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Identification of Odor-Active 3-Mercapto-3-methylbutyl Acetate in Volatile Fraction of Roasted Coffee Brew Isolated by Steam Distillation under Reduced Pressure

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In a roasted Arabica coffee brew, the potent roasty odor quality compound was identified as 3-mercapto-3-methylbutyl acetate by comparison of its Kovats gas chromatography retention index, mass spectrum, and odor quality to those of the synthetic authentic compound. 3-Mercapto-3-methylbutyl acetate has been identified for the first time in the coffee, and according to the results of the aroma extract dilution analysis, the contribution of this compound to the flavor of the roasted coffee brew varied depending on the degree of the coffee bean roasting. The concentration of this compound in the coffee brews as with 3-mercapto-3-methylbutyl formate increased with an increase in the degree of roasting. However, the slope of the amount of both esters was different, and 3-mercapto-3-methylbutyl acetate hardly increased with a low degree of roasting at more than a 21 luminosity (L)-value, but it rapidly increased when the roasting degree of the coffee beans reached the L-value of 18. These results suggested that the contribution of 3-mercapto-3-methylbutyl acetate to the overall flavor is peculiar to the flavor of the highly roasted coffee.

KEYWORDS: Coffee; 3-mercapto-3-methylbutyl acetate; roast degree; aroma extract dilution analysis

INTRODUCTION

Coffee is one of the most widely consumed beverages in the world. The high acceptability of coffee is due to many factors, one of the most contributory factors, especially, being its flavor. Therefore, the potent odorants in coffee flavor have already been the subject of much research, and 3-mercapto-3-methylbutyl formate was found as one of the most important odorants of coffee flavor (*1*−*6*). It is proposed that 3-mercapto-3-methylbutanol, which is formed from 3-methyl-2-buten-1-ol, reacts with formic acid to give 3-mercapto-3-methylbutyl formate during the roasting of the coffee beans (*7*). On the other hand, acetic acid is contained in the roasted coffee beans as well as formic acid (*8*−*9*). Therefore, if 3-mercapto-3-methylbutyl formate forms in the above reaction pathway, the formation of 3-mercapto-3-methylbutyl acetate may also be assumed to take place during the roasting of the coffee. However, this compound's contribution to the flavor of coffee has not yet been determined.

In the present paper, we report that the identification of 3-mercapto-3-methylbutyl acetate in the coffee brew and the contribution of this compound to the flavor of the roasted coffee brew were confirmed by comparative aroma extract dilution analysis (AEDA) (*10*). Furthermore, the relationships between the concentration of 3-mercapto-3-methylbutyl acetate and the degree of roasting of the coffee beans were also investigated.

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MATERIALS AND METHODS

Materials. *Coffee Brews*. The raw and roasted coffee beans were supplied by Unicafé, Inc. (Tokyo, Japan). The Arabica coffee beans were roasted at five different degrees (luminosity (L)-value 27.0, 24.1, 21.0, 18.2, and 15.8; these values were denoted as follows: 27, 24, 21, 18, and 15) and stored at −20 °C until used. Deionized hot water (ca. 80 °C, 500 mL) was poured on the ground coffee powders (50 g) in a filter. The filtrate (about 450 mL) was immediately cooled to under 20 °C in tap water.

Chemicals. Phenyl isothiocyanate was obtained from Nacalai Tesque (Kyoto, Japan). 3-Mercapto-3-methylbutyl formate and 3-mercapto-3-methylbutanol were synthesized according to the literature (*2*). 3-Mercapto-3-methylbutyl acetate was synthesized from 3-mercapto-3-methylbutanol according to the literature (*11*).

Isolation of the Volatiles. The coffee brew (800 g) was distilled under reduced pressure (40 °C, 20 mmHg). The steam distillate (about 250 mL) was passed through a column packed with 10 g of Porapak Q (Waters). The adsorbed compounds were eluted with methylene chloride (100 mL). The eluate was dried over anhydrous sodium sulfate, and the solvent was removed using a rotary evaporator to about 5 mL in volume. A further concentration was conducted with a nitrogen stream to about 100 μL. For the quantitative analysis, an internal standard solution (50 μL) prepared from phenyl isothiocyanate (1.0 mg) in methylene chloride (10 mL) was added to the eluate before the solvent was removed by an evaporator. The concentrate was used for the AEDA and gas chromatography mass spectrometer (GC-MS) analysis sample.

