Behaviors of 3-Mercaptohexan-1-ol and 3-Mercaptohexyl Acetate During Brewing Processes

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ABSTRACT

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The behaviors of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) during brewing processes were examined to establish their contributions to the distinctive beer aroma. In hops, 3MH was detected in both U.S. and European cultivars. The concentration differed between cultivars, ranging from 10 to 120 µg/kg of pellet, whereas the occurrence of 4-mercapto-4-methylpentan-2-one (4MMP) depended on the growing region as well as the cultivar—4MMP was not detected in European cultivars. The addition of copper granules to beer indicated that 4MMP was more susceptible than 3MH to binding with copper, which may corroborate the fact that the growing regions of cultivars have little effect on 3MH content. During the boiling process of hopped wort, the 4MMP content decreased, whereas the 3MH content increased at a faster rate at 100°C than at 90°C. During fermentation, the 3MH and 4MMP contents increased and peaked at an early stage of the process. 3MH also was detected in measurable amounts in the hop pellets of all cultivars and in unhopped beer but not in unhopped wort. This finding indicates that both malt and hops are a source of 3MH precursors. Little or no 3MHA was detected in hop pellets. It was revealed that 3MHA was synthesized during fermentation from 3MH and had a lower threshold value than 3MH. This study suggests that selecting yeast strains with a high ability to convert thiols from 3MH to 3MHA is a useful strategy to increase the aroma impact of beer.

Keywords: Hop, 3-Mercaptohexan-1-ol, 3-Mercaptohexyl acetate, 4-Mercapto-4-methylpentan-2-one, Thiol, Threshold value

RESUMEN

Los comportamientos de 3-mercaptohexan-1-ol (3MH) y el acetato 3-mercaptohexil (3MHA) durante los procesos de la fabricación de cerveza fueron examinados para establecer sus contribuciones al aroma distintivo de cerveza. En lúpulo, 3MH fue descubierto tanto en cultivares estadounidense y de europeo. La concentración difiere entre cultivares, en los límites de 10 a 120 µg/kg de pellet, mientras que la presencia de 4-mercapto-4-metilpentan-2-ona (4MMP) dependió de la región creciente así como el cultivar-4MMP no fue descubierta en cultivares de europeo. La adición de gránulos de cobre a la cerveza indicó que 4MMP era más susceptible que 3MH a la fijación con el cobre, que puede corroborar el hecho que las regiones crecientes de cultivares tienen poco efecto sobre 3MH el contenido. Durante el proceso de hervir el mosto con lúpulo, el contenido de 4MMP se disminuyó, mientras que el contenido de 3MH se aumentó en una velocidad más rápida en 100°C que en 90°C. Durante la fermentación, el contenido de 3MH y 4MMP aumentó y alcanzó su punto máximo en una etapa temprana del proceso. 3MH también fue descubierto en cantidades mensurables en pellets de lúpulo de todo los cultivares y en la cerveza sin lúpulo, pero no en mosto sin lúpulo. Este hallazgo indica que tanto la malta y lúpulo son una fuente de 3MH precursores. Poco o ningún 3MHA fue descubierto en pellets de lúpulo. Fue revelado que 3MHA fue sintetizado durante la fermentación de 3MH y tenía un valor de umbral inferior que 3MH. Este estudio sugiere que la selección de tensiones de levadura con una alta capacidad de convertir tioles de 3MH a 3MHA sea una estrategia útil de aumentar el impacto de aroma de la cerveza.

Palabras claves: Lúpulo, 3-Mercaptohexan-1-ol, 3-Mercaptohexil acetato, 4-Mercapto-4-methilpentan-2-ona, Tiol, Valor de umbral

To determine the components that contribute to the sensory hop aroma characteristics of beer, we previously identified several hop-derived potent odorants that persist even after fermentation (4,5,8–11); thiols were included in these odorants. Based on the results of gas chromatography-olfactometry (GC-O) analysis (5,7), we detected seven hop-derived volatile thiols with muscat or blackcurrant-like aromas, and GC mass spectrometry (GC/MS) and GC-O analyses indicated that 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA) were present. 4MMP has been found to contribute to the aroma in beer from cv. Cascade (5–7,8,11,12). Thiols are well known for their extremely low threshold values (below 100 ng/L) and contributions to the fruity aroma of beer or wine (1,12,13,16,17,20,21).

