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

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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

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 beer's composition and storage conditions (*1*−*4*). The pH appears to be a key factor influencing how beer ages. Optimizing this parameter is not an easy task for the brewer because of its impact at each step of the brewing process (*5*). A lower pH is known to enhance many malt enzyme activities during mashing, to increase the rate of bioconversion of acetolactate to diacetyl, and to limit dimethyl sulfide production during fermentation. On the other hand, increasing the pH of the boiling wort promotes better utilization of hop, a higher α-acid isomerization rate, and more efficient clarification. A higher pH at the end of fermentation also favors ester production (*6*) and yeast flocculation.

As for the effect of pH on beer stability through storage, a lowering of the final pH from 4.5 to 3.9 increases colloid and foam stability, for reasons not fully understood (*5*). Acidity is also known to enhance resistance to microbial spoilage; Simpson (*7*) recently showed that the antimicrobial activity of hop bitter compounds (contributed by uncharged forms) needs a low pH. With regard to beer's organoleptic properties, a higher pH seems to increase palate smoothness and drinkability (*5*). According to Taylor (*8*), when the pH is <4, beers become sharper and more bitter. At a pH <3.7, there is a metallic afterpalate, whereas especially when the pH is > 4.4, soapy and caustic notes are reported. A higher pH leads to comments about mouth coating and "biscuit" or "toasted" flavor. Kaneda et al. (*9*) has mentioned the influence of low pH on protonation of the superoxide radical, leading to the much more damaging perhydroxyl species.

The present paper aims to identify the influence of pH on the development of the most intense staling flavors found in aged lager beers. A first step was to determine key flavor compounds by aroma extract dilution analysis (AEDA), as proposed by Ullrich and Grosch (*10*, *11*). Then the impact of pH was assessed on the compounds quantifiable by usual chromatographic techniques.

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-

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namate Quantification]. Amberlite XAD-2 resin (2 g, maintained in methanol) was thoroughly rinsed with ultrapure water (100 mL) and poured into a 100-mL Schott flask (Vel, Leuven, Belgium) containing 50 mL of beer. This mixture was shaken on a platform shaker at 200 rpm for 2 h at 20 °C. The content of the flask was then transferred to a glass column (60 × 1 cm i.d.) ending with a PTFE tap. The lower part was filled with glass beads (3 g with a diameter of 3.5−4.5 mm and 1 g with a diameter of 0.8−1.2 mm) in order to retain the resin. The resin was first rinsed with 100 mL of ultrapure water in order to eliminate sugars and other water-soluble substances. Aroma compounds were then eluted with 40 mL of ether at a flow rate of 0.75 mL/min. The ether extract was dried with anhydrous sodium sulfate and then concentrated to 0.5 mL. The extract was analyzed by GC-MS and GC−olfactometry (GC-O). For quantification, an external standard (2-methylpentanal, 25 ppm) was added before transfer of the extract to a chromatographic vial.

***β*-Damascenone Extraction.** β-Damascenone was extracted from beer according to the method developed by Guyot et al. (*12*) for carotenoid derivatives. Instead of ether as described above, dichloromethane was used to recover β-damascenone from the XAD-2 resin. To improve quantification, 500 μL of an external standard solution (dodecane, 20 ng/μL in dichloromethane) was added to the Snyder−Kuderna vessel before concentration.

GC-O. For the GC-O analyses, we used a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250 °C, and the split vent was opened 0.5 min postinjection. The carrier gas was helium at a flow rate of 1 mL/min. Compounds were separated on a 50 m × 0.32 mm, wall-coated open-tubular (WCOT) apolar CP-SiP5 CB capillary column (1.2-μm film thickness) connected to a flame ionization detector (FID). The oven temperature was programmed to rise from 36 to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, and to 250 °C at 3 °C/min. The maximum temperature was maintained for 30 min. To assess the olfactory potential of the compounds, a T-junction was used at the end of the capillary column. Fifty percent of the eluent was sent to an FID maintained at 250 °C and connected to a Shimadzu CR6-A integrator, while the other part was directed to a GC-odor port (Chrompack-Varian) at the same temperature as the FID. In the latter case, the eluent was diluted with a large volume of air (20 mL/min) prehumidified with an aqueous copper(II) sulfate solution. A 2-μL aliquot of the beer extract was injected.

GC-MS Identifications. The chromatographic conditions and column were the same as for GC-O. The column was connected directly to an HP 5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV (filament current, 300 mA; electron multiplier voltage, 2500; scan rate, 4 s^{-1}; *m*/*z* range, 40−250). Spectral recording throughout elution was automatic using HP59970C software. Identification was achieved on the basis of peak enhancement by co-injection with authentic standard compounds and by comparison with mass spectra found in the NBS/EPA/NIH mass spectra library.

