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Effect of the Reducing Power of a Beer on Dimethyltrisulfide Production During Aging

Laurence Gijs and Sonia Collin,[1] *Unité de Brasserie et des Industries Alimentaires, Faculté d'Ingénierie biologique, agronomique et environnementale, Université catholique de Louvain, Croix du Sud, 2 bte 7, B-1348 Louvain-la-Neuve, Belgium*

ABSTRACT

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In synthetic matrices, degradation of 3-methylthiopropionaldehyde, the main precursor of dimethyltrisulfide in aged beer, is influenced by the presence of anti- and prooxidants. Surprisingly, the reducing power of the beer proved not to be a key determinant of dimethyltrisulfide production during beer aging. However, other properties of anti- and prooxidants must be taken into account, such as the ability to bind 3-methylthiopropionaldehyde (sulfitic adducts) or methanethiol (copper complexation). This binding leads, respectively, to a higher or lower dimethyltrisulfide level.

Keywords: Beer aging, Methional

RESUMEN

Efecto del poder reductor de una cerveza sobre la producción de dimetiltrisulfuro durante el envejecimiento

En matrices sintéticos, la degradación de 3-metiltiopropionaldehido (el precursor principal de dimetiltrisulfuro en cerveza envejecida) es influenciada por la presencia de anti- y prooxidantes. Sorprendentemente, se determinó que el poder reductor de la cerveza no es un factor clave en la producción de dimetiltrisulfuro durante el envejecimiento de la cerveza. Sin embargo, hay que tomar en cuenta otras propiedades de los anti- y prooxidantes, tales como su habilidad de enlazarse con 3-metiltiopropionaldehido (aducciones sulfíticos) o metanotiol (complexación con cobre). Estos tipos de enlace conducen a mayores o menores niveles de dimetiltrisulfuro, respectivamente.

Palabras claves: Envejecimiento de cerveza, Metional

S-Methylcysteine sulfoxide in hops is the postulated precursor of dimethyltrisulfide (DMTS) in fresh beers (6), but Gijs and coworkers (3) have recently proposed two additional DMTS sources in aged beers: 3-methylthiopropionaldehyde and its reduced form, 3-methylthiopropanol. These results suggest that the 3-methylthiopropionaldehyde level in the cold wort is the best indicator of the rate of DMTS synthesis during aging. Analogous to the well-known nonenal potential, it was defined as the DMTS potential (2).

Hydroxyl radicals, produced either with copper (II) and ascorbic acid or iron (II) and hydrogen peroxide, can favor decomposition of 3-methylthiopropionaldehyde, mainly when the pH is above 6 (5,8). Wainwright and coworkers (9) also reported that iron and metabisulfite increase the methanethiol and propenal levels when the pH is around 3. The aim of the present work was to assess the influence of the reducing power of wort or beer on DMTS production during beer aging.

[1] Corresponding author. Fax: +32-10-47 21 78; E-mail: collin@inbr.ucl.ac.be

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EXPERIMENTAL

Chemicals

Ethylmethylsulfide (99%) and 2-methylpentanal were purchased from Aldrich Chemicals (Bornem, Belgium), and L-(+)-ascorbic acid was supplied by Acros (Geel, Belgium). Absolute ethanol p.a. was obtained from Merck (Overijse, Belgium) and potassium metabisulfite, from UCB (Brussels, Belgium).

Beer Production

A control beer was produced with pale malt (2.8°EBC). For two other productions, 50 ppm of SO_2 or 25 ppm of ascorbic acid were added during mashing in. Mashings were performed in a 30-L mash tun (Biostat U, B. Braun, Vil Voorde, Belgium) with 8.5 kg of malt flour and 21.25 L of water (Millipore, containing 35 ppm $CaSO_4 \cdot 2H_2O$, 10 ppm $MgCl_2 \cdot 6H_2O$, and 30 ppm NaCl). The temperature regime applied was 50°C for 30 min, to 63°C at 1.3 degrees C per minute, 63°C for 30 min, to 72°C at 0.6 degrees C per minute, and 72°C for 30 min. The temperature was then raised to 80°C at 0.6 degrees C per minute, and the wort was filtered with a "2001 filter" (Meura, Louvain-la-Neuve, Belgium). The gravity of the filtered wort was adjusted to 12°P with mashing water and boiled for 75 min. After 20 min of clarification, the trub was eliminated by filtration, and 0.3 ppm of $ZnCl_2$ was added to the wort. Fermentation was conducted in 3-L EBC tubes with an ale yeast (*Saccharomyces cerevisiae*, pitching rate: 7.5×10^6 cells per milliliter) at 20°C for seven days and 7°C for seven days. Accelerated aging was carried out at 40°C for five days.

Accelerated Aging of Bottled Beers Spiked with Copper

Copper II ($CuCl_2 \cdot 2H_2O$, 10 m*M*) and ascorbic acid (10 m*M*) were injected into bottled commercial lager beers. The bottles were then closed with a silicone top (No. 5, Vel, Leuven, Belgium), crown sealed, and aged at 40°C for five days in a dark room.

