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Production of *S*-Methyl Thioacetate from Methyl Mercaptan by Brewer's Yeast*

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S-Methyl thioacetate (MeSAc) production by brewer's yeast from methyl mercaptan (MeSH) was investigated under various conditions. At optimum, 98 mg/liter of MeSAc was produced from 500 mg/liter of MeSH contained in culture broth. The MeSAc level in yeast growth medium was increased with increasing MeSH at relatively low levels (10 to 500 mg/liter). However, higher MeSH levels in medium (over 1 g/liter) inhibited yeast growth, and no MeSAc was produced. MeSAc was formed readily by incubating MeSH with yeast resting cells. Furthermore, *S*-ethyl or *S*-*n*-propyl thioacetate accumulated in yeast cell suspension when ethyl or *n*-propyl mercaptan, respectively, was incubated with resting cells. MeSAc was also produced from L-methionine by brewer's yeast, but its formation was dramatically inhibited by copper ions. This finding suggests that MeSH is an intermediate product between L-methionine and MeSAc.

Various kinds of volatile sulfur compounds, including hydrogen sulfide, thiols and dimethyl sulfide, are known to be present in beer.[1] Most of these compounds have a characteristic ill-smell, and their excess in beer gives rise to unpleasant sulfury flavors.

S-Methyl thioacetate (MeSAc), which has an objectionable smell reminiscent of rotten vegetables, was recently identified and determined in beer as another possible source of sulfury flavor.[2,3] MeSAc and its short chain thioester homologs are also shown to greatly contribute to the unique flavor of smear coated cheeses.[4] They were postulated to be associated metabolites of *Micrococcus* cheese strains and *Brevibacterium linens*.[5]

In beer, MeSAc has been reported to form during fermentation of wort,[2] and it was proposed that MeSAc was produced from methyl mercaptan (MeSH)[2] or L-methionine[3] by yeast. However, the details on the production of MeSAc by yeast have not been examined. Consequently in our laboratory, the origins and formation of MeSAc were investigated, and methods to control MeSAc content in beer were also studied. In our previous paper,[6] it has been shown that *Saccharomyces cerevisiae* (IFO 1234) produced high levels of MeSAc from MeSH in synthetic medium, with much higher yields than those from L-methionine.

In the present work, we investigated more closely the effect of MeSH on MeSAc production by brewer's yeast. The effect of some cultural conditions and MeSAc formation by yeast resting cells are also reported.

MATERIALS AND METHODS

Chemicals. MeSH (*ca.* 30% in methanol) was supplied by Nakarai Chemicals. MeSH concentration of the reagent was determined by gas chromatography with a flame photometric detector (FPD-GC), using MeSH standard solution (1 μg/μl benzene) obtained from Wako Pure Chemical Industries. The agent was further diluted with methanol before use. MeSAc was prepared by the method described in the earlier paper.[2] Other chemicals were of reagent grade.

Yeast. Seven bottom fermenting and three top fermenting strains of brewer's yeasts were used from the culture collection of our laboratories.

Medium. The basal fermentation medium for MeSAc production contained 5% glucose, 0.5% peptone, 0.05%

* Studies on Volatile Sulfur Compounds in Beer. Part V. For Part IV, see ref. 6.

yeast extract, 0.2% $(NH_4)_2SO_4$, 0.2% $Na_2HPO_4 \cdot 12H_2O$, 0.1% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.001% $CaCl_2 \cdot 2H_2O$, and 0.0005% $FeSO_4 \cdot 7H_2O$. The pH of the basal medium was 6.3 after autoclaving.

Cultural conditions. The sterilized basal medium (100 ml) was placed into a 500 ml-conical flask fitted with a stopper and a U-tube containing water. After adding 100 μl methanol solution of MeSH (0.03 mg/μl methanol), the basal medium was inoculated with yeast (10^4 cells/ml), which had been precultured by incubating in MYGP medium (0.3% malt extract, 0.3% yeast extract, 5% glucose and 0.5% peptone) at 25°C for two weeks, and cultivated on a reciprocal shaker (45 rev/min, 4 cm amplitude) at 25°C for 72 hr.

