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Volatile Compounds and the Changes in Their Concentration Levels during Storage in Beers Containing Varying Malt Concentrations

H. Tsuji and A. Mizuno

ABSTRACT: Volatile compounds in beers brewed with different amounts of malt were analyzed by using the stir bar sorptive extraction–gas chromatography-mass spectrometry method. We identified 90 compounds—25 esters, 17 terpenes, 14 alcohols, 11 acids, 6 furans, 6 aroma compounds, 5 carbonyls, and other compounds. An analysis of aged beer suggested that the concentration levels of stale flavor compounds—β-damascenone, γ-nonalactone, ethyl cinnamate, and 2-methoxy-4-vinylphenol—in nonmalt beer were different from those in all-malt and standard beer. Additionally, concentrations of these compounds did not increase during storage in most nonmalt beer analyzed in this study. Nerolidol may be a good marker candidate regardless of the malt content.

Keywords: beer, malt, SBSE, stale flavor, volatile compounds

Introduction

Beer containing a small amount of malt is produced in Japan, primarily because the Japanese government imposes taxes on beer based on malt content. Based on malt content, beer and beer-like alcohol beverages in Japan are mainly categorized into 4 types—all-malt beer (100%), standard beer (>67%), low-malt beer (<25%), and nonmalt beer (0%). Low-malt beer has gained popularity because of only its low price and a consumer preference for light taste.

Low-malt beer contains a higher amount of alcohol and sulfur compounds than does standard beer (Kondo 2005). Although these compounds contribute to the aromas of many food products, beer containing a higher amount of these compounds is sometimes regarded as off-flavor beer (Landaud and others 2008). This off-flavor is mainly attributable to nitrogen starvation conditions during yeast fermentation (Kondo 2005). The pH and total amount of polyphenol is lower in nonmalt beer than in standard beer (Shimizu and others 2002). The amount of polyphenol in beer strongly affects flavor stability (Guido and others 2007), and low pH is also responsible for a stale flavor (Gijs and others 2002). Therefore, the flavor and stability of nonmalt beer must be different from those of standard beer. However, a comprehensive study of volatiles in nonmalt beer has not been reported.

Aging of beer is a major quality problem because it changes the taste of beer, which can be sometimes unpleasant (Vanderhaegen and others 2006). Many studies have been conducted for detecting and decreasing the amount of stale flavor compounds in beer. Although most stale flavor compounds are present only in trace amounts in beer, they are extremely important for determining the flavor profile, because they have a low-odor threshold. Several sample preparation techniques such as solid phase microextraction (SPME) have been used for the analysis of trace elements in beer (Pinho and others 2006). SPME is a solvent-free extraction technique that allows for simultaneous extraction and concentration. SPME has very good reproducibility but limited sensitivity, because the amount of sorptive material that can be coated on the fibers is limited. Stir bar sorptive extraction (SBSE), on the other hand, allows us to detect compounds that are present in very small amounts. SBSE has been applied to the analysis of fatty acids (Horák and others 2007), terpenoids (Kishimoto and others 2006), and carbonyls (Ochiai and others 2003) in beer.

In this study, we compared volatile compositions of beers containing different amounts of malts by using SBSE with gas chromatography-mass spectrometry (GC-MS). Additionally, the pattern of changes in these beers during storage was also noted.

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Materials and Methods

Chemicals

Isobutyl acetate (Chemical Abstracts Service [CAS nr 110-19-0]), ethyl butyrate (CAS nr 105-54-4), ethyl isovalerate (CAS nr 108-64-5), 2-methyl-1-propanol (CAS nr 78-83-1), isopentyl acetate (CAS nr 123-92-2), myrcene (CAS nr 123-35-3), isoamyl isobutyrate (CAS nr 2050-1-3), 3-methyl-1-butanol (CAS nr 123-51-3), ethyl hexanoate (CAS nr 123-66-0), hexyl acetate (CAS nr 142-92-7), ethyl heptanoate (CAS nr 106-30-9), 1-hexanol (CAS nr 111-27-3), heptyl acetate (CAS nr 112-06-1), 2-nonanone (CAS nr 821-55-6), nonanal (CAS nr 124-19-6), ethyl octanoate (CAS nr 106-32-1), 1-heptanol (CAS nr 53535-33-4), acetic acid (CAS nr 64-19-7), 2-furaldehyde (CAS nr 98-01-1), octyl acetate (CAS nr 112-14-1), 2-ethyl-1-hexanol (CAS nr 104-76-7), 2-nonanol (CAS nr 628-99-9), ethyl nonanoate (CAS nr 123-29-5), linalool (CAS nr 78-70-6), 1-octanol (CAS nr 111-87-5), isobutyric acid (CAS nr 79-31-2), 5-methyl-2-furaldehyde (CAS nr 620-02-0), 2-undecanone (CAS nr 112-12-9), ethyl decanoate (CAS nr 110-38-3), isoamyl n-caprylate (CAS nr 2035-99-6), 3,7-dimethyl-6-octen-1-yl acetate (CAS nr 150-84-5), furfuryl alcohol (CAS nr 98-00-0), ethyl 4-decenoate (CAS nr 76649-16-6), isovaleric acid (CAS nr 503-74-2), 2-methyl-butyric acid (CAS nr 116-53-0), diethyl succinate (CAS nr 123-25-1), 2-undecanol (CAS nr 1653-30-1), 3-(methylthio)-1-propanol (CAS nr 505-10-2), α-farnesene (CAS nr 502-61-4), 1-decanol (CAS nr 112-30-1), citronellol (CAS nr 106-22-9), 9-decen-1-ol (CAS nr 13019-22-2),

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Table 1 – Compounds identified in fresh beers containing different amounts of malt.

