

Effect of pH on the Thermal Stability of Potent Roasty Odorants, 3-Mercapto-3-methylbutyl Esters, in Coffee Drink

KENJI KUMAZAWA* AND HIDEKI MASUDA

Material Research and Development Laboratories, Ogawa & Company, Limited,
15-7 Chidori Urayasushi, Chiba 279-0032, Japan

The effect of pH on the thermal stability of potent roasty odorants, 3-mercapto-3-methylbutyl esters, in coffee drinks was investigated. The concentration of 3-mercapto-3-methylbutyl formate in the coffee drink was drastically decreased during the heat processing. However, the residual ratio of this compound in the coffee drink adjusted to pH 5.0 was higher than that of pH 6.5. On the other hand, the residual ratios of 3-mercapto-3-methylbutyl acetate showed high values in the coffee drinks adjusted to both pHs. In the pH range of 3–7, the residual ratios of 3-mercapto-3-methylbutyl formate in aqueous model solutions varied depending on the pH value. It had a thermal stability maximum at pH 4.0, while 3-mercapto-3-methylbutyl acetate showed stability during heat processing over all the pH values tested. In addition, the residual ratios of the formate and acetate esters, which were composed of various alcohol derivatives, showed the same tendency as that of the 3-mercapto-3-methylbutyl esters. These results suggested that the low thermal stability and pH dependence of 3-mercapto-3-methylbutyl formate are based on the structure of the formate ester.

KEYWORDS: Coffee; roasty odorant; 3-mercapto-3-methylbutyl formate; 3-mercapto-3-methylbutyl acetate; heat processing

INTRODUCTION

Coffee is one of the most widely consumed beverages in the world. The high acceptability of coffee is due to many factors, especially, one of the most contributory factors being its flavor. Therefore, the potent odorants in coffee flavor have already been the subject of much research, and 3-mercapto-3-methylbutyl formate and 3-mercapto-3-methylbutyl acetate were found as potent odorants of coffee flavor (1–3). Canned coffee drinks are very popular in Japan. During the manufacturing process of a canned coffee drink, the coffee flavor changes. In particular, the heat processing during sterilization affects the flavor of the coffee, and the characteristic roasty odor of the fresh coffee drink was found to have significantly decreased (4). It was reported that the decrease in the roasty odor quality after heating the coffee drink was significantly caused by the decrease in the amount of three sulfur compounds (2-furfurylthiol, methional, and 3-mercapto-3-methylbutyl formate), and it was assumed that these odorants were changed by oxidation, thermal degradation, and/or hydrolysis (5).

3-Mercapto-3-methylbutyl formate significantly contributes to the sulfurous-roasty odor quality of a coffee brew (6). However, this odorant is unstable and rapidly decreased when coffee drinks are processed or stored (5, 7, 8). Recent investigations indicated the possibility that melanoidins in the coffee brew are involved in the loss of the 3-mercapto-3-methylbutyl formate

when keeping the coffee brew warm in a thermos flask (7, 8). On the other hand, adjustment of the coffee brew pH is one of the important techniques for the production of canned coffee drinks. This process is performed in order to prevent an increase in the sour taste and the cohesion of the coffee and milk components during sterilization (9). We have already reported that the pH value of a coffee drink significantly affects the thermal stability of 2-furfurylthiol during heat processing (4). However, the pH effects on the thermal stability of the important roasty odorants, 3-mercapto-3-methylbutyl formate and 3-mercapto-3-methyl acetate, during heat processing have not yet been clarified.

Therefore, in the present paper, we describe the relationships between the thermal stability of the 3-mercapto-3-methylbutyl esters and the pH conditions during heat processing of the coffee drink that were investigated.

MATERIALS AND METHODS

Materials. *Coffee Drinks.* The Arabica coffee beans were roasted (luminosity value, 18.6), sealed under vacuum, 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 about 20°C in tap water, and then citric acid or NaHCO_3 was added to change the pH to 5.0 or 6.5, respectively, and canned under a reduced pressure condition (260 mmHg). The canned coffee drink was sterilized at 120°C for 20 min followed by immediate cooling to about 10°C in tap water. Sterilization was performed in a retort pasteurizer (model PRS-02-II-VC; supplied from Nissen, Japan). The pH values after heat processing the coffee drink were 4.7 and 5.3.