3-(Methylthio)propionaldehyde Quantification. A recovery factor of 81% was applied for quantification. The GC-MS equipment described above was used with the same column. 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 250 °C at 10 °C/min, remaining at the maximum temperature for 15 min thereafter. Two ions were selected in the selected ion monitoring (SIM) mode [*m*/*z* 104 for 3-(methylthio)propionaldehyde and *m*/*z* 58 for the external standard].

***β*-Damascenone, Ethyl Butyrate, *γ*-Nonalactone, and Ethyl Cinnamate Quantification.** The β-damascenone concentration was calculated from standard addition curves obtained by spiking each beer sample with β-damascenone (0, 10, 50, 100, and 250 ppb in beer). The ethyl butyrate, γ-nonalactone, and ethyl cinnamate concentrations were calculated from standard addition curves obtained by spiking each beer sample with ethyl butyrate (0, 57, 113, and 283 ppb in beer), γ-nonalactone (0, 103, 269, and 517 ppb in beer), and ethyl cinnamate (0, 110, 258, and 515 ppb in beer). Chromatographic separation was performed on a Hewlett-Packard model 5890 gas chromatograph equipped with a Hewlett-Packard model 7673 automatic sampler, a cold on-column injector, an FID, and a Shimadzu CR4-A integrator. Analyses were carried out using the column, carrier gas, and oven temperature described for GC-O. The injector temperature was maintained 3 °C above the oven temperature. The detector temperature was 275 °C.

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 analysis. The aroma 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) precooling 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 ozone 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 20 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 Ullrich and Grosch (*10*, *11*). The dilution factor (FD) was calculated as 3^{n-1}, *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 synergic interactions. Only the major storage-induced changes are depicted in **Table 1**.

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

		dilution factors (AEDA) [b]		
RI[a]	individual odors	fresh	aged	compound
774	floral, fruity	27	81	ethyl butyrate [d]
810	hop, sulfur, onion	729	243	3-methyl-2-butene-1-thiol[d]
866	potato	3	27	3-(methylthio)propionaldehyde[d]
896	cereal, roasted	3	27	2-methoxypyrazine[d]
959	geranium, earthy, potato	27	81	dimethyl trisulfide[d]
1033	sweet, candy floss, caramel		81	2-acetylpyrazine[d]
1060	caramel, roasted	9	27	maltol[d]
1092	rose, hyacinth, floral	81	81	β-phenylethanol[c,d]
1095	roasted, floral		27	unknown
1123	butter, popcorn, cardboard	9	81	*trans*-2-nonenal[d]
1192	soap, solvent	1	27	unknown
1265	honey, hay	27	27	2′-aminoacetophenone[d]
1325	peach, fruity	3	27	γ-nonalactone[c,d]
1372	rhubarb, red fruits, strawberry	9	243	β-damascenone[d]
1426	chestnut, sweet		27	unknown
1440	fruity, sweet	1	27	ethyl cinnamate[d]
1475	plastic, grilled nuts		81	unknown
1532	dentist, smoked, vanilla	9	243	unknown

[a] RI, retention index on CP-SiP5 CB. [b] Dilution factors = 3^{n-1} (with n = number of dilutions required for no odor to be perceived). [c] Confirmation by GC-MS. [d] Confirmation by standard co-injection.

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-butene-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-methoxypyrazine, 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.

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

		concn[a] (ppb)	
RI	compound	fresh	aged
774	ethyl butyrate	184	261
866	3-(methylthio)propionaldehyde	11	45
959	dimethyl trisulfide	0.16	0.32
1123	*trans*-2-nonenal	0.05	0.30
1325	γ-nonalactone	63	77
1372	β-damascenone	3	9
1440	ethyl cinnamate	3	14

[a] Coefficient of variation between analyses duplicates ≤15%.

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.

Effect of pH on the β-Damascenone Level of Aged Beer. Chevance and co-workers (*17*) suggest that the increase in β-damascenone during beer aging could be partially due to acid-catalyzed hydrolysis of glycosides present in fresh beer.

The effect of pH on the β-damascenone concentration was investigated in lager beers aged for 5 days at 40 °C (**Figure 1**). The higher the pH of the fresh beer, the lower the concentration of this compound after aging, especially in beers in which the pH was initially adjusted to 3 and 4.2. When the pH was higher, the β-damascenone concentration tended toward the level found in fresh beer. These results support the view that β-damascenone is produced in the bottle by acid hydrolysis of precursors, especially when the pH is <4.2. Taking into account that most commercial beers have a pH range between 3.8 and 4.5, our results show how crucial could be a slight pH adjustment of the final beer.

