

3-Methylthiopropionaldehyde as Precursor of Dimethyl Trisulfide in Aged Beers

Laurence Gijs, Philippe Perpète, Aurore Timmermans, and Sonia Collin*

Unité de Brasserie et des Industries Alimentaires, Faculté des Sciences Agronomiques, Université catholique de Louvain, Croix du Sud, 2 bte 7, B-1348 Louvain-la-Neuve, Belgium

Hop *S*-methylcysteine sulfoxide has previously been postulated as the precursor of dimethyl trisulfide (DMTS) in beers. The present data point to 3-methylthiopropionaldehyde, the Strecker aldehyde issued from methionine, as another potential precursor in aged beers. Spiking either fresh beer or wort before boiling leads in all cases to higher levels of DMTS after storage. Moreover, special malts with a high level of 3-methylthiopropionaldehyde also favor polysulfide synthesis. A higher pH should increase this onion-like off-flavor, whereas a low pH is unfortunately known to enhance the cardboard flavor of aged beers. 3-Methylthiopropional, issued from yeast reducing activity, can be considered as an additional DMTS source during aging.

Keywords: *Dimethyl trisulfide; beer aging; methional; special malts*

INTRODUCTION

Dimethyl trisulfide (DMTS) is a key flavor in many food products such as cognac, whiskey, wine, and beer (Williams and Gracey, 1982a,b; Nedjma and Hoffmann, 1996). Its flavor threshold is extremely low [e.g., 0.1 ppb in beer; Peppard, 1978; Soltoft, 1985; BE-GC-LOADS (best estimated-GC-lower amount detected by sniffing) = 1.4 ng; Gijs et al., 2000]. In a synthetic matrix, its odor is fresh-onion-like (Soltoft, 1985). Although close to its threshold value in fresh beer, the DMTS level can be much higher after storage (e.g., 2.5 ppb after 4 days at 45 °C; Williams and Gracey, 1982a), enough to impart a characteristic off-flavor to aged beer.

In beer, polysulfides may derive from methanethiol either by direct oxidation or through reactions with hydrogen sulfide (Yu and Ho, 1995; Nedjma and Hoffmann, 1996; Prentice et al., 1998). 3-Methylthiopropionaldehyde is usually considered to be the main precursor of methanethiol. After Strecker degradation of methionine, 3-methylthiopropionaldehyde decomposes easily to thiol and acrolein (Ballance, 1961), especially when the pH is high (Chan and Reineccius, 1994; Meynier and Mottram, 1994). As shown with copper(II) and ascorbic acid or iron(II) and hydrogen peroxide (Lieberman et al., 1965; Pryor and Tang, 1978), hydroxyl radicals can also favor decomposition of 3-methylthiopropionaldehyde, mainly when the pH is >6. On the other hand, Wainwright et al. (1972) report that iron and metabisulfite should increase the methanethiol and propenal levels when the pH is ~3. Provided the pH is not close to 8.5, 3-methylthiopropionaldehyde might also yield methanethiol in the presence of riboflavine and light (Patton, 1954; Yang et al., 1967; Allen and Parks, 1975) through transfer of one electron from the sulfur atom to the photoactivated flavinyl moiety (Yang et al., 1972).

In the absence of methanethiol, the reaction between methanesulfenic acid and hydrogen sulfide is another way to synthesize DMTS (Peppard, 1978). Methane-

sulfenic acid is formed by β -elimination from *S*-methylcysteine sulfoxide as an unstable intermediate. This pathway was evidenced in fresh beers by Peppard (1978) when using (in the brewkettle) non-sulfur-dioxide-treated hops or sulfur-dioxide-treated hops stored for a long time. A similar reaction can also occur with methionine sulfoxide (Yu and Ho, 1995).

As beers usually contain a few parts per billion of 3-methylthiopropionaldehyde, it appeared to be of interest to determine whether this compound might be another significant source of polysulfides in aged beers, yielding them through degradation and subsequent oxidation. Moreover, as large amounts of 3-methylthiopropionaldehyde are reduced by yeast during fermentation, 3-methylthiopropional was investigated as a third potential precursor.

EXPERIMENTAL PROCEDURES

Chemicals. Ethyl methyl sulfide (99%) was purchased from Aldrich Chemicals (Bornem, Belgium). 3-Methylthiopropionaldehyde (95%) and 3-methylthiopropional (95%) were supplied by Acros (Geel, Belgium). Absolute ethanol (for analysis) was obtained from Merck (Overijse, Belgium).