MeSAc production by resting cells. Cells grown in MYGP medium were harvested by centrifugation and washed twice with distilled water. Yeast cells (5 g) were placed into 500 ml-conical flask, and suspended in 200 ml distilled water. MeSH was added to the yeast cell suspension at concentrations of 5 and 20 mg/liter. The flask was fitted with a stopper and shaken horizontally at 25°C for 22 hr.

Measurement of growth. The culture broth was centrifuged at 4000 rpm for 10 min, and the precipitate was resuspended in the same volume of distilled water. Cell concentration was determined by measuring the turbidity of the suspension at 660 nm with a Hitachi model 181 Spectrophotometer.

Determination of MeSH and MeSAc. MeSH and MeSAc in the culture broth were determined by the headspace method. After removing yeast cells from the culture broth by centrifugation (5000 rpm for 10 min), 25 ml of the supernatant was taken into a 100 ml-flask, together with 9 g NaCl and 50 μl *n*-butyl ethyl sulfide ethanol solution (500 mg/liter ethanol). The flask was fitted with a Teflon cap having a small central rubber septum, and shaken thoroughly, then immersed in a 35°C water bath for 30 min. A 1 ml gas phase sample was removed using a syringe and immediately injected into FPD-GC. Gas chromatography was performed by a Microtek Gas Chromatograph (25% TCEP on Chromosorb P AW-DMCS, 80 ~ 100 mesh, 3 mm × 3 m; nitrogen: 30 ml/min; column temp.: 70°C isothermal for 10 min, then programmed to 150°C at the rate of 5°C/min; injection and detector temp.: 180°C; detector: FPD). The retention times of MeSH, *n*-butyl ethyl sulfide and MeSAc were 6, 20 and 23 min, respectively. The amounts of MeSH and MeSAc were calculated on the basis of peak height comparisons with *n*-butyl ethyl sulfide as an internal standard.

Preparative gas chromatography. Preparative gas chromatography was performed for purification of MeSAc from the culture broth under the same conditions as described previously.[2)]

Gas chromatography. The purity of MeSAc isolated from culture broth was examined by a Hitachi Gas Chromatograph 073 with a flame ionization detector (FID-GC) (column: 10% PEG 20M on Chromosorb W AW-DMCS, 60 ~ 80 mesh, 3 mm × 3 m; nitrogen: 50 ml/min; oven temp.: 70 ~ 150°C (3°C/min); injection and detector temp.: 200°C).

Combined gas chromatography-mass spectrometry (GC-MS). The mass spectrum was measured with a Hitachi M-52 Gas chromatograph-Mass spectrometer (column: 3% PEG 20M on Chromosorb W AW-DMCS, 60 ~ 80 mesh, 3 mm × 3 m; oven temp.: 70 ~ 150°C (3°C/min); carrier gas (He): 0.3 kg/cm^2; ionization energy: 20 eV).

Infrared spectrum. The infrared (IR) absorption spectrum of the liquid film formed between sodium chloride plates was recorded with a JASCO model IRA-1 Infrared Sepctrophotometer.

RESULTS

Separation and purification of MeSAc

An attempt was made to isolate and purify MeSAc from yeast growth medium containing MeSH. Yeast and MeSH were added into the basal medium (2.5 liter) in a 5 liter-conical flask at concentrations of 5 g and 0.5 g/liter, respectively. Cultivation was then carried out at 20°C for 72 hr with gentle stirring.

