

20 Methionine: A Key Amino Acid for Flavour Biosynthesis in Beer

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Abstract The level of sulfur flavours such as methional, methionol, dimethyldisulfide, dimethyltrisulfide or methanethiol in fresh beer is mainly dependent on the yeast reduction activity during fermentation. Among these, methional was recently reported to be responsible for the worty aroma of alcohol-free beers and should be considered as a precursor of dimethyltrisulfide which is implied in the staling of beer. Methionine probably also plays a role in methional excretion. However, while the Strecker degradation is a well-known chemical mechanism transforming amino acids to aldehydes, no genetic evidence of a methionine Ehrlich-like pathway has been reported previously. The efficiency of this pathway will be compared with the C-S lysase activities of *Saccharomyces cerevisiae*. The aim of this chapter is to demonstrate that this biochemical degradation could lead to the development of sulfurous off-flavours in beer.

20.1 Introduction

Sulfur compounds from malt and hops or synthesised during the brewing process are natural components of beer. Individually, sulfur compounds usually impart an aroma or onion, rotted vegetables or cabbage (Table 20.1).

Dimethylsulfide (50 ppb in some lager beers) and sulfur dioxide (up to 10 ppm) are major sulfur compounds in beer. The synthesis pathway and parameters influencing their final level have been well documented.⁴ Often characterised by a very low detection

Table 20.1 Organoleptic qualities and detection thresholds of some sulfur compounds

Compound	Structure	Odour	Detection threshold (ppb)	Concentration in beer (ppb)
Isopentenylmercaptan	$(\text{CH}_3)_2\text{C}=\text{CH}-\text{CH}_2\text{SH}$	'Sunstruck'	0.002-0.004	100
2-Mercapto-3-methylbutanol	$\text{CH}_2\text{OH}-\text{CHSH}-\text{CH}(\text{CH}_3)_2$	Onion	<1 ppb	-
Methional	$\text{CH}_3-\text{S}-\text{CH}_2-\text{CH}_2-\text{CHO}$	Boiled potatoes, soup	0.1-250	2-50
Methionol	$\text{CH}_3-\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2\text{OH}$	Radish	1200	2-50
Methanethiol	CH_3-SH	Excrement, putrefaction	0.02-41	2-12
DMS	$\text{CH}_3-\text{S}-\text{CH}_3$			
DMDS	$\text{CH}_3-\text{S}-\text{S}-\text{CH}_3$	Cooked cabbages, onion, rubber	3-50	0.3-7.5
DMTS	$\text{CH}_3-\text{S}-\text{S}-\text{S}-\text{CH}_3$	Fresh onion, sulfurous, boiled vegetables	0.1	0.2-1.8

Adapted from Refs 1-3.

DMS: dimethylsulfide; DMDS: dimethyldisulfide; DMTS: dimethyltrisulfide.

threshold, minor sulfur compounds such as thioesters, polysulfides or thiols most probably also modify the overall organoleptic quality of beer.

Thioesters (*S*-methylthioacetate, *S*-methylthioisovalerate), which originate mainly from hops,⁵ give rise to rotted vegetable aromas⁶ in lager beers.

With a flavour threshold of 0.1 ppb, dimethyltrisulfide is known to be responsible for the onion off-flavour of aged beers. Recent data show that *S*-methylcysteine sulfoxide, methional and methionol are its main precursor in beer.^{7,8}

Although thiols are hard to analyse because of their very high reactivity and very low concentration/detection threshold, they are also very relevant compounds for brewers. Among them, isopentenylmercaptan imparts the well-known 'sunstruck' flavour⁹ and methanethiol (detection threshold: 0.1 ppb) is usually characterised with 'excrement' or 'putrefaction' descriptors. In aged beers, as in wines,¹⁰ polyfunctional thiols are probably also responsible for delicate flavours.⁸

Methional or 3-methylthiopropionaldehyde imparts a boiled potato aroma at high concentrations,² but can be described as 'soup' or 'hot wort' at lower concentrations.¹¹ This aldehyde was initially detected in Cheddar cheese,¹² corn tortillas¹³ and boiled trout,¹⁴ and has been measured in beer and alcohol-free beers, where it contributes to the warty aroma along with 2-methylbutanal and 3-methylbutanal.¹¹ More recently, methional was also proposed as an additional key compound in aged beers.¹⁵ As 2-methylbutanal and 3-methylbutanal can be synthesised by Strecker degradation of amino acids, wort methional may derive from methionine. During wort fermentation, *Saccharomyces cerevisiae* produces NADPH-dependent enzymes allowing methional reduction.^{11,16} However, because of the low temperature applied and interaction with polyphenols, this enzymic reduction is incomplete in alcohol-free beer productions, leading to a strong warty flavour in the final product.