RI	Compound	Percent (%) in total area			
		NM	LM	HM	AM
1007	Isobutyl acetate[a]	0.074 to 0.175	0.184 to 0.265	0.142 to 0.233	0.108 to 0.169
1036	Ethyl butyrate[a]	0.169 to 0.331	0.224 to 0.307	0.241 to 0.311	0.256 to 0.277
1100	Ethyl isovalerate[a]	0.005 to 0.031	nd to 0.027	nd to 0.001	nd to 0.01
1100	2,7-Dimethyl-1,6-octadiene	0.755 to 2.11	0.231 to 0.835	0.348 to 0.548	0.355 to 0.891
1105	2-Methyl-1-propanol[a]	+0.094 to 0.39	0.106 to 0.285	0.063 to 0.078	0.06 to 0.091
1143	Isopentyl acetate[a]	11 to 25.533	16.567 to 18.867	18.1 to 22.733	15.167 to 23.767
1169	Myrcene[a]	0.007 to 0.576	nd to 0.191	nd	nd to 0.329
1207	D-Limonene	nd to 0.006	nd	nd	nd to 0.005
1207	Isoamyl isobutyrate[a]	+0.006 to 0.045	nd to 0.011	nd to 0.008	nd to 0.002
1219	3-Methyl-1-butanol[a]	+7.753 to 15.733	7.74 to 12.133	6.993 to 8.853	6.04 to 10.043
1243	Ethyl hexanoate[a]	–2.18 to 4.067	3.407 to 5.617	3.523 to 5.42	4.293 to 5.637
1284	Hexyl acetate[a]	–0.014 to 0.088	0.046 to 0.121	0.107 to 0.15	0.208 to 0.257
1297	Methyl 2-methyl-heptanoate[b]	nd to 0.033	nd to 0.012	nd	nd to 0.024
1303	Ethyl 4(E)-hexenoate[b]	nd to 0.02	0.001 to 0.104	0.003 to 0.012	0.01 to 0.012
1328	3-Methyl-2-buten-1-ol[a]	+0.029 to 0.09	0.004 to 0.009	0.006 to 0.011	0.01 to 0.011
1342	Ethyl heptanoate[a]	0.032 to 0.162	0.033 to 0.086	0.052 to 0.089	0.054 to 0.077
1346	Methyl 4-methylene-hexanoate[b]	nd to 0.226	nd to 0.133	nd	nd to 0.34
1357	1-Hexanol[a]	nd to 0.03	0.004 to 0.017	0.014 to 0.014	0.016 to 0.021
1383	Heptyl acetate[a]	–0.011 to 0.057	0.033 to 0.109	0.088 to 0.127	0.1 to 0.159
1403	2-Nonanone[a]	nd to 0.013	nd to 0.011	nd to 0.003	nd to 0.007
1407	Nonanal[a]	0.004 to 0.011	0.006 to 0.009	0.003 to 0.009	nd to 0.006
1430	3-(4-Methyl-3-pentenyl)-furan[b]	0.002 to 0.01	nd to 0.008	nd to 0.001	0.002 to 0.007
1443	Ethyl octanoate[a]	–6.913 to 14.733	11.367 to 16.533	14.667 to 16.2	12.8 to 16.7
1459	1-Heptanol[a]	nd to 0.029	0.002 to 0.012	0.006 to 0.01	0.007 to 0.008
1464	Acetic acid	0.073 to 0.116	0.035 to 0.106	0.041 to 0.094	0.023 to 0.085
1464	Isopentyl hexanoate	nd to 0.003	nd to 0.007	0.002 to 0.008	0.001 to 0.006
1482	2-Furaldehyde[a]	0.011 to 0.041	nd to 0.018	nd to 0.01	0.007 to 0.011
1484	Octyl acetate[a]	0.032 to 0.295	0.119 to 0.267	0.112 to 0.23	0.171 to 0.307
1494	2-Ethyl-1-hexanol[a]	+0.039 to 0.047	0.025 to 0.044	0.013 to 0.061	0.012 to 0.03
1522	2-Nonanol[a]	0.002 to 0.057	nd to 0.032	nd	nd to 0.075
1545	Ethyl nonanoate[a]	–0.01 to 0.024	0.018 to 0.041	0.022 to 0.047	0.014 to 0.046
1549	Furfuryl acetate[a]	nd	nd to 0.002	nd to 0.005	0.003 to 0.01
1552	Linalool[a]	0.037 to 0.142	nd to 0.074	0.005 to 0.023	0.007 to 0.115
1561	1-Octanol[a]	0.06 to 0.11	0.084 to 0.156	0.076 to 0.117	0.094 to 0.17
1574	α-Ionene	nd	nd	nd	nd
1579	Isobutyric acid[a]	+0.065 to 0.123	0.036 to 0.087	0.051 to 0.063	0.048 to 0.066
1592	5-Methyl-2-furaldehyde[a]	0.004 to 0.006	nd to 0.004	0.002 to 0.002	0.002 to 0.004
1609	2-Undecanone[a]	nd to 0.039	nd to 0.05	nd	nd to 0.021
1621	2-Decanol	nd to 0.105	nd to 0.038	nd	nd to 0.066
1646	Ethyl decanoate[a]	1.067 to 1.267	0.997 to 2.407	1.084 to 3.307	1.079 to 3.953
1663	1-Nonanol	nd to 0.021	nd	nd	nd to 0.006
1667	Isoamyl n-caprylate[a]	–nd	nd to 0.027	nd to 0.035	nd to 0.041
1670	3,7-Dimethyl-6-octen-1-yl acetate[b]	0.014 to 0.039	0.009 to 0.035	0.005 to 0.041	0.004 to 0.021
1671	Furfuryl alcohol[a]	0.018 to 0.045	nd to 0.043	nd to 0.037	0.026 to 0.061
1673	Ethyl 4-decenoate[a]	nd to 0.067	nd to 0.02	nd to 0.011	nd to 0.023
1674	β-Farnesene	nd to 0.121	nd to 0.088	0.056 to 0.081	0.023 to 0.066
1680	Isovaleric acid[a]	+0.159 to 0.408	0.058 to 0.179	0.098 to 0.144	0.095 to 0.144
1681	2-Methyl-butyric acid[a]	nd to 0.226	nd to 0.052	0.029 to 0.033	0.025 to 0.06
1684	Ethyl benzoate	nd	nd to 0.127	nd	nd
1686	Diethyl succinate[a]	nd	nd	nd	nd
1699	Ethyl 9-decenoate	0.065 to 0.739	0.213 to 0.701	0.319 to 1.77	0.231 to 0.982
1705	Methyl 3,7-dimethyl-2,6-octadienoic acid	0.006 to 0.09	nd to 0.022	nd	nd to 0.038
1721	2-Undecanol[a]	0.025 to 0.287	nd to 0.106	nd	nd to 0.176
1725	3-(Methylthio)-1-propanol[a]	nd to 0.01	0.007 to 0.014	0.004 to 0.006	0.005 to 0.012
1753	α-Farnesene[a]	nd to 0.035	nd to 0.025	0.012 to 0.023	0.008 to 0.017
1764	1-Decanol[a]	0.152 to 0.258	0.088 to 0.459	0.171 to 0.289	0.228 to 0.353
1768	Citronellol[a]	+nd to 0.157	0.013 to 0.05	0.01 to 0.039	0.018 to 0.035
1778	3-Tridecanone[b]	+0.018 to 0.061	0.006 to 0.031	nd	nd
1798	Ethyl phenylacetate	–nd	nd to 0.005	0.002 to 0.007	0.002 to 0.005
1819	9-Decen-1-ol[a]	nd to 0.071	0.003 to 0.082	0.011 to 0.056	nd to 0.047
1827	2-Phenylethyl acetate[a]	2.313 to 5.367	3.933 to 6.073	5.123 to 7.803	3.91 to 7.287
1832	β-Damascenone	nd to 0.068	nd to 0.085	0.069 to 0.072	0.028 to 0.09
1846	Ethyl laurate[a]	nd to 0.06	nd to 0.056	nd to 0.009	0.01 to 0.148
1849	Geraniol	nd to 0.023	nd to 0.021	0.004 to 0.014	nd to 0.01
1852	Hexanoic acid[a]	–0.058 to 0.119	0.088 to 0.118	0.095 to 0.12	0.104 to 0.133
1874	3-Hydroxy-2,4,4-trimethylpentyl 2-methyl-propanoate	+0.028 to 0.038	0.012 to 0.031	0.004 to 0.036	nd to 0.002
1896	Ethyl-3-phenylpropionate[a]	–nd to 0.022	0.023 to 0.039	0.033 to 0.047	0.022 to 0.04
1923	2-Phenylethanol[a]	1.637 to 8.157	2.697 to 5.333	4.467 to 4.747	3.003 to 3.407
1929	α-Calacorene	nd to 0.01	nd	nd	nd to 0.002
1963	(E)-2-Methyl-2-pentenoate[b]	+0.204 to 0.69	0.087 to 0.199	0.056 to 0.103	0.079 to 0.211