Enrichment of Odorants for Identification. For the identification experiments, the coffee volatiles were isolated from the coffee brew (L-value, 18) as described above. These procedures were repeated and all the volatile fractions were combined (total, 6 L of deionized water

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Table 1. Extracted Ions and Calibration Factors for Mass Chromatography

compound	extracted ion (*m*/*z*)	calibration factor
3-mercapto-3-methylbutyl formate	148	192.8
3-mercapto-3-methylbutyl acetate	102	10.3
3-mercapto-3-methylbutanol	86	15.5
phenyl isothiocyanate[a]	135	1.0

[a] Internal standard.

was added to 600 g of ground coffee powders). The basic volatiles were removed from the volatile concentrate with 1 M hydrochloric acid (3 × 25 mL). The organic phase containing the neutral and acidic volatiles, which was washed with saturated brine (2 × 5 mL), was dried over anhydrous Na_2SO_4 and finally concentrated to 100 μL (neutral fraction). The neutral fraction was applied onto a column (20 × 0.7 cm i.d.) filled with silica gel (Wakogel C-200; Wako Pure Chemical Industries). Elution was performed using the following solvents: hexane (100 mL), hexane/methylene chloride (100 mL, 95/5, v/v, fraction A), hexane/methylene chloride (100 mL, 90/10, v/v, fraction B), hexane/methylene chloride (100 mL, 80/20, v/v, fraction C), hexane/methylene chloride (100 mL, 50/50, v/v, fraction D), hexane/methylene chloride (300 mL, 50/50, v/v, fraction E), methylene chloride (100 mL, fraction F), and ethyl acetate (100 mL, fraction G). These fractions were then concentrated to 100 μL as described above.

Gas Chromatography−Olfactometry (GC−O). A Hewlett-Packard (HP) model 5890 series II gas chromatograph equipped with a thermal conductivity detector (TCD) was used. A fused silica column (30 m × 0.25 mm i.d.; coated with a 0.25 μm film of DB-Wax; J & W) was used with splitless injection. The column temperature was programmed from 40 °C to 210 °C at the rate of 5 °C/min for all runs. The injector and detector temperatures were 250 °C and 230 °C, respectively. Helium was used as the carrier gas at the flow rate of 1 mL/min. A glass sniffing port was connected to the outlet of the TCD and heated by a ribbon heater. Moist air was pumped into the sniffing port at about 100 mL/min to quickly remove the odorant eluted from the TCD out of the sniffing port.

AEDA. The original odor concentrate of the coffee brew was stepwise diluted with methylene chloride to 1:16, 1:64, 1:256, 1:1024, 1:4096, and 1:16384 aliquots (1 μL) of each fraction, which were analyzed by capillary GC on the DB-Wax column. The odor active compounds were detected by GC eluate sniffing (GC−O). The flavor dilution (FD) factors of the odorants were determined by AEDA.

GC−MS. An Agilent 6890 N gas chromatograph coupled to an Agilent Model 5973 N series mass spectrometer was used. The column was a 60 m × 0.25 mm i.d. DB-Wax fused silica capillary column (J & W Scientific) with a film thickness of 0.25 μm. The column temperature was programmed from 80 °C to 210 °C at the rate of 3 °C/min. The injector temperature was 250 °C. The flow rate of the helium carrier gas was 1 mL/min, and the split ratio was 1:30. The mass spectrometer was used under the following conditions: ionization voltage, 70 eV (EI); and ion source temperature, 150 °C. The quantities of the odorants in the volatile fraction of the coffee brews were determined based on the extracted ion peak area using mass chromatography. The extracted ion peak areas were monitored in the ranges given in **Table 1**. The calibration factors were determined in mixtures of equal amounts of odorants and the internal standard compound (by weight) and were calculated as the ratio of the extracted ion peak area of the internal standard to the extracted ion peak area of each odorant. These extracted ion peak areas were mean values of triplicate results. These calibration factors were used to calculate the amounts of odorants on the basis of the internal standard.

Identification of Components. The identification of the components was made by comparison of their Kovats GC retention indices, mass spectra, and odor quality to those of authentic compounds. The Kovats GC retention indices (RI) of the odorants were calculated from the retention time of *n*-alkanes.