The 4MMP contents in different hop cultivars also were detected and compared (5,7) in cultivars from the United States, Australia, and New Zealand, but not in those from Europe. The behaviors of hop-derived 3MH and 3MHA have not previously been examined, although the existence of volatile thiols in beer seems to affect the overall flavors. 3MH and 3MHA initially were identified in passion fruit (2) and also have been identified in Sauvignon Blanc wines (18). Darriet et al (1) and Tominaga et al (17,20) reported the effects of 4MMP, 3MH, and 3MHA on wine flavors. Vermeulen et al (22) reported the presence of 3MH in fresh lager beers. The current study investigated the behaviors of 3MH and 3MHA during brewing processes to retain these thiols in beer and establish their contributions to the distinctive beer aroma.

EXPERIMENTAL

Chemicals

Dowex (1 × 2, Cl(–)-form, strongly basic, 50–100 mesh; Chemical Abstracts Service [CAS] no. 69011-19-4) was obtained from Sigma-Aldrich (St. Louis). *p*-Hydroxymercuribenzoic acid sodium salt (CAS no. 138-85-2) was purchased from Acros Organics (Morris Plains, NJ). 4MMP (CAS no. 19872-52-7) and 4-methoxy-2-methyl-2-mercaptobutane were obtained from San-Ei Gen FFI, Inc. (Osaka, Japan). *tert*-Butyl-4-methoxyphenol (CAS no. 25013-16-5) and L-cysteine hydrochloride monohydrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Tris(hydroxymethyl)-aminomethane (CAS no. 77-86-1), HNO_3 for inductively coupled plasma mass spectroscopy (ICP-MS) analysis, yttrium and indium for atomic-absorption analysis, and copper granules were purchased from Kanto Chemical Co., Inc. (Tokyo). 3MH (CAS no. 51755-83-0) was purchased from Avocado Research Chemicals Ltd. (Heysham, UK). 3MHA (CAS no. 136954-20-6) was purchased from Atlantic Research Chemicals Ltd. (Bude, UK).

Hops

The hop cvs. Simcoe (2005 and 2006 crops, United States) and Topaz (2007 crop, Australia) were purchased from Yakima Chief (Sunnyside, WA) and Hop Products Australia (Tasmania, Australia),

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respectively. Cvs. Hersbrucker (2005 crop, Germany), Saazer (2005 crop, Czech Republic), Fuggle (2005 crop, United Kingdom), and Perle (2006 crop, Germany) were purchased from Joh. Barth & Sohn GmbH & Co. (Nuremberg, Germany). Cvs. Summit (2006 crop, United States), Millennium (2006 crop, United States), and Nugget (2005 crop, United States) were purchased from John I. Haas, Inc. (Yakima, WA). Cvs. Apollo (2006 crop, United States), Cascade (2006 crops, United States), Willamette (2006 crop, United States), and Perle (2006 crop, United States) were purchased from S.S. Steiner, Inc. (New York). Cvs. Magnum (2006 crop, Germany), Taurus (2006 crop, Germany), Nugget (2006 crop, Germany), and Pacific Gem (2006 crop, New Zealand) were purchased from Simon H. Steiner, Hopfen, GmbH (Mainburg, Germany).

Brewing Processes

Pellets of hop cvs. Simcoe, Summit, Apollo, Willamette, and Fuggle were used in the brewing processes. Hopped and unhopped beers were brewed independently in 20-L volumes as described previously (5,7). To trace the changes in the 3MH and 4MMP concentrations during wort boiling, 50 g of cv. Simcoe pellets was added to 20 L of wort at the beginning of the brewing process, and the mixture then either was boiled at an intensity of 8% or kept at 90°C.

Determination of Difference Threshold Values

The difference threshold values of 3MHA were determined using the method of the American Society of Brewing Chemists, as described previously (11).

Isolation and Quantification of Volatile Thiols

Volatile thiols were isolated from the hop pellets, wort, and beer using a strongly basic anion-exchanger resin (Dowex 1) and *p*-hydroxymercuribenzoate (*p*HMB) with elution columns, as described previously (5,7).