* To whom correspondence should be addressed. E-mail: kumazawa.kenji@ogawa.net.

Chemicals. Hexyl formate, (*Z*)-3-hexenyl formate, benzyl formate, 2-phenylethyl formate, hexyl acetate, (*Z*)-3-hexenyl acetate, benzyl acetate, and 2-phenylethyl acetate were obtained from Wako Pure Chemical Industries (Osaka, Japan). Phenyl isothiocyanate was obtained from Nacalai Tesque (Kyoto, Japan), and methyl nonanoate was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). 3-Mercapto-3-methylbutyl formate and 3-mercapto-3-methylbutanol were synthesized according to literature procedures (10). 3-Mercapto-3-methylbutyl acetate was synthesized from 3-mercapto-3-methylbutanol according to a literature procedure (11).

Isolation of the Coffee Volatiles. The coffee drink (750 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 to about 5 mL in volume using a rotary evaporator. A further concentration to about 100 μ L was conducted using a nitrogen stream. For the quantitative analysis, an internal standard solution (50 μ L), 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 as the gas chromatography–mass spectrometry (GC–MS) analysis sample.

Model Experiments on the Thermal Stability of Formic and Acetic Esters in Aqueous Solution. At the concentration of 1 ppm each, the esters were dissolved in citric/ Na_2HPO_4 buffer solutions of pHs 3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 (mixed 1/10 M citric acid and 1/5 M Na_2HPO_4). One-half of each solution was canned under the reduced pressure condition (260 mmHg) and treated by heat processing (120 °C, 20 min; the instrument was previously described), followed by immediate cooling to about 10 °C in tap water, and the other half of the solution was not heated. Esters in the treated and untreated solutions (100 mL) were isolated by extraction with methylene chloride (50 mL \times 2). After drying over anhydrous sodium sulfate, the solvent was evaporated to a volume of about 5 mL. A further concentration to about 100 μ L was conducted with a nitrogen stream. The internal standard solution (20 μ L) prepared from methyl nonanoate (505.6 mg) in methylene chloride (100 mL) was added to the concentrate before the solvent was removed by the evaporator. Quantification of the formate and acetate esters was performed by GC, and the residual ratios (%) and yields (%) were calculated from the measured values of the esters in the thermally treated and nontreated solutions.

Gas Chromatography (GC). An Agilent 6850 gas chromatograph equipped with a flame ionization detector (FID) was used. A fused silica column (30 m \times 0.25 mm i.d.; coated with a 0.25 μ m film of DB-Wax; J&W Scientific) was used. The column temperature was programmed from 80 to 210 °C at a rate of 3 °C/min for all runs. The injector and detector temperatures were both 250 °C. The flow rate of the nitrogen carrier gas was 0.7 mL/min, and the split ratio was 1:30.

Gas Chromatography–Mass Spectrometry (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 \times 0.25 mm i.d. DB-Wax fused silica capillary column (J&W Scientific) with a film thickness of 0.25 μ m. The column temperature was programmed from 80 to 210 °C at a 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); ion source temperature, 150 °C. The quantities of the 3-mercapto-3-methylbutyl esters in the volatile fractions of the coffee drinks were determined based on the extracted ion peak areas using mass chromatography. The extracted ions were as follows: 3-mercapto-3-methylbutyl formate, m/z 148; 3-mercapto-3-methylbutyl acetate, m/z 102; phenyl isothiocyanate, m/z 150. The residual ratios (%) were calculated from the measured values (the ratio of the extracted ion peak areas of each odorant to the extracted ion peak area of the internal standard) of the odorants in the thermally treated and nontreated solutions. These residual ratios were the mean values of triplicate results.

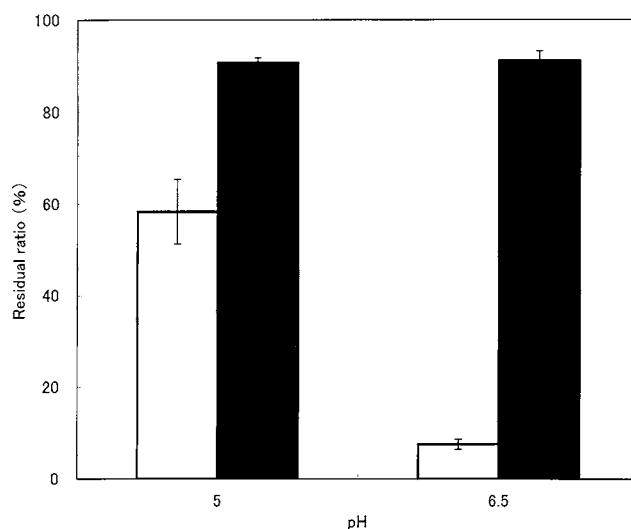


Figure 1. Residual ratios of 3-mercapto-3-methylbutyl formate (1, \square) and 3-mercapto-3-methylbutyl acetate (2, \blacksquare) after heating the coffee drinks under two pH conditions.

