed RV5 pump).

Degradation of Methionine in a Model Medium through Aging. The pH of a 50 mM phosphate buffer was adjusted to 3, 4.2, 5, 6, or 7. Each sample, maintained under argon, was spiked with 5 ppm of methionine and 2 g/100 g of glucose and kept for 5 days at 40 °C.

Interactions between 3-(Methylthio)propionaldehyde and Sulfites through Aging. The pH of a 50 mM phosphate buffer was adjusted to 3, 4.2, 5, 6, or 7. Each sample, maintained under argon, was spiked with 50 ppm of 3-(methylthio)propionaldehyde and 20 ppm of sodium bisulfite and kept for 5 days at 40 °C. Free sulfur dioxide and total sulfur dioxide were quantified as recommended by the American Society of Brewing Chemists (Methods of Analysis of the American Society of Brewing Chemists, 8th rev. ed.; American Society of Brewing Chemists: St. Paul, MN, 1992.).

RESULTS

GC-O Analyses. To determine aromatic changes in beer caused by aging, a freshly produced lager beer (pH 4.2) was stored for 5 days at 40 °C. Prior to GC-O analysis, the fresh and the 40 °C aged beer were extracted with Amberlite XAD-2 resin (procedure adapted from ref 13).

Beer odor intensities were determined by AEDA, as described by Ulrich and Grosch (10, 11). The dilution factor (FD) was calculated as \(3^n\), \(n\) being the number of 3-fold dilutions required for no odor to be perceived. Many odors were detected in both the fresh and the aged beers. To identify the highly flavor-active compounds in aged beer extracts, we compared the dilution factors of all compounds with that of isoamyl acetate. This ester occurred in aged beer at 1.08 ppm, close to its threshold concentration established as 1.60 ppm by Meilgaard (14).

As its FD value was 9 for the aged beer extract, we considered that flavor-active compounds in aged beer had to have an FD value ≥9 in the absence of synergetic interactions. Only the major storage-induced changes are depicted in Table 1.
As recently described by Lermusieau et al. (13), yeast secondary metabolites, such as ethyl butyrate and β-phenylethanol, are odor-active compounds in fresh beer. Maltol and 2′-aminoacetophenone are two other molecules perceived at the sniffing port; they are mainly derived from malt (Maillard reactions, tryptophan degradation, etc.). Hops are a third potentially significant source of odors in fresh beer extract (e.g., 3-methyl-2-buten-1-thiol, dimethyl trisulfide, and β-damascenone).

The aroma profile perceived at the sniffing port proved to be completely different for the aged beer. Although a series of compounds are inevitably partially lost (such as the hoppy thiol known, however, to be responsible for the skunky off-flavor created in beer during exposure to light), many odorants are perceived more strongly after an accelerated aging in a dark room.

As expected, the role of trans-2-nonenal among the aged beer off-flavors was confirmed by GC-O (FD = 81 as opposed to 9 in the fresh sample). In the past decade, this alkenal has been viewed by many brewers as the key staling compound (3, 15). A low pH is known to enhance this cardboard flavor by causing hydrolysis of the alkenal–protein adducts present in fresh beer (1, 2). Bech and Nyborg (16) detected 0.06 ppb of trans-2-nonenal after aging (10 days at 37 °C) of a pH-unmodified beer (pH 4.2), as opposed to 0.12 ppb at pH 3.8 and 0.03 ppb at pH 4.6.

Although more strongly perceived in the aged beer extract, trans-2-nonenal proved not to be the most interesting compound in our AEDA. Of course, due to possibly different Stevens’s slopes, conclusions about dilution factors must be drawn with caution. Nevertheless, with FD = 243, we can assume that β-damascenone and the unknown compound at RI = 1532 are probably at least as important as trans-2-nonenal. The FD was also significantly increased after aging for ethyl butyrate, dimethyl trisulfide, 2-acetylpyrazine (FD = 81), 3-(methylthio)propionaldehyde, 2-methoxy(pyrazine, maltol, γ-nonalactone, and ethyl cinnamate (FD = 27). As shown in Table 2, quantification of ethyl butyrate, 3-(methylthio)propionaldehyde, dimethyl trisulfide, γ-nonalactone, β-damascenone, and ethyl cinnamate confirmed their formation through aging. Ethyl nicotinate was also easily quantified in beer. Despite a major quantitative increase observed after aging (268 ppb in aged beer vs 92 ppb in fresh beer), ethyl nicotinate should probably not be considered to be flavor-active in aged beer (FD = 3). Other relevant compounds were not quantified in the present study due to the absence of a well-resolved peak in GC-FID or GC-MS. Other methods should be used in these cases after specific optimization.

To assess the impact of pH, three easily quantified compounds were selected as representative of the staling flavors discussed above: β-damascenone, 3-(methylthio)propionaldehyde as a Maillard reaction product, and dimethyl trisulfide.