Methionol is the reduction product of methional. Although not really pleasant, with its cauliflower or radish-like aroma, methionol is not considered an off-flavour in beer (detection threshold close to 1200 ppb).

Most of the sulfur compounds described above should be derivable from methionine, so it is very surprising that so little is known about the methionine-degrading pathway in yeast. The aim of this work was to find evidence for a catabolic pathway leading to methanethiol, either with or without a methional intermediate.

20.2 Materials and methods

20.2.1 Reagents

Methional (95%) and methionol (98%) were purchased from Acros Chemika (Brussels, Belgium). Methanethiol (99.5%) was from Aldrich (Brussels, Belgium).

20.2.2 Strains

Saccharomyces cerevisiae BRAS291 (bottom fermentation) and BRAS212 (top fermentation) were provided by the BRAS collection of the Unité de Brasserie et des Industries Alimentaires (Louvain-la-Neuve, Belgium).

20.2.3 *Culture media and sampling*

Precultures were grown in YPS medium (1% yeast extract, 0.5% peptone and 10% sucrose) at 28°C on a rotary shaker and collected in the exponential phase. After collection and washing, the yeast was pitched at 10^6 cells/ml in model media. Two media were used during these experiments: a glucose-methionine medium (citrate-buffered medium containing 3% glucose and 10 mM methionine) and a glucose-ammonium medium (citrate-buffered medium containing 3% glucose and 10 mM ammonium sulfate). Cultures were grown at 28°C on a rotary shaker. At a given time, samples were collected and centrifuged, and supernatants were immediately frozen in liquid nitrogen for methanethiol quantification.

20.2.4 *Methanethiol quantification*

Methanethiol was quantified by dynamic headspace gas chromatography. A Hewlett-Packard model 5890 gas chromatograph equipped with a Chrompack Purge and Trap Injector, a flame ionisation detector and a Shimadzu CR3A integrator was used. Samples were injected into the chromatographic column in the following three steps.

1. Precooling of the trap (CPSil 8 CB capillary column, 0.53 mm internal diameter; film thickness 5 μ m): the trap was cooled at -95°C for 2 min in a stream of liquid nitrogen;
2. Purging of the sample: the temperature of the purge vessel was set at 50°C . The sample was purged with helium gas (12 ml/min) for 15 min. The gas stream was passed through a condenser kept at -15°C by means of a cryostat (Colora WK 15) to remove water vapour and then through an oven at 200°C . The volatiles were finally concentrated in the cold trap maintained at -95°C (liquid nitrogen);
3. Desorption of the volatiles: cooling was stopped, and the surrounding metal capillary was immediately heated to 220°C for 5 min. The carrier gas swept the trapped compounds into the analytical column. Analysis of samples was carried out on a $50\text{ m} \times 0.32\text{ mm}$, wall-coated, open tubular (WCOT) CP-Sil5 CB capillary column (Chrompack, Antwerp, Belgium) (film thickness 1.2 μ m). Oven temperature, initially kept at 36°C for 15 min, was programmed to rise from 36 to 120°C at $2^\circ\text{C}/\text{min}$ then to 200°C at $10^\circ\text{C}/\text{min}$, remaining at the maximum temperature for 10 min thereafter. Helium carrier gas was used at a flow rate of 1.0 ml/min. Injection and detection temperatures were 200 and 220°C , respectively.

All analyses were done in duplicate. The assessment of the reproducibility of this technique has been described previously (coefficients of variation under 10% for five analyses of the same standard mixture).¹⁷

20.3 Results and discussion

During mashing, methionine can be transformed to methional by Strecker degradation (Fig. 20.1) and further oxidation can lead to methanethiol.^{18,19} Because of its high volatility, this thiol is easily stripped out of the wort in the boiling kettle.

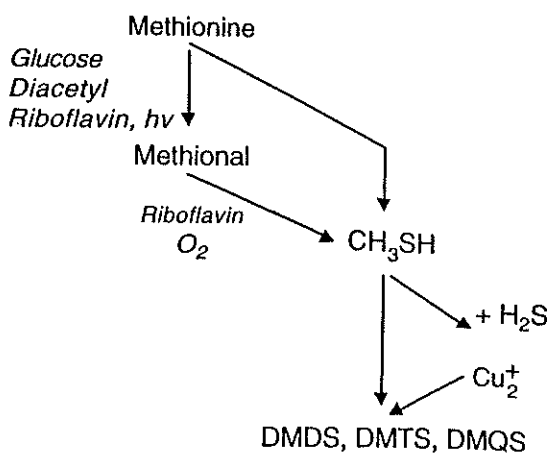
Mashing – Boiling – Clarification – Staling → chemical synthesis

Fig. 20.1 Potential mechanisms of methional and methanethiol synthesis during mashing processes.