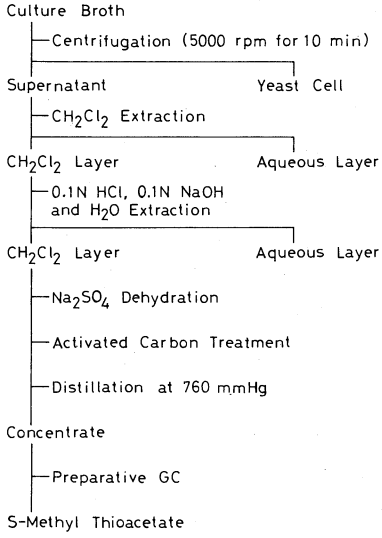

FIG. 1. Separation and Purification of *S*-Methyl Thioacetate.

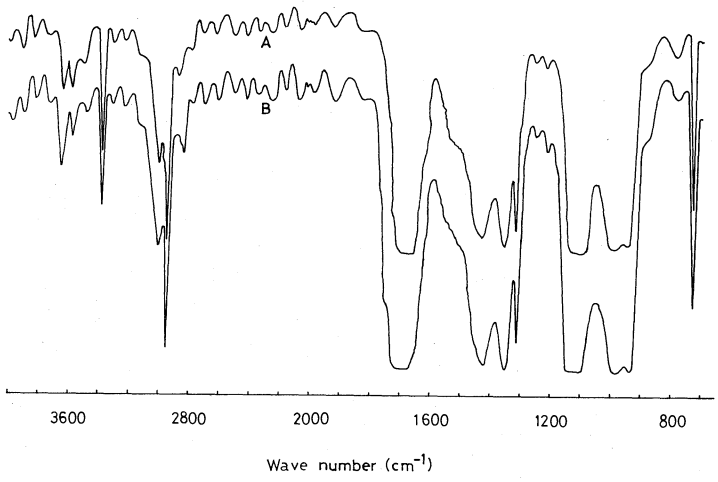

FIG. 2. Infrared Spectra of Sulfur Compound from Culture Broth (A), and Authentic *S*-Methyl Thioacetate (B).

Following the procedure shown in Fig. 1, MeSAc separation was started with 5 liter of the broth which contained 70 mg/liter of MeSAc, as determined by FPD-GD. After centrifugation, the supernatant was extracted twice with 2.5 liter of CH_2Cl_2, and the extract was washed with 1 liter of 0.1 N HCl, 0.1 N NaOH and H_2O, successively. After being dehydrated with anhyd. Na_2SO_4 and decolorized with activated carbon, the CH_2Cl_2 extract was concentrated by distillation under atmospheric pressure. MeSAc in the concentrate was fractionated by preparative GC to give 280 mg of colorless liquid with a rotten vegetable-like odor characteristic of authentic MeSAc. IR (Fig. 2) and MS spectra, as well as retention times on FID and FPD gas chromatogram of this liquid were identical with those of authentic MeSAc. The purity was estimated to be 99% in FID-GC. The overall recovery by this procedure was around 80%.

MeSAc production by brewer's yeasts

Ten strains of brewer's yeasts were cultivated in basal medium containing 10 mg/liter of MeSH to examine differences among brewer's yeasts to produce MeSAc. As shown in Table I, all yeast strains examined produced MeSAc, and the produced levels were somewhat higher than by *S. cerevisiae* IFO 1234.[6]

TABLE I. PRODUCTION OF *S*-METHYL THIOACETATE FROM METHYL MERCAPTAN BY TEN YEAST STRAINS

Cultivation was carried out under the standard conditions described in MATERIALS AND METHODS, except that the concentration of methyl mercaptan was 10 mg/liter.

Yeast strain	Medium (pH)	Yeast growth ($OD^{660\ nm}$)	MeSAc (mg/liter)	Yield (%)
Bottom fermenting				
No. 1	3.2	1.63	1.1	5.9
2	3.2	1.63	1.2	6.4
3	3.3	1.61	1.2	6.4
4	3.2	1.65	1.0	5.3
5	3.2	1.67	0.9	4.8
6	3.2	1.66	1.0	5.3
7	3.2	1.65	0.8	4.3
Top fermenting				
No. 8	3.2	1.60	1.5	8.0
9	3.1	1.59	1.5	8.0
10	3.2	1.57	1.2	6.4

There was no distinct difference in the amount of MeSAc between the top and bottom fermenting yeasts. Strain No. 1 was used in experiments hereafter as a representative yeast.