Quantification of Thiols by Multidimensional GC/MS

The 3MH and 3MHA contents in the wort and beer were quantified using multidimensional (MD)-GC/MS as described previously (5,7).

Quantification of Divalent Metal Ions

Divalent metal ions were quantified, as described previously (5,7), using ICP-MS (model 7500c, Agilent Technologies, Santa Clara, CA). Prior to the ICP-MS analysis, the samples were digested in closed polytetrafluoroethylene vessels using a microwave sample-digestion system (Multiwave 3000, PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA).

Lead Conductance Values of Hop Pellets

The lead conductance values of the hops studied were determined using European Brewing Convention (EBC) method 7.4 (3).

RESULTS AND DISCUSSION

In our previous studies (5,7), we investigated the thiols found in beers hopped with cultivars from the United States and Europe, using an extraction method involving solvent-assisted flavor evaporation, a strongly basic anion-exchanger resin (Dowex 1), and *p*HMB. Seven odorants that contributed to a blackcurrant-like aroma, which is often attributed to the presence of thiols, were detected by GC-O analysis. Three of these seven odorants were identified as 4MMP, 3MH, and 3MHA through comparison of their mass spectra, Kovats retention indices, and odor qualities with those of authentic compounds on both DB-WAX and HP-5 capillary columns by GC-O and GC-MS. The 17 hop cultivars examined in the current study and their origins, crop years, and abbreviations, plus the lead conductance values of α-acids in the hop pellets, are shown in Table I.

3MH and 3MHA Concentrations in Hop Pellets

The 3MH content in hop pellets (Fig. 1) was determined as described previously (5,7). 3MH was detected in hop pellets from both U.S. and European cultivars (Fig. 1), whereas 3MHA content was below the detection limit. The results are presented as the mean values of duplicated analyses, with the coefficients of variation (3MH = 23.2% and 3MHA = 5.4%) and detection limits (3MH = <1.50 and 3MHA = <0.17 μg/kg of pellet, where the height of the signal was threefold that of the noise). The results showed that the 3MH concentration differed between cultivars, ranging from 10 to 120 μg/kg of pellet. The highest 3MH content was detected in the cv. Simcoe (2006, United States) hop pellets, followed by cvs. Topaz (Australia), Fuggle (United Kingdom), and Cascade (Unites States). We previously reported (5,7) that the content of 4MMP depended on the growing region as well as the cultivar. 4MMP was detected in the hop pellets of cultivars from the United States, Aus-

TABLE I
Abbreviations, Origins, Crop Years, and Lead Conductance Values (LCV) of α-Acids for Hop Cultivars Studied

Cultivar	Origin	Abbreviation	Crop Year	LCV of α-Acids (%)
Simcoe	United States	US-SIM	2005	10.4
Simcoe	United States	US-SIM	2006	10.4
Summit	United States	US-SUM	2006	16.3
Apollo	United States	US-APO	2006	17.1
Millennium	United States	US-MIL	2006	15.1
Cascade	United States	US-CAS	2006	5.5
Willamette	United States	US-WIL	2006	3.4
Topaz	Australia	AU-TPZ	2007	13.6
Pacific Gem	New Zealand	NZ-PGM	2006	14.0
Perle	United States	US-PEL	2006	7.0
Perle	Germany	GE-PEL	2006	10.7
Nugget	United States	US-NUG	2005	11.9
Nugget	Germany	GE-NUG	2006	11.3
Magnum	Germany	GE-MAG	2006	12.5
Taurus	Germany	GE-TAU	2006	13.7
Hersbrucker	Germany	GE-HER	2005	2.9
Saazer	Czech Republic	CZ-SAZ	2005	5.4
Fuggle	United Kingdom	UK-FGL	2005	4.6