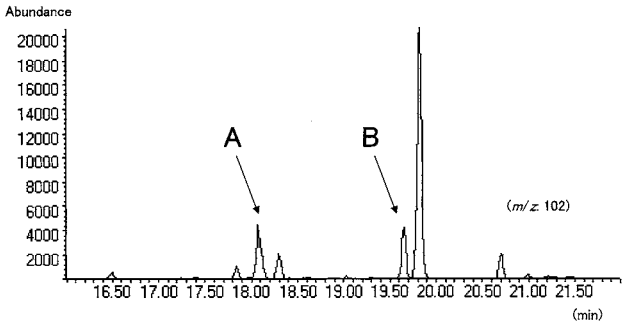

Figure 1. Mass chromatogram of the volatile concentrate of a coffee brew (L 18) showing the extracted ion (A, 3-mercapto-3-methylbutyl formate; B, 3-mercapto-3-methylbutyl acetate).

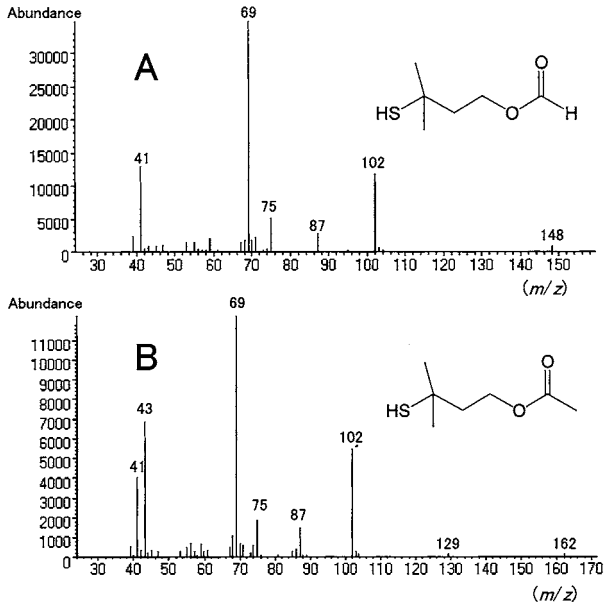

Figure 2. Mass spectra of 3-mercapto-3-methylbutyl formate (A) and 3-mercapto-3-methylbutyl acetate (B), which were obtained from enrichment fractions C and D, respectively.

RESULTS AND DISCUSSION

Identifying New Volatile Thiol in Coffee Brew. The flavor extract of the coffee brew, which was extracted from the roasted beans (L-value, 18), was prepared by steam distillation under reduced pressure. The steam distillate was then concentrated by the adsorptive column method. The volatile fraction isolated from the coffee brew was screened by GC-MS for a new volatile thiol. The mass chromatogram of the coffee brew volatile concentrate was recorded for the typical fragment ion at *m*/*z* 102 (**Figure 1**), and several peaks appeared. For identification of peaks A and B, these components were enriched, and the odorants were compared to their Kovats retention indices on the DB-Wax stationary phase and their mass spectra to those of the authentic compounds. As a consequence, peaks A and B could be identified as 3-mercapto-3-methylbutyl formate (**1**, RI = $1518_{(DB-Wax)}$) and 3-mercpto-3-methylbutyl acetate (**2**, RI = $1562_{(DB-Wax)}$), respectively (**Figure 2**). On the silica gel column chromatography, odorants **1** and **2** were eluted in the fractions from C to E, and **1** was eluted earlier than **2**. Of the two volatile thiols, **1** was reported as a potent odorant for the sulfurous/roasty note of the roasted coffee flavor (*4*). On the other hand, **2** is reported here for the first time as a component of the roasted coffee flavor. The first identification of **2** was from passion fruit