(Continued).

Table 1 — Continued.

RI	Compound	Percent (%) in total area			
		NM	LM	HM	AM
1973	1-Dodecanol[a]	+0.232 to 0.316	0.128 to 0.273	0.034 to 0.236	0.042 to 0.096
1974	Maltol[a]	nd to 0.008	0.004 to 0.038	0.013 to 0.016	0.005 to 0.016
2039	γ-Nonalactone[a]	nd to 0.308	0.032 to 0.165	0.116 to 0.174	0.122 to 0.179
2048	Nerolidol[a]	nd to 0.09	0.02 to 0.122	0.076 to 0.101	0.078 to 0.154
2054	Ethyl tetradecanoate	nd to 0.033	nd to 0.032	nd to 0.016	nd
2058	Caryophyllenyl alcohol	+0.034 to 0.067	nd to 0.028	nd to 0.018	nd to 0.016
2069	Octanoic acid[a]	–1.35 to 2.597	2.037 to 2.977	2.32 to 3.41	2.713 to 3.627
2140	Ethyl cinnamate[a]	–nd to 0.015	0.01 to 0.04	0.027 to 0.066	0.023 to 0.062
2165	1,5,5,8-Tetramethyl-3,7-cycloundecadien-1-ol	0.073 to 0.198	0.037 to 0.061	0.024 to 0.068	0.03 to 0.143
2175	Nonanoic acid[a]	0.006 to 0.035	nd to 0.035	nd to 0.039	nd to 0.021
2206	2-Methoxy-4-vinylphenol[a]	–0.006 to 0.034	0.031 to 0.181	0.122 to 0.157	0.165 to 0.368
2222	α-Bisabolol[a,b]	nd	nd	nd	nd to 0.001
2236	α-Cadinol	+0.084 to 0.124	0.015 to 0.058	0.016 to 0.029	0.017 to 0.059
2273	3,7,11-Trimethyl-6,10-dodecadien-1-ol	nd to 0.071	nd to 0.193	0.077 to 0.1	0.019 to 0.13
2280	Decanoic acid[a]	1.293 to 1.763	1.092 to 4.783	1.39 to 4.09	1.537 to 5.367
2342	9-Decenoic acid[a]	0.021 to 0.219	0.054 to 0.242	0.088 to 0.615	0.099 to 0.308
2362	Farnesol[a]	–0.104 to 0.199	0.071 to 0.516	0.222 to 0.34	0.21 to 0.353
2436	δ-Dodecalactone[a,b]	nd to 0.04	0.012 to 0.021	0.014 to 0.023	0.009 to 0.024
2492	Lauric acid	nd to 0.095	nd to 0.148	nd to 0.019	0.017 to 0.307
2520	5-Hydroxymethyl-2-furaldehyde[a]	0.006 to 0.051	nd to 0.025	0.008 to 0.015	0.008 to 0.008