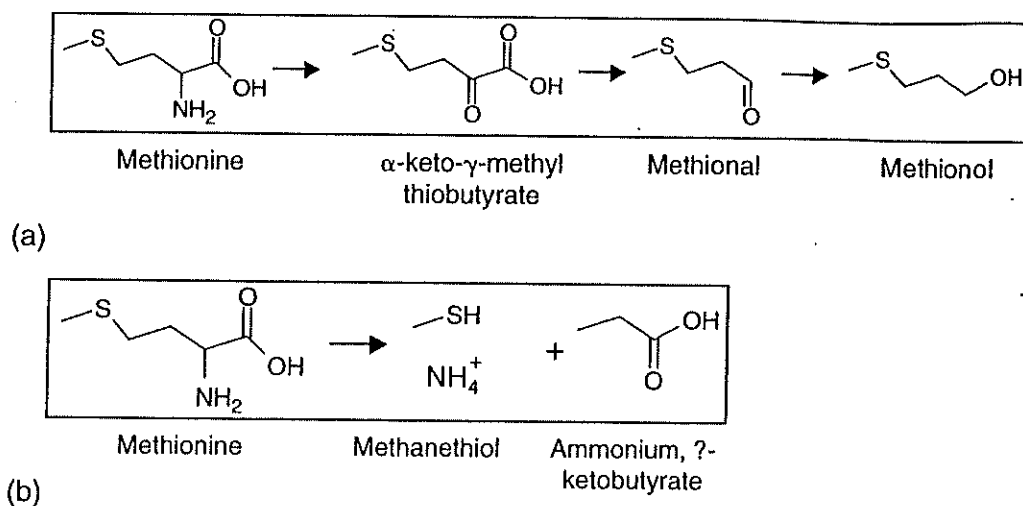


Fig. 20.2 Hypothetical methionine degradation pathways in *Saccharomyces cerevisiae*: (a) Ehrlich-like; (b) C-S lyase.

During fermentation, *S. cerevisiae* can consume both methionine and methional. Methionine uptake by yeast is well documented in the literature. At least five amino acid permeases are involved: MUP1p and MUP3p (for methionine uptake) seem to be the most specific transporters,²⁰ while MUP2p/AGP1p (initially defined as an asparagine glutamine permease) and BAP2 and BAP3 (branched amino acid permease) were initially believed to be dedicated to translocating other amino acids such as isoleucine or leucine.²¹ Once in the yeast cell, methionine may be further degraded via two enzymic routes (Fig. 20.2). The 'Ehrlich-like' pathway would lead to α -keto- γ -methylthiobutyrate, methional and methionol (Fig. 20.2a). The end of this biochemical pathway has been demonstrated in *S. cerevisiae* strains: methional and methionol are detected in beer¹¹ and an NADPH-dependent enzyme can reduce the aldehyde to its primary alcohol.^{7,11}