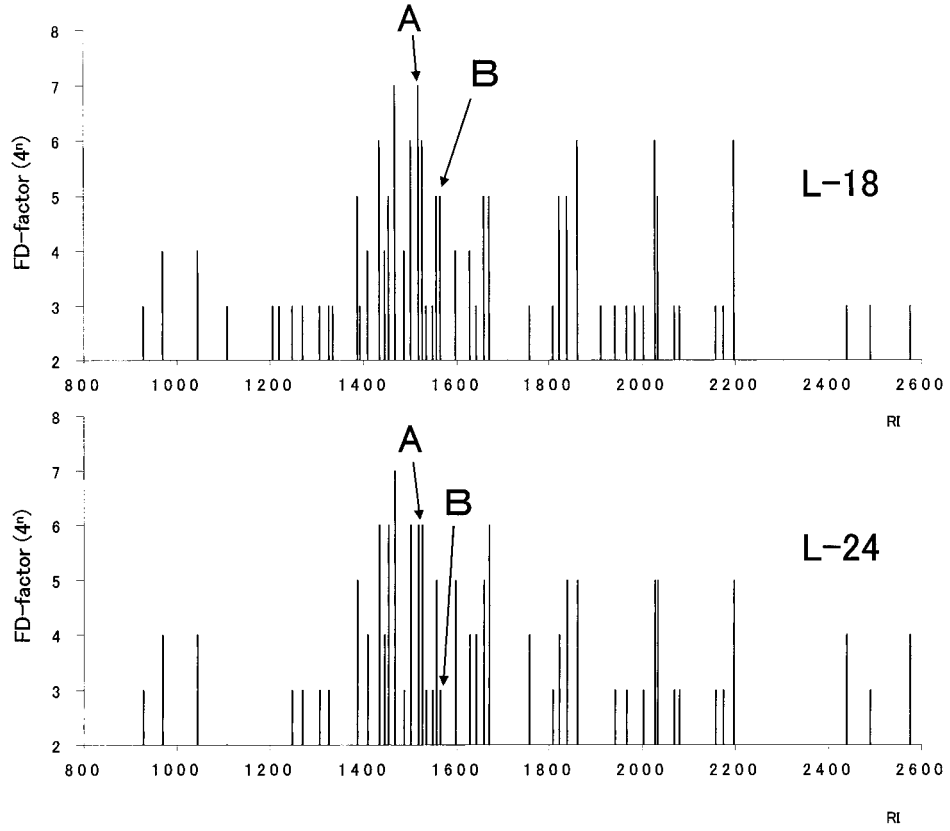

Figure 3. Flavor dilution chromatograms (DB-Wax stationary phase column) of coffee brews, which were prepared from the beans with different degrees of roasting L 18 (top) and L 24 (bottom) (A, 3-mercapto-3-methylbutyl formate; B, 3-mercapto-3-methylbutyl acetate).

juice (*11*), however, this thiol has not yet been identified in other kinds of aromas arising from natural sources.

It is proposed that 3-mercapto-3-methylbutanol (**3**), which was formed from 3-methyl-2-buten-1-ol, reacts with formic acid to give **1** during the roasting of coffee (*7*). On the other hand, acetic acid is contained in large amounts as a volatile acid in the coffee beans as well as formic acid (*8*−*9*). Therefore, it is possible that the esterification of **3** with acetic acid gave **2** as well as the formation of **1** during the coffee roasting.

The Effects of 3-Mercapto-3-methylbutyl Esters on the Coffee Flavors with Different Roasting Degrees. Application of the comparative AEDA using the volatile fraction of the freshly filtered coffee brews, which were prepared from the beans with different degrees of roasting (L-value, 24 and 18), revealed 46 and 52 odor-active peaks with FD factors between 4^3 and 4^7. The results in **Figure 3** reveal **1** with a high FD factor as the predominant odorant of the coffee brews. Comparative AEDA indicated that this compound had the highest FD factor (4^7) for the highly roasted (L 18) coffee brew. However, the differences in the FD factor of this compound between L 18 and L 24 were comparatively small. On the other hand, the AEDA of the highly roasted coffee brew (L = 18) also revealed **2** as a potent odorant (FD factor, 4^5), but its FD factor was low when compared to that of **1**. However, there was a remarkable difference in the FD factor of **2** between L 18 and L 24 as compared with the difference in the FD factor of **1**. On the basis of these results, for these two odorants, **2** suggests the possibility of being related to the difference in the overall flavor between the coffee brews of different roasting degrees rather than **1**.