RI = Kovats retention index on HP-INNOWAX column.
NM = nonmalt (0%), LM: low-malt (<25%), HM: high-malt (>67%), and AM: all-malt (100%).
[a]Identified on the basis of both mass spectral data and referential compounds.
[b]Compound identified for the 1st time in beer.
+ = higher concentration in nonmalt beer than in all-malt beer, as confirmed by ANOVA ($P < 0.01$).
– = lower concentration in nonmalt beer than in all-malt beer, as confirmed by ANOVA ($P < 0.01$).
nd = not detected.

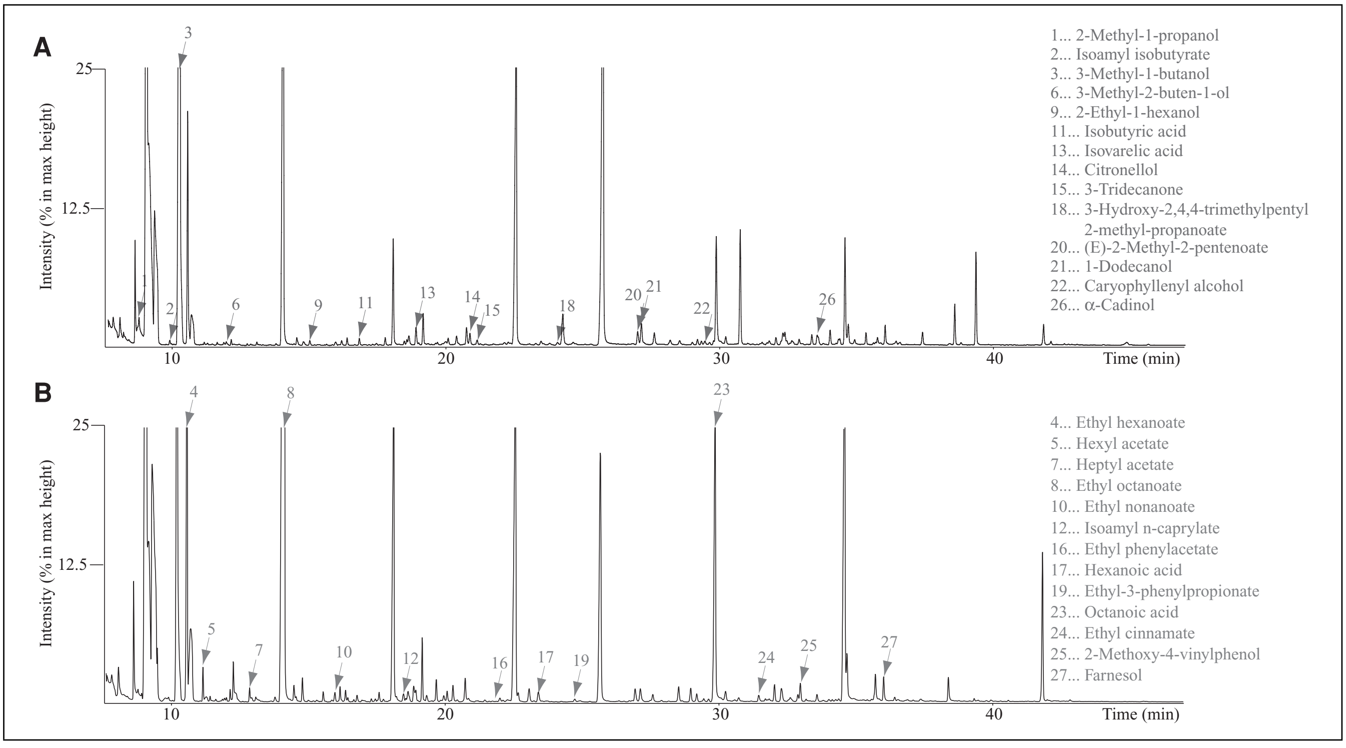

Figure 1 — Total ion chromatograms of (A) nonmalt beer and (B) all-malt beer obtained by SBSE with GC-MS. Peaks indicated by arrows represent compounds whose concentrations are high in (A) nonmalt beer or in (B) all-malt beer.