Accelerated Aging of Bottled Beers Spiked with 3-Methylthiopropionaldehyde or 3-Methylthiopropional. Twenty microliters of ethanol (control) or of an ethanolic solution (1000 ppm of 3-methylthiopropionaldehyde or 3-methylthiopropional) was injected with a glass syringe into bottled commercial lager beers through a silicone top (Vel no. 5). The bottles were then crown-sealed and the beers aged at 40 °C for 5 days in a dark room.

Boiling with Addition of 3-Methylthiopropionaldehyde. 3-Methylthiopropionaldehyde [0 (control), 1, or 10 ppm] was added to an industrial 12 °Plato wort (15 L) 30 min after the wort began to boil (total boiling time = 1.25 h). Fermentations were conducted in duplicate in 3-L EBC tubes with a lager yeast (*Saccharomyces carlsbergensis*, pitching rate = 15×10^6 cells/mL) at 12 °C for 10 days and at 3 °C for 10 days. Yeast cells were removed from beer by centrifugation (10000 rpm, 10 min). Accelerated aging was carried out at 40 °C for 5 days.

Beer Production. A control beer was produced with pale malt (2.8 °EBC) and a special one with 10% caramel malt (152

* Author to whom correspondence should be addressed [e-mail collin@inbr.ucl.ac.be; fax +32-(0)10-47 21 78].

°EBC). Mashings were performed in a 30-L mash tun (Biostat U) with 8.5 kg of malt flour and 21.25 L of water (Millipore water containing 35 ppm of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 10 ppm of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and 30 ppm of NaCl). The temperature regime applied was 50 °C for 30 min, to 63 °C at 1.3 °C/min, 63 °C for 30 min, to 72 °C at 0.6 °C/min, and 72 °C for 30 min. The temperature was then raised to 80 °C at 0.6 °C/min, and the wort was filtered with a "filtre 2001" (Meura, Belgium). The gravity of the filtered wort was adjusted to 12 °Plato with mashing water, and the wort was boiled for 1.25 h. 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/mL) at 20 °C for 7 days and at 7 °C for 7 days. Yeast cells were removed from beer by continuous centrifugation (15000 rpm; Contifuge 17RS, Heraeus Sepatech). Accelerated aging was carried out at 40 °C for 5 days.

Polysulfide Quantification by Dynamic Headspace and GC-SCD. *Extraction Method.* Two hundred and fifty milliliters of 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 Tenax cartridge (90 mg, 25–30 mesh) was fitted to the gas vent branch of the Drechsel head, 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 flow for 3 min and transferred to the Chrompack TCT/PTI 4001 GC unit for analysis. Desorption/injection was carried out in four steps: (1) The trap was precooled [CP-Sil8 CB capillary column, 0.53-mm internal diameter; film thickness = 5 μm ; the trap was cooled (–95 °C) for 4 min in a steam of liquid nitrogen]. (2) The first desorption was performed by heating the Tenax cartridge to 230 °C; the cartridge remained at this temperature for 10 min with a helium gas flow of 10 mL/min. (3) The second desorption was performed by stopping the cooling of the cold trap, and the surrounding metal capillary was immediately heated to 200 °C. (4) The Tenax cartridge was heated to 275 °C for 45 min, with a 10 mL/min reversed helium flow for reconditioning.

Gas Chromatography Analytical Conditions. GC analyses were carried out on a 50 m \times 0.32 mm, wall-coated open tubular (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 a vacuum (150–275 Torr obtained by an Edwards oil-sealed RV5 pump).

3-Methylthiopropionaldehyde Quantification by Liquid-Liquid Extraction and GC-FID. *Extraction Method.* Each 25-mL sample was extracted in the dark by 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. Twenty-five parts per million of external standard was added before the extract was transferred to a chromatographic vial.

Gas Chromatography Analytical Conditions. A Hewlett-Packard model 5890 gas chromatograph was used, equipped with a Hewlett-Packard model 7673 automatic sampler, a cold on-column injector, a flame ionization detector, and a Shimadzu CR3A integrator. Analyses were carried out on a 50 m \times 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 °C/min, then to 200 °C at 1 °C/min, and to 250 °C at 20 °C/min. The carrier gas was helium at a flow rate of 1.5 mL/min. The injector temperature was maintained at 3 °C above the oven temperature. The detector temperature was 275 °C.