Polysulfide Quantification by Dynamic Headspace and Gas Chromatography-Sulfur Chemiluminescence Detection

Extraction method. Sample (250 ml) was poured into a 500-ml flat-bottomed flask fitted with a sintered Drechsel head. The flask was placed in a thermostatic bath maintained at 30°C. A Tenax cartridge (90 mg, 25–30 mesh; Chrompack, Sint-Katelijne-Waver, Belgium) was fitted to the gas vent branch of the Drechsel head, and another was 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 an inversed 15-ml/min nitrogen current for 3 min and transferred to the gas chromatographic (GC) unit (TCT/PTI 4001, Chrompack, Antwerp, Belgium) for analysis.

Desorption/injection was carried out in four steps: 1) precooling of the trap (CP-Si18 CB [Chrompack] capillary column, 0.53-mm i.d.; film thickness, 5 µm): the trap was cooled (–95°C) for 4 min in a steam of liquid nitrogen; 2) first desorption: the Tenax cartridge was 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 was stopped, and the surrounding metal capillary was immediately heated to 200°C; and 4) reconditioning: the Tenax cartridge was heated to 275°C for 45 min, with a 10 ml/min inversed helium flow for reconditioning.

Gas chromatography analytical conditions. GC analyses were carried out on a 50-m × 0.32-mm, wall-coated open tubular (WCOT) CP-Si15 CB (Chrompack,) 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 degrees C per minute then to 200°C at 10 degrees C per minute, 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 (model 355, Sievers, Boulder, CO), the air and hydrogen flows were maintained at 40 and 100 ml/min, respectively. A 6-psi airflow was applied in the ozone generator under a vacuum (150–275 torr obtained by an Edwards oil-sealed RV5 pump; Sievers).

3-Methylthiopropionaldehyde Quantification by Liquid/Liquid Extraction and Gas Chromatography-Flame Ionization Detection

Extraction method. Each 25-ml sample was extracted in the dark and under stirring with, successively, 20, 15, and 15 ml of bidistilled dichloromethane. The organic phase was concentrated to 0.5 ml in a Kuderna Danish vessel. External standard (25 ppm) was added before transferring the extract to a chromatographic vial.

Gas chromatography analytical conditions. A gas chromatograph (model 5890, Hewlett-Packard, Golden, CO) equipped with an automatic sampler (model 7673, Hewlett-Packard), a cold on-column injector, a flame ionization detector, and an integrator (CR3A, Shimadzu, Kyoto, Japan), was used.

Analyses were made on a 50-m × 0.32-mm WCOT CP-SIL5 CB capillary column (film thickness, 1.2 µm). The oven temperature was programmed to rise from 36 to 50°C at 20 degrees C per minute, then to 200°C at 1 degree C per minute and to 250°C at 20 degrees C per minute. The carrier gas was helium at a flow rate of 1.5 ml/min. The injector temperature was maintained at 3 degrees C above the oven temperature. The detector temperature was 275°C.

RESULTS AND DISCUSSION

Impact of Sulfite

The favorable influence of sulfites on the *trans*-2-nonenal level in aged beer is well known. They act either by masking aldehydes in the final product or as antioxidants during mashing or boiling (4). Unknown, however, is the effect of sulfites on the 3-methylthiopropionaldehyde content of wort and subsequent production of DMTS during aging.

Figure 1 shows the evolution of 3-methylthiopropionaldehyde when sulfite (50 ppm) is added during the mashing-in. The first difference between this production and the sulfite-free reference appeared at the filtration (wort separation) step (80°C). In the control, as is the case for 3- and 2-methylbutanal (1), 3-methylthiopropionaldehyde is produced in the 2001 filter by Maillard reactions. More chemical synthesis occurs during boiling. Compared to 3- and 2-methylbutanal, evaporation losses are reduced due to the compound's lower volatility (7). In the presence of sulfites, which mask the reducing sugars and diketone precursors in the wort, Strecker degradation of methionine is inhibited (Fig. 2; mainly in the spent grains and in the kettle). Hence, a very low DMTS potential characterized our production with added sulfite.

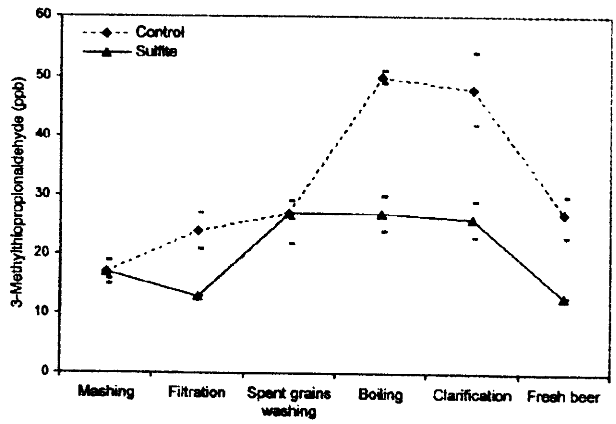

Fig. 1. Evolution of 3-methylthiopropionaldehyde during beer production with addition of 50 ppm sulfite at the mashing-in. Comparison with a control production.