RESULTS AND DISCUSSION

Comparison of Thermal Stability of 3-Mercapto-3-methylbutyl Esters in the Coffee Drinks under Different pH Conditions. 3-Mercapto-3-methylbutyl formate (1) and 3-mercapto-3-methylbutyl acetate (2) are assumed to be the key contributors to the roasty odor quality of a coffee brew. Of the two volatile thiols, 1 was reported as a potent odorant for the sulfurous/roasty note of a roasted coffee flavor (6), and very recently, 2 was found as a peculiar odorant for the flavor of a highly roasted coffee brew (3). The thermal stability of these mercapto compounds is expected to significantly influence the roasty odor quality of the coffee drink products. In general, the canned coffee drinks are sterilized after adjusting the pH to between 5 and 7. Therefore, the thermal stability of the 3-mercapto-3-methylbutyl esters in the coffee drinks with different pHs were compared. The pH of the coffee drinks adjusted to pH 5.0 and 6.5 decreased to pH 4.7 and 5.3 during the heat processing, respectively. The flavor extracts of the thermally treated and nontreated coffee drinks were prepared by steam distillation under reduced pressure. The steam distillate was then concentrated by the adsorptive column method. The volatile fractions isolated from the coffee drinks were measured by GC–MS for the 3-mercapto-3-methylbutyl esters. The residual ratios of 1 and 2 in the after-heating coffee drinks, under different pH conditions, are shown in Figure 1. The concentration of 1 in the after-heating coffee drinks had drastically decreased, but 2 was hardly lost. This finding suggested that 1, which is significantly lost during the heat processing, is more important for the roasty odor quality of the coffee drinks than 2. In addition, the residual ratio of 1 in the coffee drink adjusted to pH 5.0 was higher than that of pH 6.5, while the residual ratio of 2 in the after-heating coffee drinks was only slightly affected by the pH conditions. These results suggested that the thermal stability of 1 not only has to be lower than 2 but also the possibility that it depends on the pH conditions.

Thermal Stability of Formate and Acetate Esters in Aqueous Model Solutions. The additional experiments focused on the difference in the structure of the formate and acetate esters and investigated the difference in the thermal stabilities of 1 and 2 in aqueous solutions. Figure 2 shows the residual ratios of 1 and 2 in aqueous model solutions at various pH values. Both esters were hydrolyzed and produced 3-mercapto-

Table 1. Residual Ratios of 3-Mercapto-3-methylbutyl Formate and Yields of Main Degradation Products (3-Mercapto-3-methylbutanol) after Heating Model Solutions at Different pH Values

		pH						
		7.0	6.5	6.0	5.5	5.0	4.0	3.0
3-mercapto-3-methylbutyl formate	residual ratio ^a	0.0	0.0	0.0	1.9	20.7	48.0	7.4
3-mercapto-3-methylbutanol	yield ^a	94.8	93.7	94.4	89.4	72.9	48.1	85.0

^a Residual ratios and yields are denoted by percentage.