2-phenylethyl acetate (CAS nr 103-45-7), ethyl laurate (CAS nr 106-33-2), hexanoic acid (CAS nr 142-62-1), ethyl-3-phenylpropionate (CAS nr 2021-28-5), 2-phenylethanol (CAS nr 60-12-8), 1-dodecanol (CAS nr 112-53-8), maltol (CAS nr 118-71-8), γ-nonalactone (CAS nr 104-61-0), trans-nerolidol (CAS nr 40716-66-3), octanoic acid (CAS nr 124-07-2), ethyl cinnamate (CAS nr 103-36-6), nonanoic acid (CAS nr 112-05-0), 2-methoxy-4-vinylphenol (CAS nr 7786-61-0), α-bisabolol (CAS nr 72691-24-8), decanoic acid (CAS nr 334-48-5), 9-decenoic acid (CAS nr 14436-32-9), δ-dodecalactone (CAS nr 713-95-1), and 5-hydroxymethyl-2-furaldehyde (CAS nr 67-47-0) were purchased from Wako Pure Chemical Industry, Ltd. (Tokyo, Japan). 3-Methyl-2-buten-1-ol (CAS nr 556-82-1), furfuryl acetate (CAS nr 623-17-6), farnesol (CAS nr 4602-84-0), and paraffin standard mixtures (C7-11, C12-16, C17-20, and C21-25) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Beer samples

Fifteen commercial beers—4 nonmalt beers, 5 low-malt beers, 3 standard beers, and 3 all-malt beers—were analyzed in this study. These beers are produced by major breweries in Japan and supplied in 350 mL cans.

Aged samples of beers were prepared by storing fresh beers at 30 °C for 1, 2, and 4 wk. After being stored, they were maintained at 2 °C until further analysis.

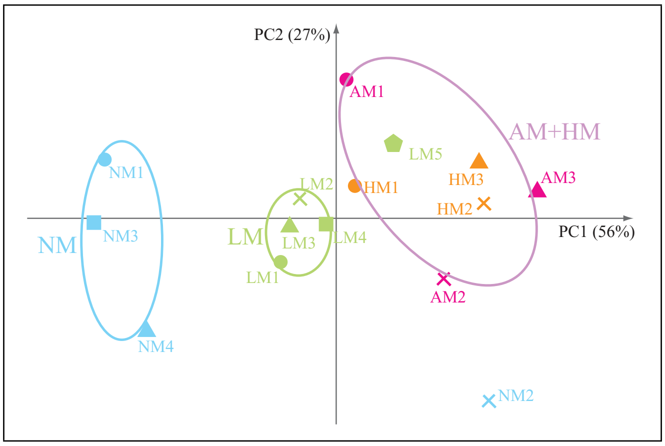

Figure 2–Plots of beer samples onto PCA component axes.

SBSE

SBSE was performed by using a "Twister" with dimensions of 10 × 0.5 mm. (Gerstel, Mulheim an der Ruhr, Germany). Ten milliliters of each beer sample were poured into a 10 mL vial by using the Twister and sealed with a silicone septum. Then, the Twister was operated at 750 rpm for 1 h for extraction at room temperature.

Analysis of compounds in beer

A thermal desorption system (TDS2, Gerstel) was used for desorption of compounds from the Twister. The desorption tube was introduced into a thermodesorption unit. Then, the stir bar was thermally desorbed by heating TDS2 from 20 °C (for 1 min) to 230 °C (for 4 min) at a rate of 60 °C/min. The desorbed compounds were cryofocused in the CIS 4 at −150 °C. Then, the temperature of the CIS 4 was increased from −150 to 230 °C at a rate of 12 °C/min and maintained for 4 min. The trapped compounds were injected into a GC column in the splitless mode.

A gas chromatograph (Agilent Technologies, Santa Clara, Calif., U.S.A.; N6890) and a mass spectrometer (Agilent Technologies; 5973N) were used for separation and detection. The gas chromatograph was equipped with an HP-INNOWAX capillary column (Agilent Technologies; length = 30 m; i.d. = 0.25 mm; film thickness = 0.25 μm), and helium was used as the carrier gas. The velocity of the carrier gas was 1 mL/min. The temperature program was as follows: 30 °C (for 4 min) to 100 °C (for 1 min) at a rate of 15 °C/min,