Effect of pH on the 3-(Methylthio)propionaldehyde Level in Aged Beer. 3-(Methylthio)propionaldehyde is produced by Strecker degradation of methionine. For 2,3-isomerization of

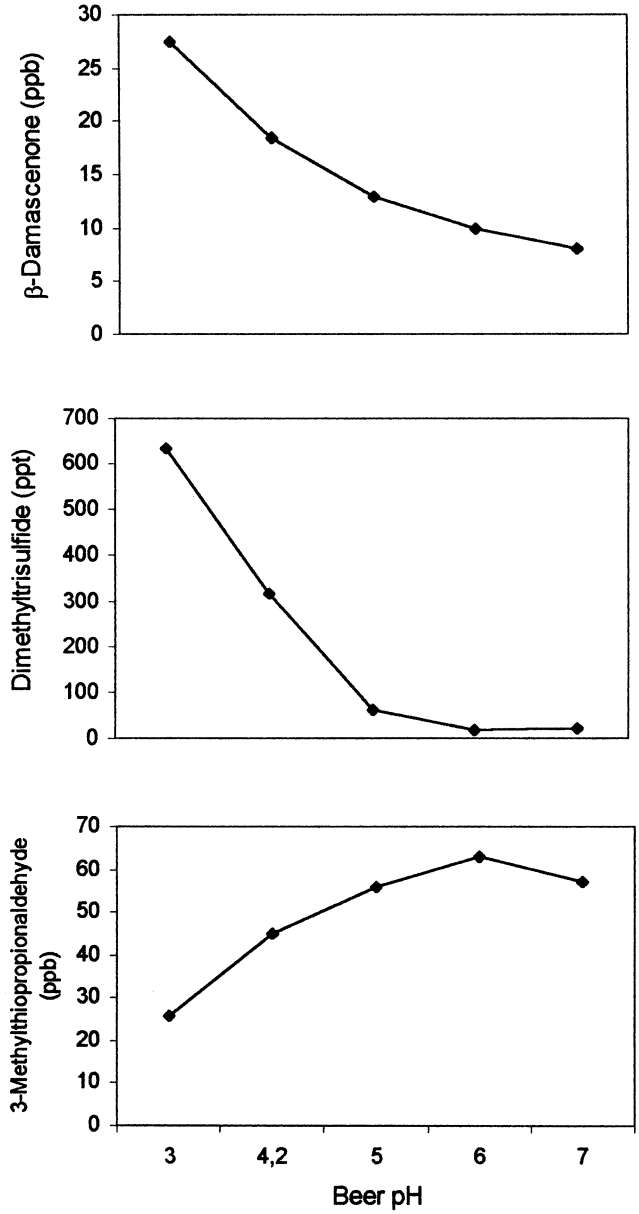

Figure 1. Concentration of β-damascenone (ppb), dimethyl trisulfide (ppt), and 3-(methylthio)propionaldehyde (ppb) after accelerated aging (5 days at 40 °C) of beers when the pH was adjusted to 3, 4.2, 5, 6, or 7.

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

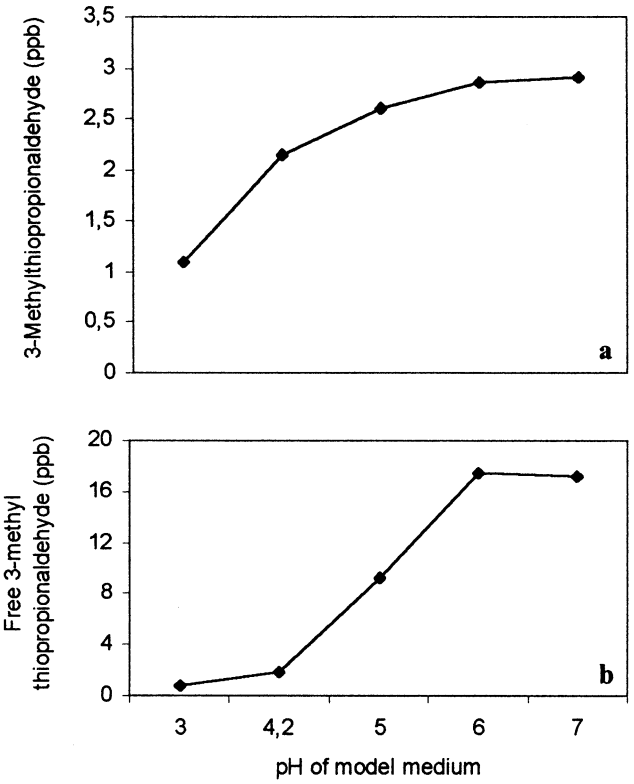

Figure 2. (a) Concentration of 3-(methylthio)propionaldehyde (ppb) produced during accelerated aging (5 days at 40 °C) of phosphate buffer (50 mM) at various pH values (3, 4.2, 5, 6, and 7) spiked with methionine (5 ppm) and glucose (2% w/w). (b) Concentration of free 3-(methylthio)-propionaldehyde (ppb) after accelerated aging (5 days at 40 °C) of a phosphate buffer (50 mM) adjusted to various pH values (3, 4.2, 5, 6, and 7) and spiked with 3-(methylthio)propionaldehyde (20 ppm) and sodium bisulfite (20 ppm).

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.

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