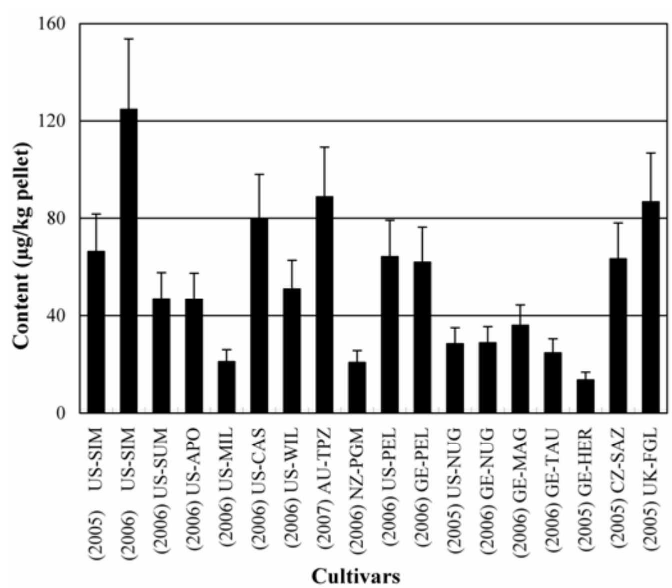

Fig. 1. 3-Mercaptohexan-1-ol content in hop pellets of different cultivars. Cultivar abbreviations are listed in Table I.

tralia, and New Zealand, but not from Europe. Our previous studies (5,7) suggested that this phenomenon could be caused not only by a genetic effect but also by the use of copper sulfate (Bordeaux mixture) in Europe to protect against downy mildew.

3MH content did not correlate with that of 4MMP. The reasons for little or no effect of growing region on 3MH content were unclear. The effect of copper was examined through the addition of excess copper granules to beer. Copper granules (50 g) were added to beer (250 mL) hopped with cvs. Apollo and Simcoe and then left overnight at 4°C. The 4MMP content decreased by 50% following the addition of copper granules (Fig. 2), whereas the 3MH and 3MHA contents remained unchanged. These results confirmed that 4MMP is adsorbed by copper ions and is more susceptible to copper ions than 3MH. They also corroborate the fact that 3MH content was not affected by growing region or copper content (Fig. 1).

Even within a single cultivar, 3MH content differed greatly between crop years (Fig. 1). This was particularly evident in cv. Simcoe hop pellets, in which the 3MH content of the 2006 crop was twice that of the 2005 crop, whereas the α-acid and copper ions were relatively unchanged.

3MH and 3MHA Contents in Beers

The 3MH and 3MHA contents in worts and beers hopped with cvs. Simcoe, Summit, Apollo, Willamette, and Fuggle are shown in Figure 3A and B, respectively. The results are presented as the mean values of duplicated analyses, with the coefficients of variation: 3MH = 7.0% in wort and 6.3% in beer (Fig. 3A) and 3MHA = 4.9% in wort and 8.4% in beer (Fig. 3B). The detection limits were 3MH = <12.0 and <10.2 ng/L in wort and beer, respectively, and 3MHA = <4.4 and <3.9 ng/L in wort and beer, respectively, where the height of the signal was threefold that of the noise. To investigate the aroma quality of hop-derived components that can survive even after fermentation, without causing temperature effects on thiols with low boiling points, the hops were added after the wort-cooling process as described in previous studies (5,7).

3MH was detected in the beers of all cultivars in the range of 35 to 59 ng/L, with the highest found in cv. Simcoe hopped beer, followed by cv. Willamette and cv. Apollo hopped beers. The contents were close to or below the difference threshold value (55 ng/L) (11).

Behavior of Volatile Thiols During the Brewing Process

During the wort-boiling process, 4MMP content decreased by 91% after boiling at 100°C for 60 min and by 23% after heating at 90°C (Fig. 4A). Interestingly, with hopped wort, 3MH content increased (Fig. 4B) at a faster rate at 100°C (at which the content increased threefold) than at 90°C. Furthermore, 3MH was not detected in unhopped wort. These results indicate that 3MH was formed thermally during the wort-boiling process from precursors that existed in the hops. During fermentation, the 3MH (Fig. 5A) and 4MMP