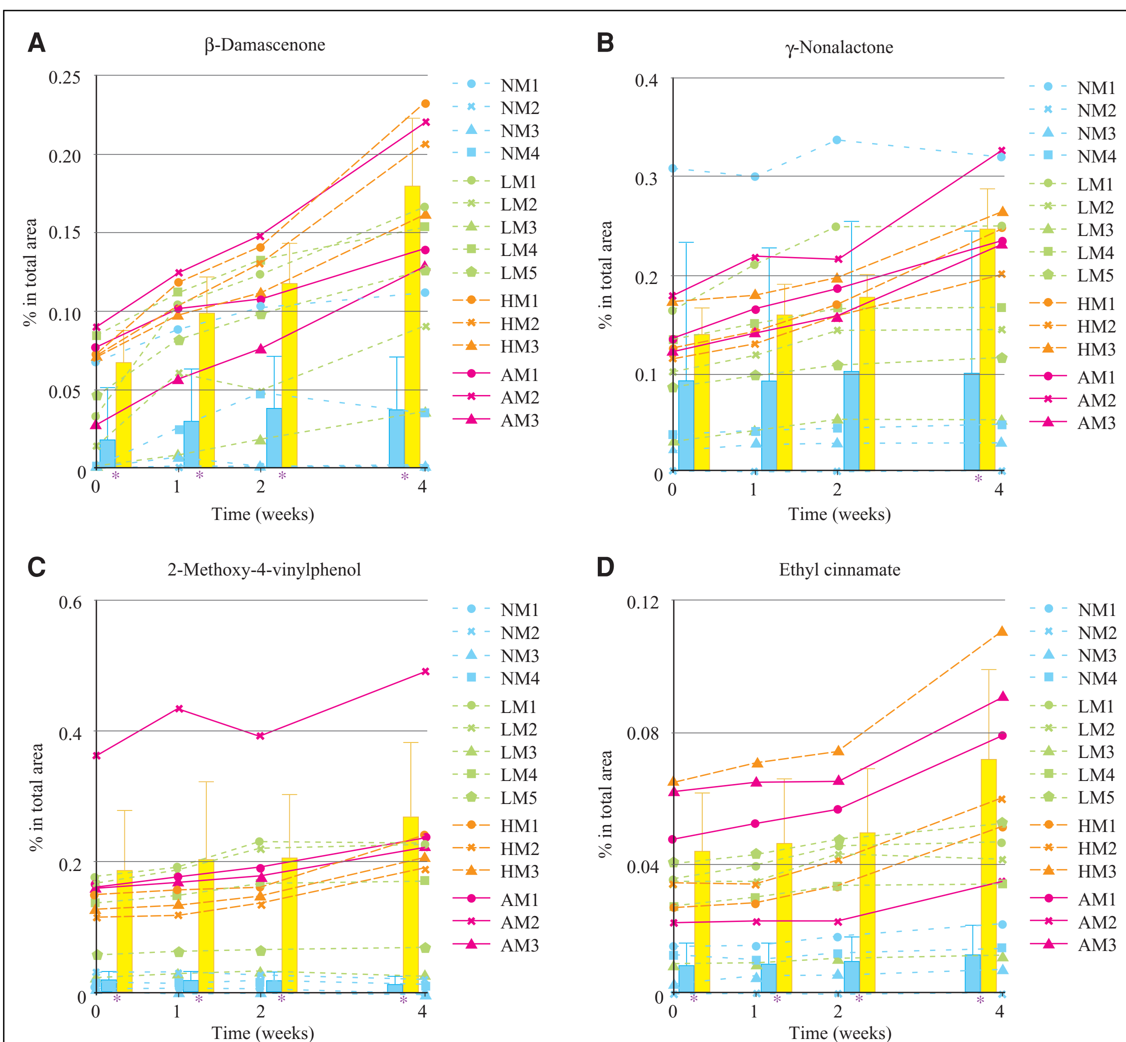

Figure 3–Changes in concentrations of stale flavors in beer samples during storage at 30 °C. (A) β-damascenone, (B) γ-nonalactone, (C) 2-methoxy-4-vinylphenol, and (D) ethyl cinnamate.

100 to 150 °C (for 5 min) at a rate of 5 °C/min, and 150 to 240 °C (for 5 min) at a rate of 15 °C/min.

The mass spectrometer was set to detect ions with a mass-to-charge ratio (m/z) of 35 to 400 and was operated in the electron impact mode at 70 eV. All analyses were performed 3 times, and blank analyses were performed to eliminate the contamination peaks.

Identification and quantitative analysis

Compounds were identified by comparing their spectra with those in the NIST library and their GC retention indices with those of the standard compounds. The GC retention indices were calculated by using paraffin samples (C10-C25) as reference compounds, in accordance with the procedure reported in the literature (Halang and others 1978).

Peak area percentages were determined from the total ion chromatogram by using AMDIS (NIST). The mean peak area and standard deviations were calculated from these results by replicate analyses. Comparisons using a one-way analysis of variance (ANOVA) and principal component analyses were performed with JMP 6 (SAS Inst. Inc., Cary, N.C., U.S.A.).

Results and Discussion

Identification of volatile compounds

Fifteen commercial beers—4 nonmalt beers, 5 low-malt beers, 3 standard beers, and 3 all-malt beers—were analyzed by using SBSE with GC-MS. Ninety compounds—25 esters, 17 terpenes, 14 alcohols, 11 acids, 6 furans, 6 aroma compounds, 5 carbonyls, and 6 other compounds—were identified (Table 1). The primary group is an ester, including important flavor compounds, such as isopentyl acetate, ethyl octanoate, and ethyl hexanoate. The secondary major group is terpene. Terpene is also important flavor compounds, commonly originated by hop; for example, myrcene, prenol, and linalool, imparts peppery, fruity, and tangy flavors to beer. Many other compounds imparting beer flavor including isobutyrate, isovalerate, and 2-phenylethanol could be detected by using this method. Nine compounds were detected for the 1st time in beer—methyl 2-methyl-1-heptanoate, ethyl 4(E)-hexenoate, methyl 4-methylene-hexanoate, 3-(4-methyl-3-pentenyl)-furan, 3,7-dimethyl-6-octen-1-yl acetate, 3-tridecanone, (E)-2-methyl-2-pentenoate, alpha-bisabolol, and delta-dodecalactone. The sensitivity of this method facilitates the detection of such trace components.