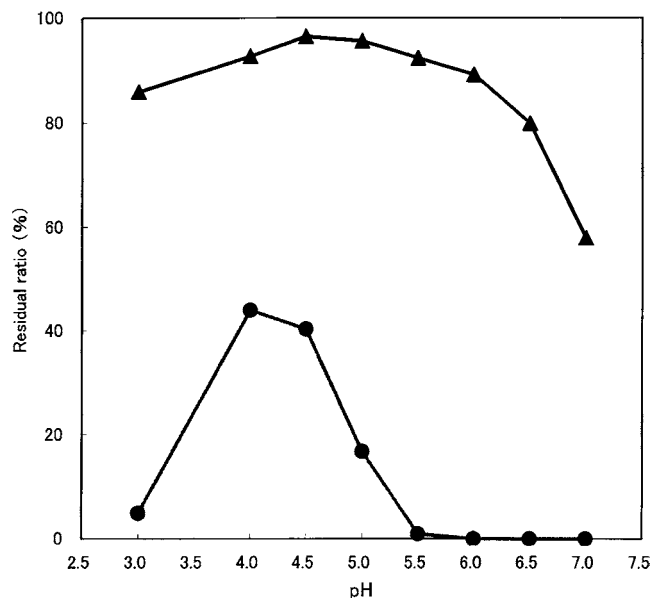


Figure 2. Changes in the residual ratios of 3-mercapto-3-methylbutyl formate (**1**, ●) and 3-mercapto-3-methylbutyl acetate (**2**, ▲) after heating model solutions at various pH values.

3-methylbutanol during the heat processing. In all of the pH ranges, the residual ratio of **1** was much lower than that of **2** and was influenced by the pH in the aqueous model solutions far more clearly than that of **2**. However, the residual ratios of **1** were not as simple as 2-furfurylthiol, which decreased with the increasing pH value (4). Namely, the residual ratios of **1** showed a maximum value at pH 4.0, and it decreased even if the pH was more than 4 or less than 4. After heating, **1** was almost lost above pH 6.0, but in the pH range of 4.0–6.0, the residual ratio of **1** increased with the decreasing pH values. These data clearly indicate that the very unstable **1** remains though it is in the range of the general pH condition used for the production of canned coffee drinks. Furthermore, it can account for the different residual ratios of **1** in the coffee drinks between pH 6.5 and 5.0 (The pH of the coffee drinks decreased during the heat processing. Therefore, it seems that the residual ratios of **1** in the coffee drinks gave a higher value than the residual ratio of the model experiment at the corresponding pH values).

In the pH range of 3.0–7.0, the residual ratios of the formate and acetate esters, which are composed of four kinds of alcohol derivatives, are shown in **Figures 3** and **4**. These esters were hydrolyzed during the heat processing and gave the corresponding alcohols, which were detected by GC and GC–MS. The residual ratios of the formate esters were much lower than that of the corresponding acetate esters over the pH range of 3.0–7.0. These results suggested that there is a more pronounced difference in the reactivity of both esters, due to the steric hindrance influence, electron release, and hyperconjugation effects of the methyl group which comprise the acetate esters. Moreover, the residual ratios of all the formate esters showed

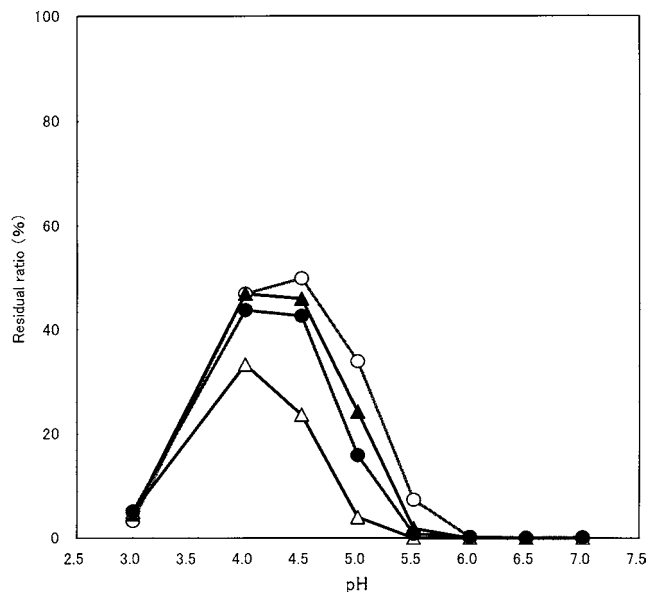


Figure 3. Changes in the residual ratios of various formate esters after heating model solutions at various pH values (○, hexyl formate; ▲, (Z)-3-hexenyl formate; ●, 2-phenylethyl formate; △, benzyl formate).