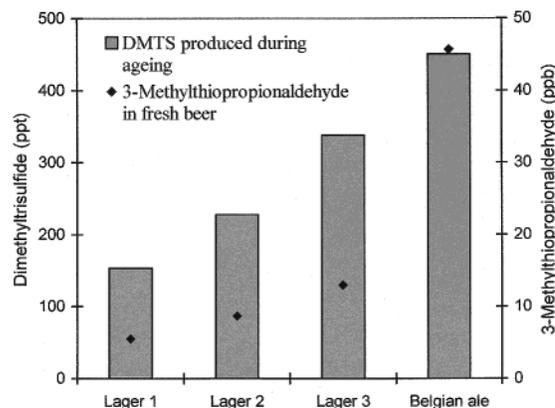


Figure 1. Concentration of DMTS produced during aging of commercial beers (three lagers and one Belgian ale) and level of 3-methylthiopropionaldehyde in the fresh beers. Initial DMTS concentrations in fresh beers are 53, 140, 8, and 38 ppt for lagers 1, 2, and 3 and Belgian ale, respectively (with a variation coefficient under 6%).

Table 1. Concentration of DMTS after an Accelerated Aging of a Control Beer and the Corresponding One to Which 3-Methylthiopropionaldehyde (101 ppb) or 3-Methylthiopropylol (124 ppb) Had Been Added before Aging

type of beer	DMTS (ppt)	
control	49 ^a	
spiked with 3-methylthiopropionaldehyde	216	185 ^{a,b} 244 ^{a,b}
spiked with 3-methylthiopropylol	287	287 ^{a,b} 288 ^{a,b}

^a Variation coefficient between analysis duplicates $\leq 6\%$. ^b Duplicates of the spiking experiment.

RESULTS

On the basis of the literature, we logically suspected that 3-methylthiopropionaldehyde might be a precursor of DMTS during beer aging. To test this hypothesis, we first quantified 3-methylthiopropionaldehyde and DMTS in various commercial beers. Three lager beers and one Belgian ale beer were analyzed before and after aging. As shown in Figure 1 and as suspected, the higher the 3-methylthiopropionaldehyde content of the fresh beer, the higher the concentration of DMTS produced during aging. Given the low threshold of this polysulfide, this production is sufficient to impart an off-flavor to the aged beers.

A second experiment further strengthened our hypothesis. When fresh commercial beers were spiked with 3-methylthiopropionaldehyde (101 ppb) before aging, higher amounts of DMTS were again detected in the aged beer (216 versus 49 ppt, Table 1) despite the inevitable introduction of oxygen into the bottle (source of aldehyde oxidation through aging). 3-Methylthiopropionaldehyde thus does emerge as a potential precursor of DMTS in aged beer.

However, only $\sim 0.1\%$ of the 3-methylthiopropionaldehyde (if given in DMTS equivalents) appeared here to be degraded to DMTS, against 2–6% for the first experiment, suggesting that another DMTS source should also be considered. As most aldehydes are reduced by yeast into alcohols, 3-methylthiopropylol was suspected to be this additional precursor. Therefore, a fresh commercial beer was spiked with 124 ppb of 3-methylthiopropylol. From this experiment (287 ppt of DMTS against 49; Table 1), we could conclude that

Table 2. Concentration of DMTS in Fresh and Aged Beers Produced from Wort Spiked, or Not, with 3-Methylthiopropionaldehyde during Boiling

type of beer	DMTS (ppt)			
	beer A		beer B	
	fresh	aged	fresh	aged
control	0.1	49	0.5	18
1 ppm of 3-methylthiopropionaldehyde added	0.3	585	0.6	168
10 ppm of 3-methylthiopropionaldehyde added	17	4759	47	1034