Comparison among beers containing different amounts of malt

We compared the volatile compositions among samples using the one-way ANOVA test ($P < 0.01$) (Table 1). The total ion chromatogram of the fresh nonmalt or all-malt beer is shown in Figure 1. 3-Tridecanone was detected only in nonmalt beer. As per our knowledge, this is the 1st time that 3-tridecanone was

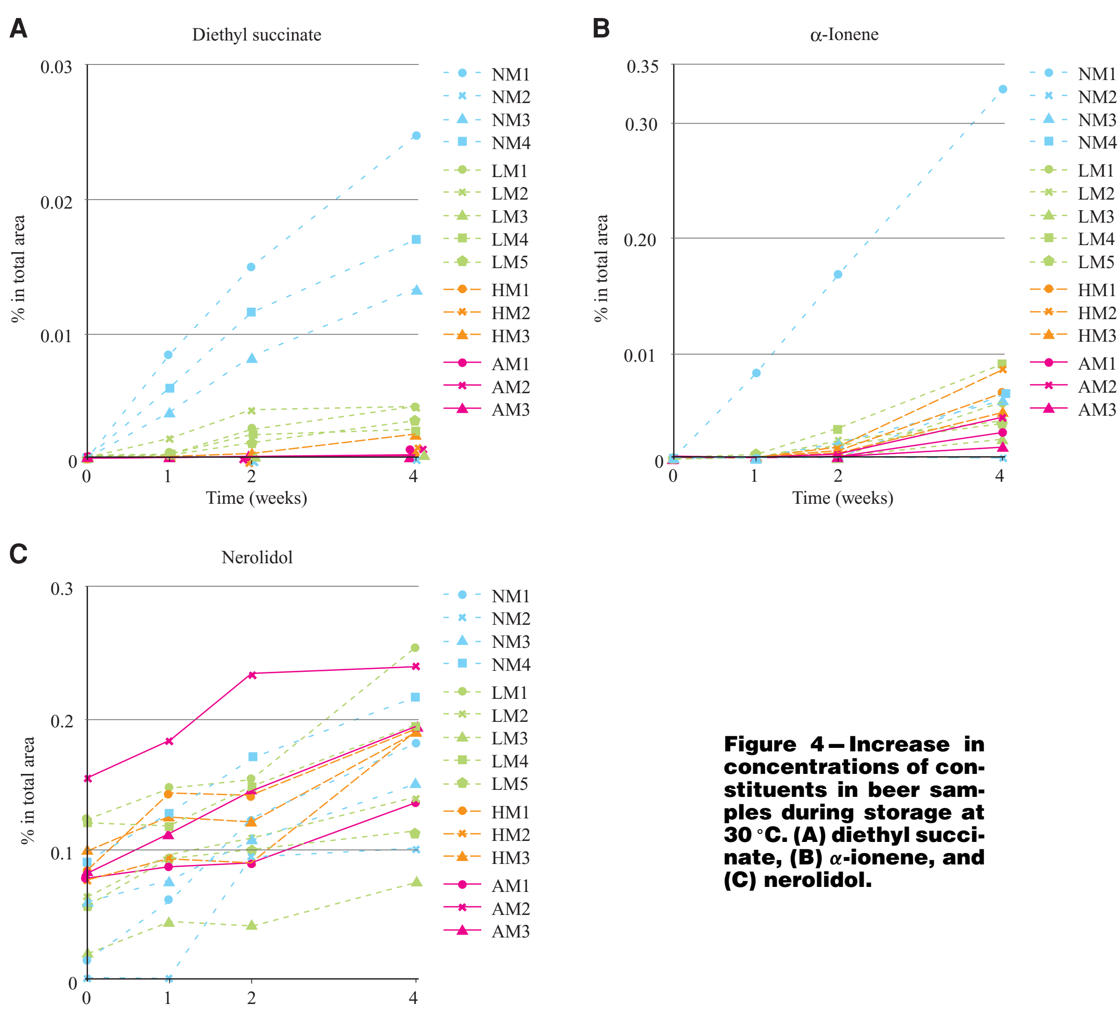

Figure 4–Increase in concentrations of constituents in beer samples during storage at 30 °C. (A) diethyl succinate, (B) α-ionene, and (C) nerolidol.

detected in beer. Concentrations of 15 compounds in nonmalt beer were higher than those in all-malt beer. High concentrations of isobutyric acid and isovaleric acid may affect the sensory profile of nonmalt beer because of their low-odor threshold. Further, concentrations of 13 compounds in nonmalt beer were lower than those in all-malt beer. Most of these compounds were esters. The flavor that originated from ferulic acid was 2-methoxy-4-vinylphenol, which is delivered from malt (Coghe and others 2004).

The concentrations of 2-nonanol, 2-decanol, 2-undecanol, 2-nonanone, and 2-undecanone in 4 samples—NM1, NM4, LM3, AM1—are significantly higher than those in the other 11 samples. Compounds contributing to hop bitterness would be degraded to 2-alkanones, which could be further reduced to 2-alkanols by yeast (Vanderhaegen and others 2006). Hence, these results may depend on the type of hops present in them.

Principal component analysis

Principal component analysis was performed to determine the similarities in volatile compounds between the samples. Only the 1st and 2nd principal components accounted for 83% of the total variance. On the basis of variables correlated with the 1st principal component, 3 groups corresponding to nonmalt beers, low-malt beers, and standard or all-malt beers were formed, with only 2 exceptions (Figure 2). The 1st principal component, which accounts for 56% of the total variance, is positively correlated with isopentyl acetate and 2-phenylethyl acetate, but negatively correlated with 3-methyl-1-butanol and 2-phenylethanol. Alcohol acetyltransferase of yeast produces 3-methyl-1-butanol from isopentyl acetate (Verstrepen and others 2003b). The 2nd principal component, which accounts for 27% of the total variance, is well correlated with ethyl octanoate and ethyl decanoate.