BREWING YEAST FERMENTATION PERFORMANCE

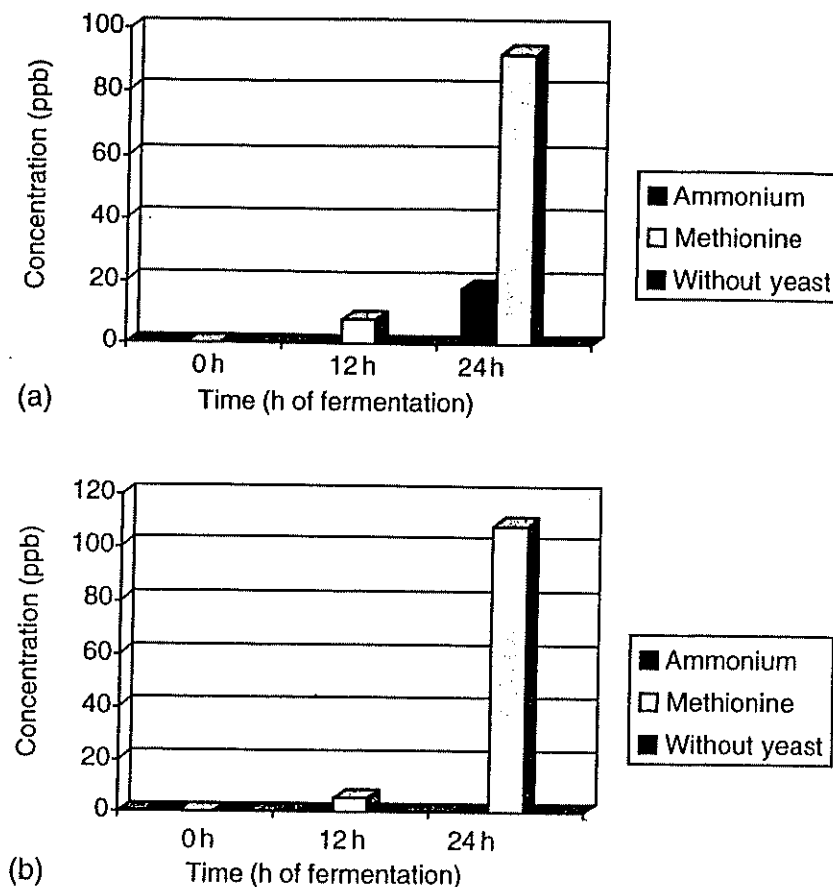


Fig. 20.3 Methanethiol production by *Saccharomyces cerevisiae* BRAS291 (bottom yeast) and BRAS212 (top yeast) in glucose-ammonium and methionine-ammonium culture media. Fermentation was carried out at 28°C during 24 h under agitation, with a pitching rate of 10^6 cells/ml.

A second methionine-degrading pathway hypothetically present in *S. cerevisiae* is one observed in several fungi, including some yeasts. In *Geotrichum candidum*, methionine is directly degraded to methanethiol, ketobutyrate and ammonium (Fig. 20.2b). The enzymes responsible for this degradation are C-S-lyases-like enzymes or γ -demethylase.^{22,23} Some *S. cerevisiae* enzymes involved in or associated with the sulfydrylation pathway²⁰ should be able to catalyse these reactions.

To provide evidence for such a mechanism in *S. cerevisiae*, glucose-methionine and glucose-ammonium media were pitched with bottom-fermenting (*S. cerevisiae* BRAS291) or top-fermenting (*S. cerevisiae* BRAS212) strains. After 24 h of fermentation, a significant increase in methanethiol level was observed in the glucose-methionine medium (Fig. 20.3a,b), resulting in concentrations near 100 ppb for both yeast strains. No trace of this sulfur compound was detected in the same glucose-methionine medium without pitching, showing that methionine was not chemically degraded in this control. Slight methanethiol production (18 ppb after 24 h) was also measured in the glucose-ammonium medium after pitching with the top-fermenting yeast BRAS212. This low level could reasonably be explained by the low initial internal methionine pool.

Another possibility to be considered is the chemical degradation of methional or methionol to methanethiol. However, when both model media were spiked with 50 ppm

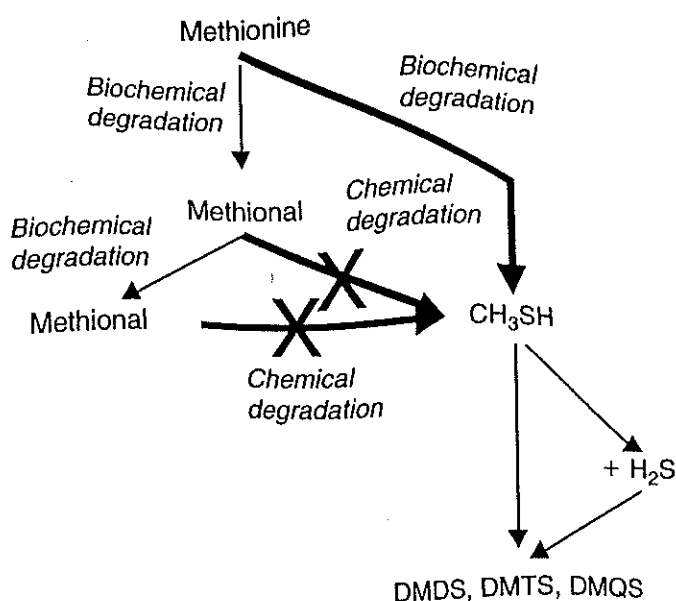


Fig. 20.4 Potential mechanisms of methional and methanethiol biosynthesis during fermentation.

methional or methionol, not even a trace of methanethiol was detected. As depicted in Fig. 20.4, the results suggest that *S. cerevisiae* catalyses the degradation of methionine into methanethiol.

These initial experiments, however, do not exclude the existence of an additional 'Ehrlich-like' pathway. To clarify this point, new experiments are being conducted with labelled methionine and intermediates.