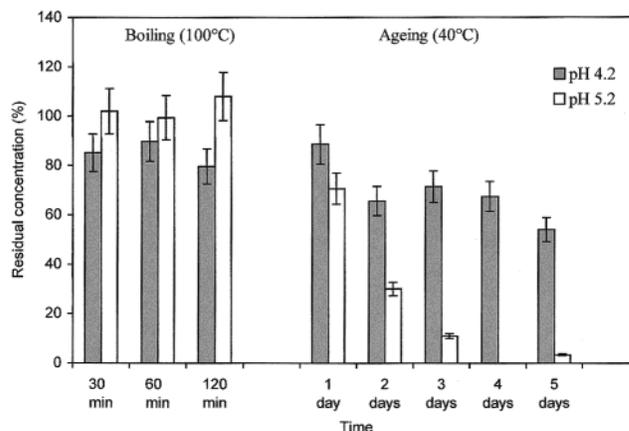
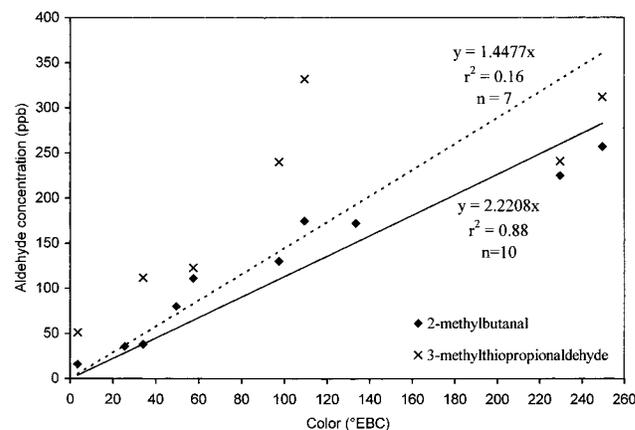
both the aldehyde and its corresponding alcohol were degraded during storage.

To be a precursor in itself, 3-methylthiopropionaldehyde must be able to partially survive the fermentation step. As recently shown by Perpète and Collin (1999), up to 15% of the 3-methylthiopropionaldehyde present in the wort is usually left unreduced by yeast; the level rises to 40% when the fermentation is conducted at low temperature, owing to interactions with polyphenols (Perpète and Collin, 2000a). Another source of 3-methylthiopropionaldehyde could be the yeast, because deuterated Strecker aldehydes are excreted when deuterated amino acids are added to the fermentation medium (Perpète and Collin, 2000b).

To assess the relative contributions of raw materials against yeast itself to DMTS production in beer, we added 1 or 10 ppm of 3-methylthiopropionaldehyde during boiling to mimic its presence in the wort. As shown in Table 2, the DMTS level was higher in the aged beer when 3-methylthiopropionaldehyde was added. Again, only 0.03–0.10% of the 3-methylthiopropionaldehyde (in DMTS equivalents) added to the wort in the kettle was transformed to DMTS, confirming that raw materials (3-methylthiopropionaldehyde and derived 3-methylthiopropional) are much more important than aldehyde excretion by yeast. Small losses might obviously occur in the kettle through steam distillation, but such losses should be limited by the low volatility of the aldehyde, as shown by its extremely poor recovery factor in dynamic headspace analyses (Perpète and Collin, 2000c,d). Furthermore, Buckee et al. (1982) detected no 3-methylthiopropionaldehyde in boiling condensates.

Thermal degradation in the kettle is another possible way for losses to occur, so we investigated the stability of 3-methylthiopropionaldehyde during boiling in a model medium. A 50 mM phosphate buffer at pH 5.2 (wortlike) and a 50 mM phosphate buffer at pH 4.2 (beerlike) were each spiked with 500 ppb of 3-methylthiopropionaldehyde and allowed to boil. As shown in Figure 2, degradation of the aldehyde was not significant after 2 h at 100 °C, whatever the pH value. When the same media were subjected to accelerated aging (5 days at 40 °C), the measured levels of degradation were 46% at pH 4.2 and 100% at pH 5.2, respectively. These results suggest that no significant 3-methylthiopropionaldehyde degradation should occur during boiling but that aging should lead to degradation, especially when the pH is high. Improving the flavor stability of packaged beer by optimizing the pH will be, however, hazardous because another well-known defect of aged beers, the cardboard flavor, is intensified at low pH (Noël et al., 1999; Lermusieau et al., 1999).

As it appears from this work the 3-methylthiopropionaldehyde level in raw material is a determinant of beer aging, its concentration was measured in various malts.

**Figure 2.** 3-Methylthiopropionaldehyde degradation during boiling and aging.**Figure 3.** Relationships between EBC wort aldehyde concentrations and color of pale and caramel malts.