During fermentation, as expected, 3-methylthiopropionaldehyde reduction was drastic. Surprisingly, however, much higher amounts of DMTS were measured in the aged beer despite its low DMTS potential (Table I).

Therefore, we suggest that sulfites act, here, through their capacity to bind 3-methylthiopropionaldehyde (Fig. 3a). This protection could avoid its "premature" oxidation to 3-methylthiopropionic acid (Fig. 3c). The gradual release of methanethiol (Fig. 3b) during aging would then increase polysulfide synthesis.

To confirm this hypothesis, we added 10 ppm sulfite directly to fresh commercial beer before accelerated aging. The consequence of this spiking was again an increase in the concentration of DMTS in the aged beer (151 ppt in the control and 264 ppt in the beer containing sulfite). The sulfites again appeared to protect 3-methylthiopropionaldehyde from oxidation, making it more available for methanethiol production. It should further be determined, however, whether 3-methylthiopropionaldehyde in its bound form can release methanethiol (Fig. 3d) without losing the sulfite function.

Impact of Ascorbic Acid

Addition of 20 ppm ascorbic acid during mashing-in leads to amounts of DMTS in the aged beer similar to those found in the

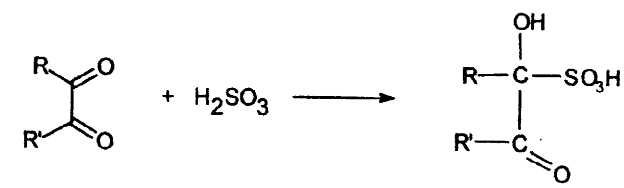

Fig. 2. Reaction between sulfite and diketone or a reducing sugar.

TABLE I
Dimethyltrisulfide Concentration in Aged Beers from the Control Production and the Production with Sulfite or Ascorbic Acid Added at Mashing-In

Production	Dimethyltrisulfide (ppt) in Aged Beer: Average	Dimethyltrisulfide (ppt) in Aged Beer: Results of Two Trials
Control	98	98
		98
With 50 ppm of SO_2	321	309
		332
With 20 ppm of ascorbic acid	104	99
		108

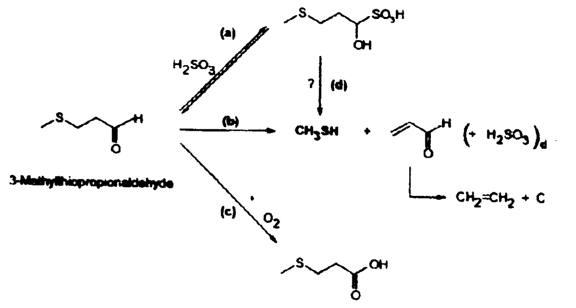

Fig. 3. Hypothetical degradations of 3-methylthiopropionaldehyde.

TABLE II
Dimethyltrisulfide Concentration in Aged Beers (5 days at 40°C) Spiked or Not with Copper II and Ascorbic Acid Before Aging

Beer	Dimethyltrisulfide (ppt) Average	Results of Two Trials
Control	49	47
		50
With copper II (10 m*M*) and ascorbic acid (10 m*M*)	0	0
		0

aged control (Table I). The reducing power of the wort thus appears not to be a key parameter for controlling the polysulfide content.

Impact of Copper

Copper I is known to generate hydroxyl radicals from hydrogen peroxide through the Fenton reaction:

$$2\,Cu^+ + 2\,H^+ + O_2 \dashrightarrow 2\,Cu^{++} + H_2O_2$$

$$H_2O_2 + Cu^+ \rightarrow OH^{\cdot} + OH^- + Cu^{++}.$$

Therefore, as previously shown by Lieberman and coworkers (4) with copper II and ascorbic acid (the source of copper I) in an aqueous model medium, we could suspect that copper would increase radical degradation of 3-methylthiopropionaldehyde to ethylene.

To assess the impact of copper I on beer aging, we applied accelerated aging to commercial beers spiked with copper II (10 m*M* $CuCl_2$) and ascorbic acid (10 m*M*).

Surprisingly, despite its prooxidant properties, copper completely inhibited DMTS synthesis (Table II). Again, this experiment confirms how slightly the reducing power of the medium influences polysulfide synthesis. In our spiked beer, of course, copper did strongly modify the flavor composition, but its ability to complex methanethiol or hydrogen sulfide is clearly the property to be taken into account to explain our results.

CONCLUSIONS

Surprisingly, the reducing power of the medium proves not to be a key determinant of DMTS production during beer aging. Sulfites protect 3-methylthiopropionaldehyde against "premature" oxidation to 3-methylthiopropionic acid through their capacity to bind the aldehyde, thus making it available for methanethiol release during aging. Addition of ascorbic acid during the mashing-in does not affect the DMTS potential. Despite its prooxidant properties, copper completely inhibits DMTS synthesis, probably through its ability to complex methanethiol and hydrogen sulfide.

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