MeSH effect for yeast growth

Figure 3 shows the effect of MeSH on growth of yeast. The yeast grew at a normal

rate in the presence of 30 mg/liter of MeSH. However, the initiation of growth was delayed with increasing concentrations above 30 mg/liter. Growth inhibition was proportional to concentration in the range of 70 ~ 500 mg/liter. MeSH completely inhibited growth at high concentration (1000 mg/liter), where a number of dead cells were observed.

Effect of MeSH on MeSAc production

The effect of MeSH concentration on MeSAc production was examined. Table II shows the results of cultivation at various MeSH concentrations. No detectable MeSAc was produced in the control. The amount of MeSAc increased with increasing MeSH up to 500 mg/liter. However, no MeSAc accumulated with MeSH at 1000 mg/liter due to the inhibitory effect (Fig. 3). The maximum yield was obtained at around 70 ~ 100 mg/liter of MeSH. But the following experiments were performed at a concentration of 30 mg/liter, at which normal yeast growth was observed.

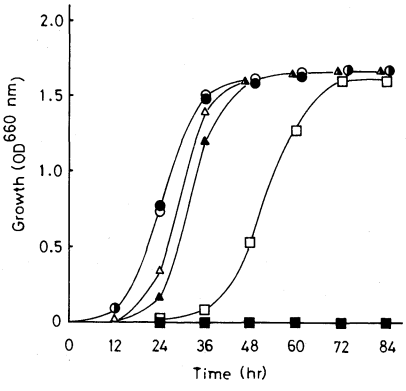

FIG. 3. Effect of Methyl Mercaptan Concentration on Yeast Growth.

Addition of methyl mercaptan: —●—, 30 mg/liter; —△—, 70 mg/liter; —▲—, 100 mg/liter; —□—, 500 mg/liter; —■—, 1000 mg/liter; —○—, control.

TABLE II. EFFECT OF METHYL MERCAPTAN CONCENTRATION ON PRODUCTION OF *S*-METHYL THIOACETATE

MeSH was added to 100 ml of basal medium at concentrations of 10 ~ 1000 mg per liter, and cultivation was carried out under standard conditions.

Addition of MeSH (mg/liter)	MeSAc (mg/liter)	Yield (%)
0	0	—
10	1.1	5.9
30	8.3	14.8
50	11.2	11.9
70	24	18.3
100	35	18.6
500	98	10.5
1000	0	0

Effect of initial pH

Incubations were carried out at various initial pH to investigate the relation between pH and MeSAc production.

As described in MATERIALS AND METHODS, the pH of the basal medium was 6.3 after preparation and autoclaving. The pH was adjusted to 2 ~ 9, and MeSAc production by yeast was examined (Fig. 4). In alkaline pH range, the amount of MeSAc was greatly reduced, although yeast growth was observed. In pH 3 ~ 7, sufficient yeast growth took place, and almost the same levels of MeSAc accumulated in the broth. Therefore, the following experiments were performed without pH adjustment.

Course of MeSAc production

The production of MeSAc from MeSH by

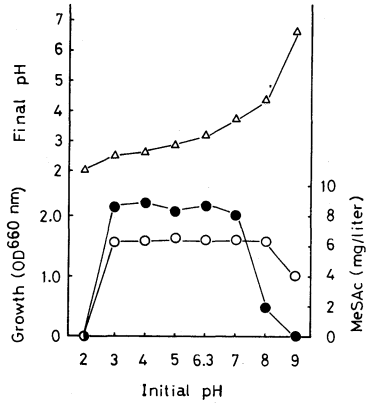

FIG. 4. Effect of Initial pH on Production of *S*-Methyl Thioacetate.