Another Strecker aldehyde, 2-methylbutanal, was also investigated because it is easily detected by the usual headspace technique (not possible for DMTS precursor; Perpète and Collin, 1999). As depicted in Figure 3, the higher the color of malts, the higher the 2-methylbutanal and 3-methylthiopropionaldehyde concentrations. Although only small amounts of DMTS precursor were found in pale malts (under 100 ppb in an EBC wort), concentrations up to 312 ppb were detected with caramel malts (50% special malts in the EBC wort). However, a linear model appeared to be appropriate only in the case of 2-methylbutanal ($r^2 = 0.88$ against $r^2 = 0.16$). Therefore, color could be used only as a first risk indicator of DMTS release during beer aging. The greatest care should particularly be taken in the case of torrefied malts, in which low aldehyde levels were detected despite a strong color (e.g., 60 ppb of 3-methylthiopropionaldehyde in the EBC wort for a 600 °EBC malt).

As shown in Figure 4, a significant fraction of 3-methylthiopropionaldehyde is also synthesized by Strecker degradation both during wort filtration and boiling (~30 ppb with a pale malt). When mashing is conducted with 10% of a caramel 152 °EBC malt, much higher amounts of 3-methylthiopropionaldehyde were detected throughout wort production, despite a more limited methionine Strecker degradation in boiling. After fermentation, 3-methylthiopropionaldehyde levels were lowered due to yeast reducing activity. Despite similar DMTS and 3-methylthiopropionaldehyde levels in the two fresh beers, much higher amounts of DMTS were detected in

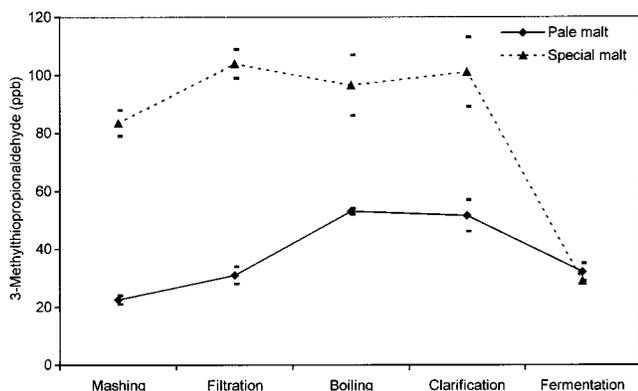


Figure 4. 3-Methylthiopropionaldehyde concentration during beer production with 100% pale malt or 90% pale malt/10% special malt.

Table 3. Concentration of DMTS in Fresh and Aged Beers Produced with 100% Pale Malt or 90% Pale Malt/10% Special Malt

grist	DMTS (ppt)			
	fresh beer	aged beer		
pale malt	8	6 ^{a,b}	98	98 ^a
special malt	15	10 ^{a,b}	171	165 ^{a,b}
		19 ^{a,b}		

^a Variation coefficient between analysis duplicates $\leq 6\%$. ^b Duplicates of the spiking experiment.

the aged beer produced with special malt, confirming the key role of 3-methylthiopropional (Table 3).

CONCLUSION

3-Methylthiopropionaldehyde and its reduced form 3-methylthiopropional are DMTS sources in aged beers. Around 0.1% of 3-methylthiopropionaldehyde initially present in malt (if given in DMTS equivalents) can be degraded to DMTS, especially when the pH is high. Because very little 3-methylthiopropionaldehyde is degraded during boiling, the choice of raw materials should have a major influence. The malt color could be used only as a first risk indicator of DMTS release during beer aging. Both the filtration and boiling steps emerged as additional sources of 3-methylthiopropionaldehyde in pitching wort.

LITERATURE CITED

- Allen, C.; Parks, O. W. Evidence for Methional in Skim Milk Exposed to Sunlight. *J. Dairy Sci.* **1975**, *58*, 1609–1611.
- Ballance, P. E. Production of volatile compounds related to the flavour of foods from the Strecker degradation of DL-methionine. *J. Sci. Food Agric.* **1961**, *12*, 532–536.
- Buckee, G. K.; Malcolm, P. T.; Peppard, T. L. Evolution of volatile compounds during wort-boiling. *J. Inst. Brew.* **1982**, *88*, 175–181.
- Chan, F.; Reineccius, G. A. Kinetics of the formation of methional, dimethyl disulfide, and 2-acetylthiophene via the Maillard reaction. *ACS Symp. Ser.* **1994**, No. 564 (*Sulfur Compounds in Foods*), 127–137.
- European Brewery Convention. *Analytica EBC, Brauerei-und-getränke-rundschau*, 4th ed.; 1987.
- Gijs, L.; Piraprez, G.; Perpète, P.; Spinnler, E.; Collin, S. Retention of sulfur flavours by food matrix and determination of sensorial data independent of the medium composition. *Food Chem.* **2000**, *69*, 319–330.