References

1. Vermeulen, C., Pellaud, J., Gijs, L. and Collin, S. (2001) Combinatorial synthesis and sensorial properties of polyfunctional thiols. *J. Agric. Food Chem.* **49**, 5445–5449.
2. Meilgaard, M. (1975) Flavour chemistry of beer: Part II: Flavour and threshold of 239 aroma volatiles. *Tech. Q. Master Brew. Assoc. Am.* **12**, 151–168.
3. Olsen, A., Christiansen, B.W. and Madsen, J.O. (1988) Onion-like off-flavour in beer: isolation and identification of the culprits. *Carlsberg Res. Commun.* **53**, 1–9.
4. Anness, B.J. and Bamforth, C.W. (1982) Dimethylsulphide – a review. *J. Inst. Brew.* **88**, 244–252.
5. Suggett, A., Moir, M. and Seaton, J.C. (1979) The role of sulphur compound in hop flavour. *Proc. Eur. Brew. Cong.* **17**, 79–89.
6. Stewart, G.G. and Russel, I. (1981) The influence of yeast on volatile sulphur compounds in beer. *Eur. Brew. Conv. Monogr. VII*, pp. 173–187.
7. Gijs, L., Perpète, P., Timmermans, A. and Collin, S. (2000) 3-Methylthiopropionaldehyde as precursor of dimethyltrisulphide in aged beers. *J. Agric. Food Chem.* **48**, 6196–6199.
8. Williams, R.S. and Gracey, D.E.F. (1982) Beyond dimethylsulphide: the significance to flavour of thioesters and polysulphides in Canadian beers. *J. Am. Soc. Brew. Chem.* **40**, 68–71.
9. Gunst, F. and Verzele, M. (1978) On the sunstruck flavour in beer. *J. Inst. Brew.* **84**, 291–292.
10. Maga, J.A. (1976) The role of sulphur compounds in food flavour. Part III: Thiols. *Crit. Rev. Food Sci. Nutr.* January, 147–192.
11. Perpète, P. and Collin, S. (1999) Contribution of 3-methylthiopropionaldehyde to the worty flavour of alcohol-free beers. *J. Agric. Food Chem.* **47**, 2374–2378.
12. Christensen, K.R. and Reineccius, G.A. (1995) Aroma extract dilution analysis of aged Cheddar cheese. *J. Food Sci.* **60**, 218–220.

13. Buttery, R.G. and Ling, L.C. (1995) Volatile flavour components of corn tortillas and related products. *J. Agric. Food Chem.* **43**, 1878–1882.
14. Milo, C. and Grosch, W. (1993) Changes in the odorants of boiled trout as affected by the storage of the raw material. *J. Agric. Food Chem.* **41**, 2076–2081.
15. Chevance, F., Gijs, L., Jerkovic, V. *et al.* (2001) Influence of pH on beer flavour stability during storage. *4th Pangborn Sensory Sci. Symp.*, 22–26 July, Dijon, France.
16. Laurent, M., Geldorf, B., Van Nederveelde, L. *et al.* (1995) Characterisation of the aldoketoreductase yeast enzymatic systems involved in the removal of wort carbonyls during fermentation. *Proc. Eur. Brew. Conv.* **25**, 337–344.
17. Collin, S., Osman, M., Delcambre, S. *et al.* (1993) Investigation of volatile flavour compounds in fresh and ripened Domiati cheeses. *J. Agric. Food Chem.* **41**, 1659–1663.
18. Balance, P.E. (1961) Production of volatile compounds related to the flavour of foods from the Strecker degradation of DL-methionine. *J. Sci. Food Agric.* **12**, 532–536.
19. Yu, T.H. and Ho, C.T. (1995) Volatile compounds generated from thermal reaction of methionine and methionine sulphoxide with or without glucose. *J. Agric. Food Chem.* **43**, 1641–1646.
20. Thomas, D. and Surdin-Kerjan, Y. (1997) Metabolism of sulphur amino acids in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **61**, 503–532.
21. Regenber, B., During-Olsen, L., Kielland-Brandt, M. and Holmberg, S. (1999) Substrate specificity and gene expression of the amino acid permeases in *Saccharomyces cerevisiae*. *Curr. Genet.* **36**, 317–328.
22. Helinck, S., Spinnler, H.E., Parayre, S. *et al.* (2000) Enzymatic versus spontaneous S-methyl thioester synthesis in *Geotrichum candidum*. *FEMS Microbiol. Lett.* **193**, 237–241.
23. Spinnler, H.E., Berger, C., Lapadatescu, C. and Bonnarme, P. (2001) Production of sulfur compounds by several yeast of technological interest for cheese ripening. *Int. Dairy J.* **11**, 245–252.