—○—, growth (OD at 660 nm); —●—, *S*-methyl thioacetate (mg/liter); —△—, final pH. The pH of medium was adjusted with 2 N HCl or 2 N NaOH.

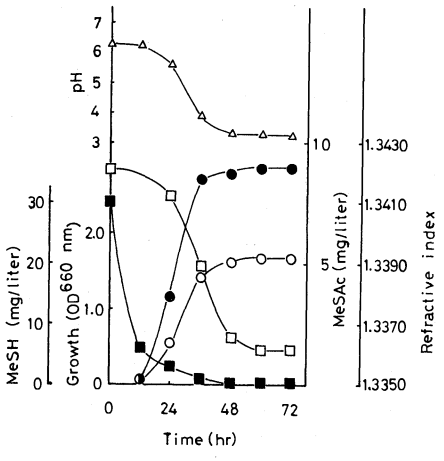

FIG. 5. The Course of *S*-Methyl Thioacetate Production.

—○—, growth (OD at 660 nm); —●—, *S*-methyl thioacetate (mg/liter); —□—, refractive index; —■—, methyl mercaptan (mg/liter); —△—, pH.

TABLE III. EFFECTS OF VARIOUS COMPOUNDS ON YEAST GROWTH AND PRODUCTION OF *S*-METHYL THIOACETATE

The agents were added to sterilized medium at a concentration of 0.1 mg/ml. Inoculation and cultivation were carried out under standard conditions.

Compound	Medium (pH)	Growth ($OD^{660\ nm}$)	MeSAc (mg/liter)
EDTA	5.1	0.28	7.6
α,α′-Dipyridyl	6.2	0	0
o-Phenanthroline	6.2	0	0
8-Oxyquinoline	6.2	0	0
NaN_3	6.2	0	0
KCN	3.3	1.57	1.9
CH_2ICOOH	5.1	0.56	8.8
PCMB	6.2	0	0
$NH_2OH \cdot HCl$	3.3	1.58	6.6
o-Phenyl phenol	6.2	0	0
$CuSO_4$	4.9	0.48	0
Control	3.3	1.63	8.3

growing yeast was followed during incubation, along with other parameters (Fig. 5). The yeast grew exponentially for 36 hr after a short lag to reach the maximum cell concentration after 60 hr of cultivation. MeSH in the medium decreased sharply from 30 to 6 mg/liter after 12 hr incubation, then gradually declined to 0.1 mg/liter after 36 hr. Although the accumulation of MeSAc after 12 hr of cultivation was only 0.2 mg/liter, a marked increase occurred during the next 24 hr in proportion to cell growth, reaching a maximum level of 8.6 mg/liter. In this period, the pH and refractive index values also decreased significantly.

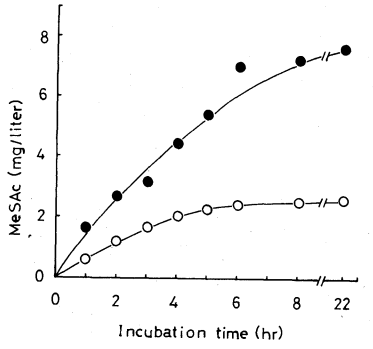

FIG. 6. The Course of *S*-Methyl Thioacetate Formation by Resting Cells.

Methyl mercaptan concentration: —○—, 5 mg/liter; —●—, 20 mg/liter.

Effect of various agents on MeSAc production

The effect of various agents listed in Table III was examined on yeast growth and MeSAc production. The concentration of the agents in medium was 0.1 mg/ml. In the presence of NaN_3, PCMB, *o*-phenylphenol and chelating agents other than EDTA (α,α′-dipyridyl, *o*-phenanthroline and 8-oxyquinoline), the yeast did not grow and MeSAc was not produced. On the other hand, the yeast grew and produced MeSAc in medium containing EDTA, KCN, CH_2ICOOH and $NH_2OH \cdot HCl$; however, yeast growth and MeSAc production were more or less inhibited. It was noted that $CuSO_4$ permitted yeast growth to some extent, but no MeSAc accumulation took place.