The Influence of the Roasting Degree on the Concentration of the 3-Mercapto-3-methylbutyl Esters. The concentration of **1**, **2**, and **3** in the coffee brews, which were prepared from different degrees of roasted beans, were investigated. The results in **Figure 4** and **Figure 5** show that the concentration of these mercapto compounds significantly changed with the degree of roasting. With an increase in the roasting degree, the levels of both esters increased. However, a difference was recognized in the amounts of the increase in these esters in each coffee brew, which were prepared from different degrees of roasted beans. Namely, the amounts of **2** sharply increased between the L-value range of 21 and 18, whereas the increase

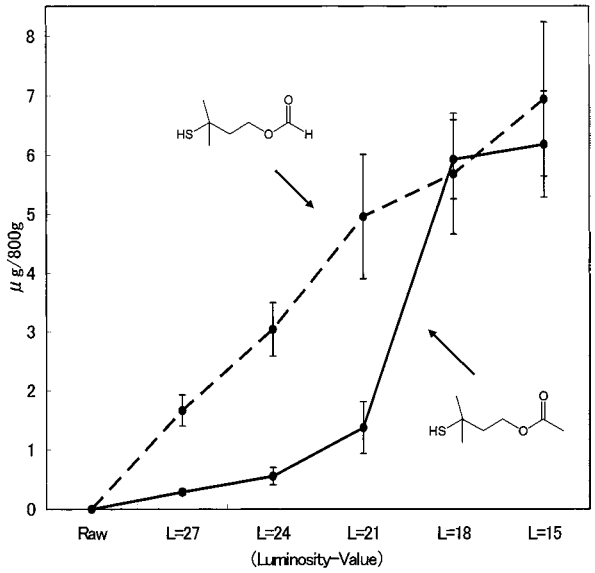

Figure 4. Changes in the amounts of 3-mercapto-3-methylbutyl formate and 3-mercapto-3-methylbutyl acetate in the coffee brews, which were extracted from different degrees of roasted beans (L-value refers to Materials).

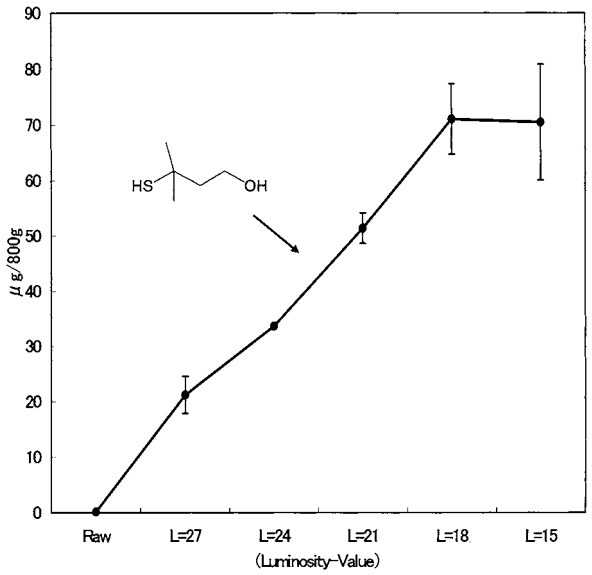

Figure 5. Changes in the amounts of the 3-mercapto-3-methylbutanol in the coffee brews, which were extracted from different degrees of roasted beans (L-value refers to Materials).

in the amounts of **1** remained constant from the raw bean to the L 15 (**Figure 4**). The amounts of **3**, which is capable of producing **1** and **2**, in the coffee brew increased with an increase in the roasting degree (**Figure 5**), and it was always contained in large amounts when compared to **1** and **2**. On the other hand, it is reported that the acetic acid and formic acid contents in the roasted coffee beans increase with an increase in the roasting temperature, and acetic acid is slightly more abundant than formic acid at all temperatures up to 240 °C (*8*−*9*). Therefore, it can be assumed that there is a smaller amount of **2** than **1** when the roast degree is low, because the difference in the reactivity of both acids is an influence, rather than the difference in the amounts of these acids. In the highly roasted condition (L 18), **1** and **2** were formed in the same way. It can be assumed that a difference in reactivity between the acetic acid and formic acid became small with the rise in the roasting degree. However, as for the esterification of the volatile compounds during the coffee roasting, it is hardly known. Therefore, it would be necessary to investigate in detail the formation mechanism and condition of these esters. These findings are in good agreement with the results of the comparative AEDA in which **2** showed a high contribution, only with the flavor of the brew using a highly roasted coffee bean.

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