### Table 1. Odors Detected by GC-O in Fresh and Aged Beers (pH 4.2)

<table>
<thead>
<tr>
<th>RI</th>
<th>individual odors</th>
<th>fresh</th>
<th>aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>774</td>
<td>floral, fruity</td>
<td>27</td>
<td>81</td>
</tr>
<tr>
<td>810</td>
<td>hop, sulfur, onion</td>
<td>729</td>
<td>243</td>
</tr>
<tr>
<td>866</td>
<td>potato</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>896</td>
<td>cereal, roasted</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>959</td>
<td>geranium, earthy, potato</td>
<td>27</td>
<td>81</td>
</tr>
<tr>
<td>1033</td>
<td>sweet, candy floss, caramel</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>1060</td>
<td>caramel, roasted</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>1092</td>
<td>rose, hyacinth, floral</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td>1095</td>
<td>roasted, floral</td>
<td>27</td>
<td>unknown</td>
</tr>
<tr>
<td>1123</td>
<td>butter, popcorn, cardboard</td>
<td>9</td>
<td>81</td>
</tr>
<tr>
<td>1192</td>
<td>soap, solvent</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>1265</td>
<td>honey, hay</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>1325</td>
<td>peach, fruity</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>1372</td>
<td>rhubarb, red fruits, strawberry</td>
<td>9</td>
<td>243</td>
</tr>
<tr>
<td>1426</td>
<td>chestnut, sweet</td>
<td>27</td>
<td>unknown</td>
</tr>
<tr>
<td>1440</td>
<td>fruity, sweet</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>1475</td>
<td>plastic, grilled nuts</td>
<td>81</td>
<td>243</td>
</tr>
<tr>
<td>1532</td>
<td>dentist, smoked, vanilla</td>
<td>9</td>
<td>243</td>
</tr>
</tbody>
</table>

- RI retention index on CP-SiP5 CB.  
- Dilution factors = 3^n (with n = number of dilutions required for no odor to be perceived).  
- Confirmation by GC-MS.  
- Confirmation by standard co-injection.

### Table 2. Concentration, in Fresh and Aged Beer (pH 4.2), of Compounds with FD Values >9 in Aged Beer

<table>
<thead>
<tr>
<th>RI</th>
<th>compound</th>
<th>fresh</th>
<th>aged</th>
</tr>
</thead>
<tbody>
<tr>
<td>774</td>
<td>ethyl butyrate</td>
<td>184</td>
<td>261</td>
</tr>
<tr>
<td>866</td>
<td>3-(methylthio)propionaldehyde</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>959</td>
<td>dimethyl trisulfide</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>1123</td>
<td>trans-2-nonenal</td>
<td>0.05</td>
<td>0.30</td>
</tr>
<tr>
<td>1325</td>
<td>γ-nonalactone</td>
<td>63</td>
<td>77</td>
</tr>
<tr>
<td>1372</td>
<td>β-damascenone</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>1440</td>
<td>ethyl cinnamate</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

- Coefficient of variation between analyses duplicates ±15%.

As pH decreases, the formation, or more accurately, the stability of β-damascenone and 3-(methylthio)propionaldehyde increases. This effect is especially pronounced during aging at lower pH, in agreement with the results of Ougier et al. (18). The effect of pH on the 3-(methylthio)propionaldehyde level of fresh beer is shown in Figure 1. At pH 3.8, the concentration is about 30% of the level found at pH 4.2, while at pH 4.2, the concentration is about 60% of the level found at pH 4.2. The effect of pH on the β-damascenone level of fresh beer is shown in Figure 1. At pH 3.8, the concentration is about 50% of the level found at pH 4.2, while at pH 4.2, the concentration is about 80% of the level found at pH 4.2.
the Amadori compound, a high pH is preferred (18). Further dehydrations lead to diketones used for Strecker degradation of amino acids.

In a lysine−ribose model medium, Meynier and Mottram (19) detected, as might be expected, a higher level of pyrazines as the pH increased. As depicted in Figure 1, the concentration of 3-(methylthio)propionaldehyde in our aged beers also increased with the pH of the fresh beer. Our results were further confirmed in a model medium composed of methionine and glucose: up to 3 ppb was found at pH 7 as opposed to only 1 ppb at pH 3 (Figure 2a).

Effect of pH on the Dimethyl Trisulfide Level in Aged Beer. Although a high pH enhances Strecker degradation of methionine to 3-(methylthio)propionaldehyde (Figure 2a), lower levels of dimethyl trisulfide are detected in aged beer when the pH of the fresh beer is raised (Figure 1). This is very surprising, because 3-(methylthio)propionaldehyde is recognized as the main precursor of dimethyl trisulfide during aging (4). Gijs et al. (20), however, have recently shown that sulfites can significantly enhance dimethyl trisulfide formation by producing adducts with aldehydes. We therefore investigated the impact of pH on the level of free 3-(methylthio)propionaldehyde in a model medium containing sulfites. As depicted in Figure 2b, very little free 3-(methylthio)propionaldehyde was detected at pH 3. Sulfitic adducts, preferably formed at lower pH, protect the aldehyde against premature oxidation to 3-(methylthio)propionic acid, thus making it available for dimethyl trisulfide formation during aging. This higher level of sulfitic adducts may explain the high dimethyl trisulfide concentration found in aged beer.

CONCLUSIONS

We have compared the GC-O profiles of a beer when fresh and after accelerated aging, confirming the role of trans-2-nonenal as a key contributor to the odor variation observed during aging. With FD = 243, β-damascenone and the unknown compound at RI = 1532 are at least as important as trans-2-nonenal. Ethyl butyrate, dimethyl trisulfide, 2-acetylpyrazine, 3-(methylthio)propionaldehyde, 2-methoxypyrazine, maltol, γ-nonalactone, and ethyl cinnamate are also relevant to the odor of aged beer.

When fresh beers at different pH values were aged and analyzed, the β-damascenone concentration was found to decrease with increasing pH. This supports the view that β-damascenone is produced by acid-catalyzed hydrolysis of glycosides during beer aging.
On the other hand, a higher 3-(methylthio)propionaldehyde concentration is measured at high pH. This is partly due to the Strecker degradation of methionine, enhanced by a higher pH, but raising the beer pH also allows release of 3-(methylthio)-propionaldehyde from sulfitic adducts. These adducts, more stable at lower pH, protect the aldehyde against premature oxidation to 3-(methylthio)propionic acid, thus making it available for dimethyl trisulfide formation during aging. Hence, lower dimethyl trisulfide levels are detected in beers aged at higher pH.

LITERATURE CITED


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