MeSAc formation by resting cells

The formation of MeSAc from MeSH by resting cells was examined. Yeast cells in the stationary growth phase were washed twice in succession by centrifuging the suspension in distilled water, and 5 g of centrifuged cells were resuspended into 200 ml of distilled water

TABLE IV. FORMATION OF *S*-METHYL THIOACETATE FROM METHYL MERCAPTAN BY RESTING CELLS

Yeast cells[a]	MeSH (5 mg/liter)	Residual substrate and product (mg/liter)					
		MeSH			MeSAc		
		2 hr	5 hr	20 hr	2 hr	5 hr	20 hr
No cells	+	4.4	4.2	4.2	0	0	0
Heat-treated cells[b]	+	3.1	2.2	2.0	0	0	0
Untreated cells	+	1.4	0.9	0.1	0.9	2.2	2.6
Untreated cells	−	0	0	0	0	0	0

[a] Resting cells were prepared by collecting yeast cells grown in MYGP medium at the stationary phase and washing twice with distilled water.
[b] Five grams of yeast was heated at 120°C for 30 min.

containing MeSH. The cell suspension was horizontally shaken at 25°C and subjected to MeSAc analysis. Figure 6 shows the course of MeSAc formation in yeast cell suspensions into which MeSH was added at concentrations of 5 and 20 mg/liter. The amounts of MeSAc in both suspensions were increased almost linearly with incubation time. After 22 hr of incubation, MeSAc levels reached 2.6 and 7.6 mg/liter. MeSAc was formed only when MeSH and intact resting cells were incubated together, and MeSAc was not produced by incubating: (a) MeSH alone, (b) MeSH and heat-treated cells, and (c) intact cells alone (Table IV). The results strongly suggest that MeSAc formation in yeast cell suspension was due to assimilation of MeSH by yeast.

These results led us to investigate the yeast ability to convert the thiol group of lower mercaptans into thioacetates. Thus, ethyl mercaptan (EtSH) and *n*-propyl mercaptan (*n*-PrSH) were independently incubated with resting yeast cells at a concentration of 10 mg/liter under the same conditions as MeSH. After 8 hr incubation, the suspension was centrifuged to remove yeast cells, and the supernatant (200 ml) was extracted with the same volume of CH_2Cl_2. The concentrates obtained by distilling CH_2Cl_2 under atmospheric pressure were analyzed by FPD-GC and GC-MS. Prominent peaks were detected on FPD-gas chromatogram at the retention times of 25 min (**I**) and 29 min (**II**). Mass spectra of these peaks were: (**I**) *m/z* (%): 104 (M^+, 17), 74 (24), 43 (100), 42 (62), 41 (45), 33 (51), 31 (48), and (**II**) *m/z* (%): 118 (M^+, 21), 106 (10), 91 (14), 76 (10), 43 (100), 42 (18), 41 (11). Each spectrum had a base peak at *m/z* 43, indicative of the presence of acetyl group in the molecule. Furthermore, molecular ion peaks and other fragment ion peaks supported the structure of $C_2H_5SCOCH_3$ for (**I**) and *n*-$C_3H_7SCOCH_3$ for (**II**). Since the compounds disappeared by incubating under alkaline conditions (pH 10 for 5 hr), it was also suggested that these sulfur compounds had an ester group in the molecule. From these results, it was concluded that the yeast was able to convert EtSH and *n*-PrSH into their acetyl derivatives.

MeSAc production from various sulfur compounds

Table V shows the production of MeSAc from various volatile and non-volatile sulfur compounds. Although MeSAc was produced from MeSH in quantity, and from dimethyl disulfide (DMDS) in small amounts, no accumulation of MeSAc occurred from other compounds. Since *S. cerevisiae* is known to have the ability to reduce the disulfide bond,[7] MeSAc accumulated in the broth with DMDS was probably attributed to the acetylation of MeSH formed as a result of DMDS reduction by yeast.