Changes during storage

The concentration of 7 compounds was found to increase during storage (Figure 3 and 4). These compounds had coefficients of variation (CVs) of less than 10%, with the exception of β-damascenone (CV = 20%) and nerolidol (CV = 12%). β-Damascenone, γ-nonalactone, 2-methoxy-4-vinylphenol, and ethyl cinnamate are the major compounds contributing to the aged flavor of beer (Gijs and others 2002). The concentration of these compounds increased during storage in all-malt and standard beers, but barely increased in nonmalt beer (Figure 3). These results strongly suggest that changes in the sensory profile of nonmalt beer during storage are different from all-malt and standard beer. Additionally, the concentration of these compounds except for γ-nonalactone is lower in nonmalt beer before aging, the difference may increase during storage. Because the formation of β-damascenone and γ-nonalactone depends on temperature, nonmalt beer may be relatively resistant to temperature.

Diethyl succinate and α-ionene were detected only in the aged samples, and their concentrations increased during storage (Figure 4A and 4B). It has been reported that the concentration of diethyl succinate increases during storage (Vanderhaegen and others 2006).

β-damascenone, γ-nonalactone, and ethyl cinnamate were not detected in NM3, and 2-methoxy-4-vinylphenol was not detected in NM2 during storage (Figure 3). Therefore, these compounds cannot be used as markers to indicate the storage period of nonmalt beer. Additionally, nerolidol is a good marker candidate that can be used for indicating the storage period of any type of beer (Figure 4C). The difference between concentrations of nerolidol before and after aging was significant in all groups of beers supported with t-value test ($P < 0.05$).

Conclusions

In this study, we identified 90 compounds in beer containing various amounts of malt by using SBSE with GC-MS. Compared to those of all-malt beer, concentrations of 14 compounds, including higher alcohols, terpene alcohol, and short chain fatty acids, were higher, whereas concentrations of 13 compounds, including ethyl esters, acetate esters, and middle chain fatty acids, were lower in nonmalt beer. Three groups corresponding to nonmalt beers, low-malt beers, and standard or all-malt beers were formed by principal component analysis.

β-damascenone, γ-nonalactone, ethyl cinnamate, and 2-methoxy-4-vinylphenol can be detected at the same time using with SBSE-GC-MS. The results of a study on the aging property of beers revealed that the concentrations of these compounds except for γ-nonalactone were lower for nonmalt beers. Because these compounds impart stale flavors, it seems that changes during storage in nonmalt beer are considerably different from those in standard beer.

References

Coghe S, Benoot K, Delvaux F, Vanderhaegen B, Delvaux FR. 2004. Ferulic acid release and 4-vinylguaiacol formation during brewing and fermentation: indications for feruloyl esterase activity in Saccharomyces cerevisiae. J Agric Food Chem 52(3):602–8.

Gijs L, Chevance F, Jerkovic V, Collin S. 2002. How low pH can intensify beta-damascenone and dimethyl trisulfide production through beer aging. J Agric Food Chem 50:5612–6.

Guido LF, Curto AF, Boivin P, Benismail N, Goncalves CR, Barros AA. 2007. Correlation of malt quality parameters and beer flavor stability: multivariate analysis. J Agric Food Chem 55:728–33.

Halang WA, Langlais R, Kugler E. 1978. Cubic spline interpolation for the calculation of retention indices in temperature-programmed gas-liquid chromatography. Anal Chem 13:1829–32.

Horák T, Kellner V, Čulík J, Jurková M, Čejka P. 2007. Determination of some beer flavours by stir bar sorptive extraction and solvent back extraction. J Inst Brew 113(2):154–8.

Kishimoto T, Wanikawa A, Kono K, Shibata K. 2006. Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. J Agric Food Chem 54:8855–61.

Kondo H. 2005. Brewing yeast fermentation and performance in beer and Happoshu. J Brew Soc Jpn 100:787–95.

Landaud S, Helinck S, Bonnarme P. 2008. Formation of volatile sulfur compounds and metabolism of methionine and other sulfur compounds in fermented food. Appl Microbiol Biotech 77:1191–205.

Ochiai N, Sasamoto K, Daishima S, Heiden AC, Hoffman A. 2003. Determination of stale-flavor carbonyl compounds in beer by stir bar sorptive extraction with in-situ derivatization and thermal desorption-gas chromatography-mass spectrometry. J Chromatogr A 986:101–10.

Pinho O, Ferreira IM, Santos LH. 2006. Method optimization by solid-phase microextraction in combination with gas chromatography with mass spectrometry for analysis of beer volatile fraction. J Chromatogr A 1121:145–53.

Shimizu C, Ohno M, Araki S, Furusho S, Watari J, Takashio M. 2002. Effect of reduction of carbonyl compounds by yeast flavor stability of Happoshu. J Am Soc Brew Chem 122–9.

Vanderhaegen B, Neven H, Verachtert H, Derdelinckx G. 2006. The chemistry of beer aging. Food Chem 95:357–81.

Verstrepen KJ, Derdelinckx G, Dufour JP, Winderickx J, Thevelein JM, Pretorius IS, Delvaux FR. 2003a. Flavor-active esters: adding fruitiness to beer. J Biosci Bioengr 96:110–8.

Verstrepen KJ, Van Laere SD, Vanderhaegen BM, Derdelinckx G, Dufour JP, Pretorius IS, Winderickx J, Thevelein JM, Delvaux FR. 2003b. Expression levels of the yeast alcohol acetyltransferase genes ATF1, Lg-ATF1, and ATF2 control the formation of a broad range of volatile esters. Appl Environ Microbiol 69:5228–37.