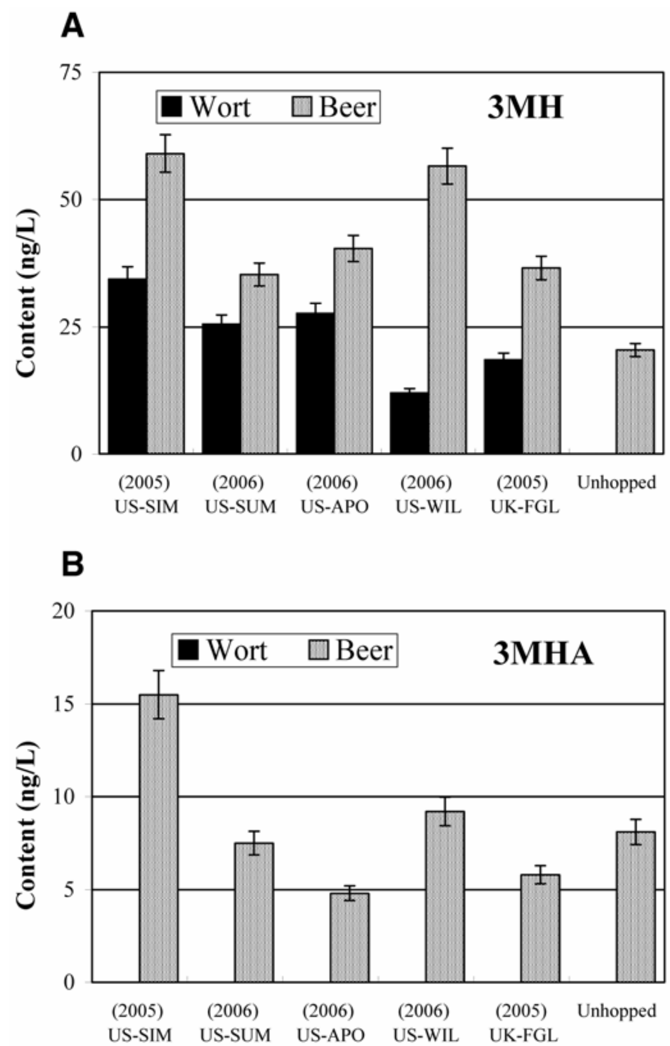

Fig. 3. A, 3-Mercaptohexan-1-ol (3MH) and **B,** 3-mercaptohexyl acetate (3MHA) contents in beers and worts hopped with five cultivars. Cultivar abbreviations are listed in Table I.

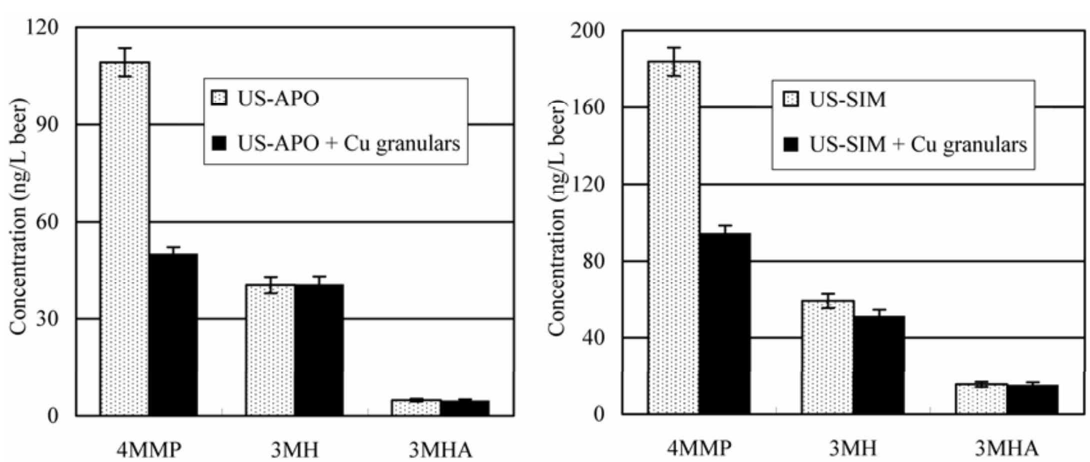

Fig. 2. Comparison of 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA) contents in beers hopped with cvs. Apollo (US-APO) and Simcoe (US-SIM), with and without added copper granules.

contents also increased (5,7) and peaked at early stages of fermentation (Fig. 5A), as previously observed in wine (15).