In the case of the culture media with EtSH

TABLE V. PRODUCTION OF *S*-METHYL THIOACETATE FROM VARIOUS SULFUR COMPOUNDS

Sulfur compound, volatile or non-volatile, was added into sterilized basal medium at a concentration of 30 mg/liter. Inoculation of yeast and cultivations were carried out under standard conditions.

Sulfur compound	Medium (pH)	Growth ($OD^{660\ nm}$)	MeSAc (mg/liter)
Methyl mercaptan	3.3	1.61	8.6
Ethyl mercaptan	3.2	1.59	0
n-Propyl mercaptan	3.1	1.58	0
Dimethyl sulfide	3.3	1.59	0
Dimethyl disulfide	3.1	1.59	0.2
3-Methylthiopropanal	3.3	1.58	0
3-Methylthiopropanol	3.2	1.60	0
M-Analog[a]	3.3	1.60	0
S-Methyl-L-cysteine	3.3	1.54	0
L-Cysteine	3.2	1.63	0

[a] 2-Hydroxy-4-methylthio butanoic acid.

TABLE VI. EFFECT OF $CuSO_4$ ON THE AMOUNT OF *S*-METHYL THIOACETATE PRODUCED FROM L-METHIONINE BY YEAST

Various amounts of L-methionine and $CuSO_4$ were added to sterilized basal medium (100 ml). Inoculation of yeast and cultivation were carried out under standard conditions.

Addition of L-methionine (mg/ml)	Yeast growth ($OD^{660\ nm}$)			MeSAc (mg/liter)		
	Addition of $CuSO_4$ (mg/liter)			Addition of $CuSO_4$ (mg/liter)		
	0	10	30	0	10	30
0	1.63	1.45	1.10	n.d.	n.d.	n.d.[a]
1.0	1.64	1.51	1.18	0.06	n.d.	n.d.
5.0	1.67	1.60	1.12	0.13	0.05	n.d.
10.0	1.64	1.56	1.36	0.14	0.04	n.d.

[a] Not detected: under 0.04 mg/liter.

and *n*-PrSH, the respective peaks of *S*-ethyl thioacetate (EtSAc) and *S*-*n*-propyl thioacetate were detected on FPD-gas chromatogram of headspace gas of the broth.

S. cerevisiae has already been shown to be capable of producing MeSAc from methionine.[3,6] In the present investigation, the production of MeSAc from L-methionine by *S. carlsbergensis* (strain No. 1) and the effect of copper ions on its production were examined. As shown in Table VI, yeast propagated at a high L-methionine level of 10 mg/ml and accumulated MeSAc in broth, though yields based on L-methionine were much lower than those from MeSH. These results were almost the same as in the case of *S. cerevisiae* IFO 1234. However, the amount of MeSAc was significantly reduced by adding copper ions into the medium.

DISCUSSION

Volatile esters such as ethyl and isoamyl acetate are important flavor components in beer and are produced by brewer's yeast during fermentation of wort.[8] The biosynthesis of these esters in yeast has been proposed to occur *via* alcoholysis of acyl-CoA.[9] Howard and Anderson[10] succeeded in synthesis *in vitro* of ethyl acetate from ethanol and acetyl-CoA using a cell free preparation from *S. cerevisiae*. Similar results were also reported on *sake* yeast[11] and *Cladosporium* sp.[12]

In spite of many studies on ester production by microorganisms, little was known about microbial production of thioesters until the reports on MeSAc production by *Brevibacterium linens*[13] and *Pseudomonas fluorescens*.[14] Cuer *et al.*[5] reported on MeSAc production by *Micrococcus* in dairy medium enriched with MeSH (2 g/liter). We have reported in the previous paper[6] that *S. cerevisiae* (IFO 1234) was able to produce a noticeable amount of MeSAc from MeSH, with much higher yields than those by *Micrococcus* cheese strains.