- Lermusieau, G.; Noël, S.; Liégeois, C.; Collin, S. Non oxidative mechanism for development of *trans*-2-nonenal in beer. *J. Am. Soc. Brew. Chem.* **1999**, *57*, 29–33.
- Lieberman, M.; Kunishi, A. T.; Mapson, L. W.; Wardale, D. A. Ethylene Production from Methionine. *Biochem. J.* **1965**, *95*, 449–459.
- Meynier, A.; Mottram, D. S. The effect of pH on the formation of volatile compounds in meat-related model systems. *Food Chem.* **1994**, *52*, 361–366.
- Nedjma, M.; Hoffmann, N. Hydrogen sulfide reactivity with thiols in the presence of copper(II) in hydroalcoholic solutions cognac brandies: Formation of symmetrical and unsymmetrical dialkyltrisulfides. *J. Agric. Food Chem.* **1996**, *44*, 3935–3938.
- Noël, S.; Liégeois, C.; Lermusieau, G.; Bodart, E.; Badot, C.; Collin, S. Release of deuterated nonenal during beer aging from labeled precursors synthesized in the boiling kettle. *J. Agric. Food Chem.* **1999**, *47*, 4323–4326.
- Patton, S. The mechanism of sunlight flavor formation in milk with special reference to methionine and riboflavin. *J. Dairy Sci.* **1954**, *37*, 446–452.
- Peppard, T. L. Dimethyltrisulphide, its mechanism of formation in hop oil and effect on beer flavour. *J. Inst. Brew.* **1978**, *84*, 337–340.
- Perpète, P.; Collin, S. Fate of the warty flavours in a cold contact fermentation. *Food Chem.* **1999**, *66*, 359–363.
- Perpète, P.; Collin, S. How to improve the enzymatic flavour reduction in a cold contact fermentation. *Food Chem.* **2000a**, *70*, 457–462.
- Perpète, P.; Collin, S. Evidence of Strecker aldehyde excretion by yeast in cold contact fermentation. *J. Agric. Food Chem.* **2000b**, *48*, 2384–2386.
- Perpète, P.; Collin, S. Influence of beer ethanol content on the wort flavour perception. *Food Science and Technology COST ACTION 96: Interaction of Food Matrix with Small Ligands Influencing Flavour and Texture*; Office for official publications of the European Communities: Luxembourg, 2000; in press.
- Perpète, P.; Collin, S. Influence of beer ethanol content on the wort flavour perception. *Food Chem.* **2000d**, *71*, 379–385.
- Prentice, R. D. M.; McKernan, G.; Bryce, J. H. A Source of Dimethyl Disulfide and Dimethyl Trisulfide in Grain Spirit Produced with a Coffey Still. *J. Am. Soc. Brew. Chem.* **1998**, *56* (3), 99–103.
- Pryor, W. A.; Tang, R. H. Ethylene formation from methional. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 498–503.
- Soltoft, M. Flavour active sulphur compounds in beer. *Brygmesteren* **1988**, *2*, 18–24.
- Wainwright, T.; McMahon, J. F.; McDowell, J. Formation of Methional and Methanethiol from Methionine. *J. Sci. Food Agric.* **1972**, *23*, 911–914.
- Williams, R. S.; Gracey, D. E. F. Beyond Dimethyl Sulfide: The Significance to Flavor of Thioesters and Polysulfides in Canadian Beers. *J. Am. Soc. Brew. Chem.* **1982a**, *40* (2), 68–71.
- Williams, R. S.; Gracey, D. E. F. Factors Influencing the Levels of Polysulfides in Beer. *J. Am. Soc. Brew. Chem.* **1982b**, *40* (2), 71–74.
- Yang, S. F.; Ku, H. S.; Pratt, H. K. Photochemical Production of Ethylene from Methionine and Its Analogues in the Presence of Flavin Mononucleotide. *J. Biol. Chem.* **1967**, *242*, 5274–5280.
- Yu, T. H.; Ho, C. T. Volatile Compounds Generated from Thermal Reaction of Methionine and Methionine Sulfoxide with or without Glucose. *J. Agric. Food Chem.* **1995**, *43*, 1641–1646.

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