Although 4MMP was not detected in both unhopped wort and beer (5,7), measurable amounts of 3MH were detected in hop pellets of various cultivars (Fig. 1) and even in unhopped beer, but not in unhopped wort (Fig. 3A), indicating that malt and hops both contain 3MH precursors.

Previous studies (15,19) have reported on cysteine conjugate precursors that lead to the release of 3MH and 4MMP during fermentation processes. Vermeulen et al (22) proposed that the synthetic pathways of 3MH from precursors are formed by the biolysis of cysteine conjugate or by Michael addition of H_2S on (*E*)-2-hexenal followed by a reduction. In the present study, it was unclear why 3MH increased or released into wort during boiling (Fig. 4B). Further research is required to clarify the formation of 3MH from precursors such as cysteine conjugate or glycosides.

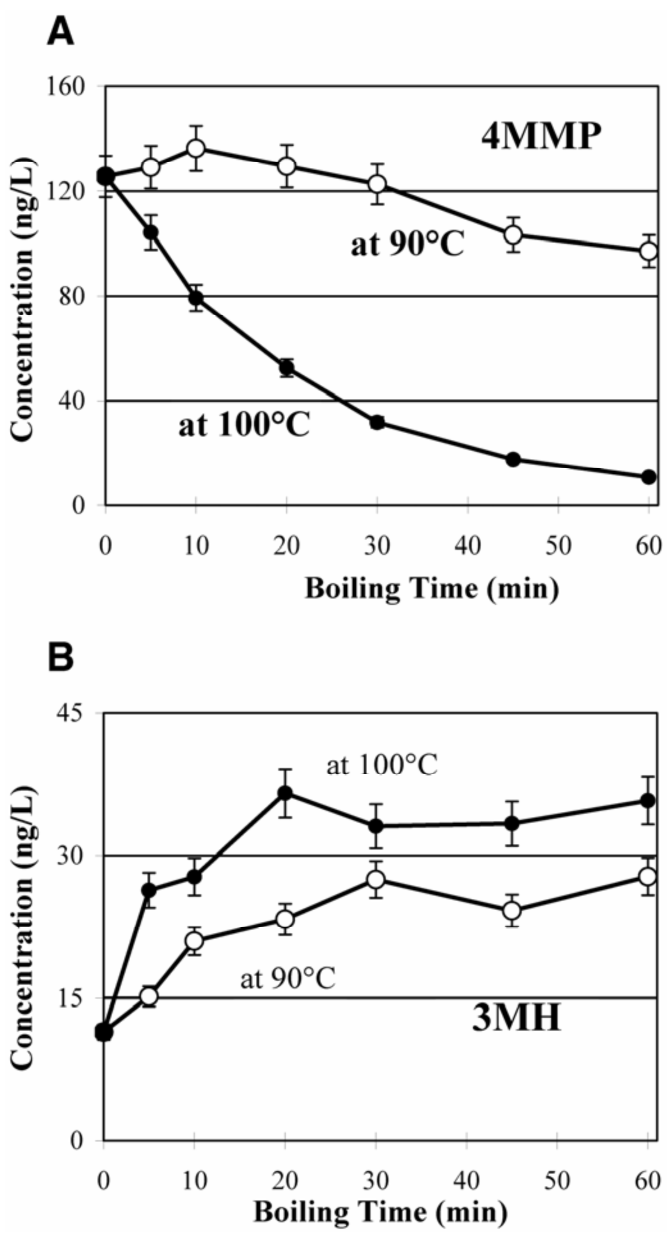

Fig. 4. Changes in **A,** 4-mercapto-4-methylpentan-2-one (4MMP) and **B,** 3-mercaptohexan-1-ol (3MH) concentrations during boiling of wort hopped with cv. Simcoe.

3MHA was not detected in the worts (Fig. 5B) and was newly synthesized (Fig. 3B) from 3MH by the action of a yeast ester-forming alcohol acetyltransferase (14,15). Yeasts with a high capacity to convert 3MH into 3MHA were studied in the literature (14,15). The difference threshold value of 3MHA was newly determined in the current study as 5.0 ng/L, which was lower than that of 3MH (55 ng/L) (11). Therefore, selecting yeast strains with a higher capacity for bioconversion of 3MH into 3MHA is a useful strategy to increase the aroma impact of beer.