It has been confirmed in the present work that a number of different strains of *Saccharomyces* brewer's yeasts were able to produce MeSAc from MeSH, and that under the optimum condition, yeast (strain No. 1) produced 98 mg MeSAc per liter in medium containing 0.5 g/liter of MeSH. Yeast could not grow in a high concentration of MeSH (over 1 g/liter), and no MeSAc was accumulated in medium. On the other hand, the yeast grew in medium with a larger amount of L-

methionine and produced MeSAc. However, the yield of MeSAc from L-methionine was extremely low compared with MeSH.

Yeast metabolizes methionine into several sulfur compounds, such as 3-methylthiopropanol, 2-hydroxy-4-methylthio butanoic acid, 2-methyl-tetrahydrothiophene-3-one,[15,16] and the presence of MeSH in culture broth of *S. cerevisiae* with L-methionine is already known.[3,6] Microbial production of MeSH from L-methionine is well known,[17] and methioninase catalyzing the conversion of L-methionine into α-ketobutyrate, MeSH and ammonia has been demonstrated in bacterial strains, *Pseudomonas putida*,[18] *Ps. ovalis*[19] and *Clostridium sporogenes*.[20] Although the presence of this enzyme in *Saccharomyces* yeast has not been reported as far as we know, the present work confirmed that a small amount of MeSH existed in yeast growth media with L-methionine added. It is, therefore, reasonable to assume that MeSAc in broth is produced from MeSH formed by the enzymatic degradation of L-methionine. The use of this pathway might also be supported indirectly by the observation that the addition of copper ions into the medium containing L-methionine significantly reduced the amount of MeSAc produced. Since growing *Saccharomyces* yeast incorporates copper ions,[21] a decrease of MeSAc in the culture medium might be ascribed to the formation, either intra- or extracellular, of copper methylmercaptide from copper ions and MeSH derived from L-methionine.

In the brewing process, the MeSH level in wort ($2 \sim 4\ \mu g$/liter) is sharply reduced to as low as $0.1\ \mu g$/liter after yeast pitching, and then gradually increases to $0.6 \sim 0.8\ \mu g$/liter toward the end of fermentation.[22] The rapid reduction of MeSH in wort during the first $2 \sim 3$ days of fermentation is due to absorption by yeast of MeSH in wort and its probable conversion into MeSAc. However, MeSH in wort does not account for all the MeSAc contained in beer, since the average level of MeSAc in beer has been reported to be $4\ \mu g$/liter[2] and $17\ \mu g$/liter.[3] Yeast would also metabolize methionine in wort to give MeSAc. In short, it appears reasonable that MeSAc in beer originates mainly from MeSH and L-methionine in wort. Further studies are underway to control MeSAc formation in the brewing processes.

It is of interest that acetyl derivatives of MeSH, EtSH and *n*-PrSH were easily formed by growing and resting yeast cells. A small amount of EtSAc has recently been shown to be present in beer.[3] Since EtSH is produced in green beer during fermentation,[22] EtSAc contained in finished beer might be produced from EtSH by yeast activity. These considerations suggest the general ability of *Saccharomyces* yeast to acetylate lower mercaptans into thioacetates, although a more elaborate study is necessary to demonstrate this assumption. This biological reaction seems to be a self-defense mechanism of yeast against mercaptans toxic to its viability.

The formation of ethyl acetate in fermentation by *S. cerevisiae* has been demonstrated to be an energy-requiring process.[23] However, since MeSAc is synthesized not only by growing cells, but it also occurs in cellular suspension containing MeSH, co-factors such as acetyl-CoA are already accumulated in resting cells. Further work on cell free synthesis of MeSAc from MeSH and/or possible participation of enzymes in the reaction will provide valuable information on yeast thioester synthesis.

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