CONCLUSIONS

The behaviors of 3MH, 3MHA, and 4MMP were examined during brewing processes to retain these thiols, which give beers their

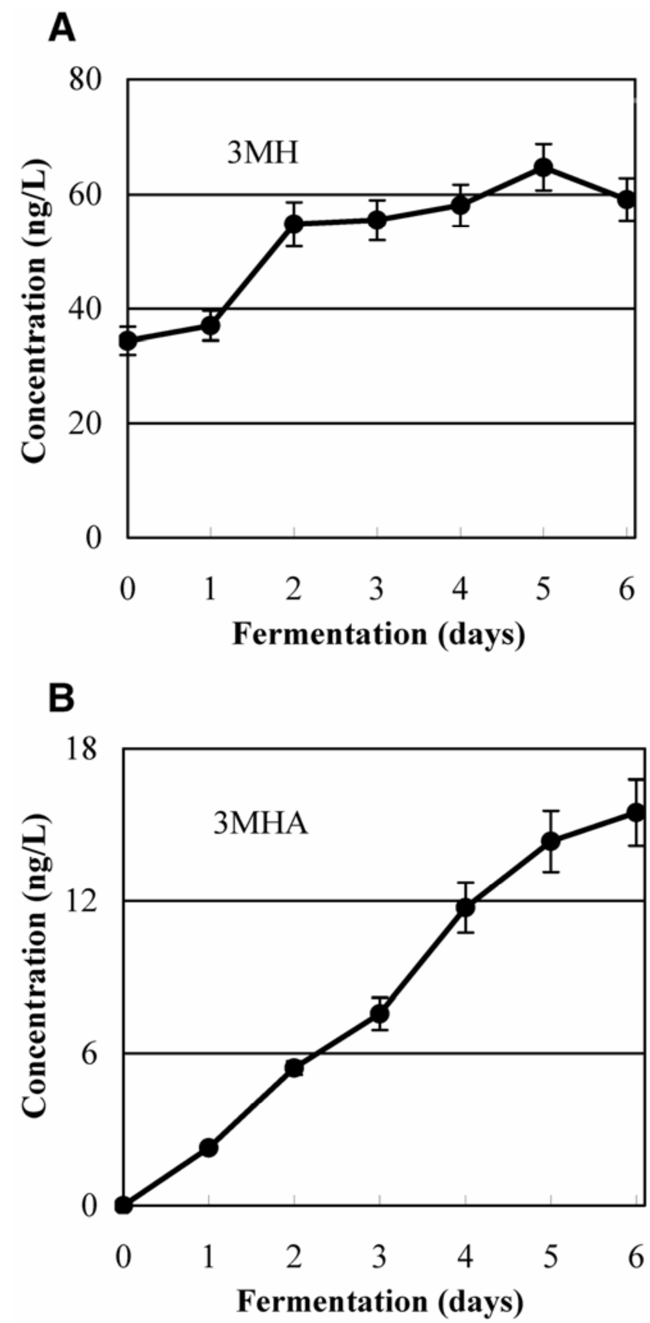

Fig. 5. Changes in **A,** 3-mercaptohexan-1-ol (3MH) and **B,** 3-mercaptohexyl acetate (3MHA) concentrations during fermentation of wort hopped with cv. Simcoe.

characteristic fruity aroma. 3MH was detected in hop cultivars from both the United States and Europe, whereas 4MMP was not detected in European cultivars. During the boiling process of hopped wort, 4MMP content decreased, whereas 3MH content increased at a faster rate at 100°C than at 90°C. During fermentation, the 3MH and 4MMP contents increased and peaked at an early stage of the process. 3MH was not detected in unhopped wort but was present in unhopped beer. These findings indicate that malt and hops both may contain 3MH precursors. It is known that 3MHA is synthesized during fermentation. We determined that 3MHA had a lower threshold value than 3MH. Thus, the selection of yeast strains with a higher capacity to convert thiols from 3MH to 3MHA might be a useful, efficient strategy to increase the aroma impact of beer.

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