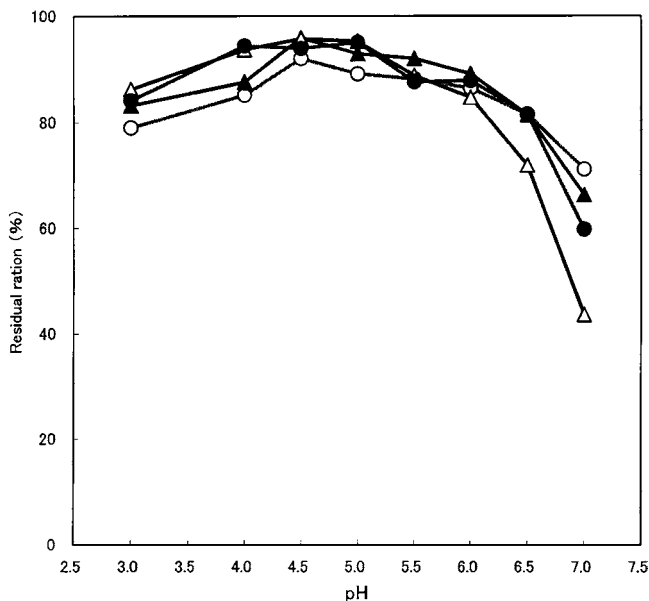


Figure 4. Changes in the residual ratios of various acetate esters after heating model solutions at various pH values (○, hexyl acetate; ▲, (Z)-3-hexenyl acetate; ●, 2-phenylethyl acetate; △, benzyl acetate).

the highest value as well as that of **1** around pH 4; therefore, the characteristic pH dependence of **1** is assumed to be caused by the effect of the formate ester structure which comprises **1**. In the after-heating model solutions, only 3-mercapto-3-methylbutanol (**3**) was detected by GC as the degradation product of **1**. The formation amounts of **3** for all the pH values

corresponded to about the amount that **1** lost during the heat processing (Table 1); therefore, it is thought that the major portion of **1** was lost by hydrolysis in all the tested pH ranges. However, the hydrolysis of **1** cannot be explained by only the acid-catalyzed mechanism due to the hydrogen ion, because that residual ratio showed a maximum value at pH 4.0. It is known that the hydrolysis in neutral aqueous solutions takes place via the following several reaction pathways: general base catalysis by water itself, the so-called water-catalyzed hydrolysis (12), alkaline hydrolysis with hydroxide ion whose concentration increases with a rise in temperature (13), and nucleophilic attack on the carbonyl carbon of the ester by the phosphate dianion in the buffer solution (14). Therefore, the hydrolysis of **1** may influence the direct nucleophilic attack mechanism by the above-described species. For instance, below pH 4.0, it can be assumed that the effect of the hydrogen-ion-catalyzed mechanism is significant and the direct nucleophilic attack mechanism is hardly involved. On the other hand, as the pH increased above 4.0, it can be presumed that the effect of the direct nucleophilic attack mechanism increased with the increasing pH, due to the concentration of the nonprotonated water and hydroxide ion, which will increase with the rise in the pH. The residual ratios of **1** that are observed with the model solutions may be assumed to be caused by the complex interaction of the different reaction mechanisms. Therefore, in the pH range of 5–7, which is used for the manufacturing process of the canned coffee drink, the hydrolysis of **1** may suffer from the influence of the direct nucleophilic attack mechanism more than the hydrogen-ion-catalyzed mechanism during the heating conditions for sterilization. However, as for the hydrolysis of the volatile esters during the coffee drink sterilization, the determining factors are unknown. Therefore, it would be necessary to investigate in detail the hydrolysis mechanism of these esters.

CONCLUSIONS

During the sterilization process of a canned coffee drink, **1** was much more unstable than **2** and the pH dependence of the thermal stability was recognized to have a maximum at pH 4.0. As for this phenomenon, the formate ester structure, which composed **1**, was assumed to be the cause. An effective method for reducing the decline in the roasty odor quality in the coffee drink products is to make the pH as low as possible during heat processing.

LITERATURE CITED

- (1) Grosch, W. Volatile compounds. In *Coffee recent developments*; Clarke, R. J., Vitzthum, O. G., Eds.; Blackwell Science: Oxford, 2001; pp 68–89.
- (2) Holscher, W.; Vitzthum, O. G.; Steinhart, H. Identification and sensorial evaluation of aroma-impact-compounds in roasted Colombian coffee. *Café Cacao Thé* **1990**, *34*, 205–212.
- (3) Kumazawa, K.; Masuda, H. Identification of odor-active 3-mercapto-3-methylbutyl acetate in volatile fraction of roasted coffee brew isolated by steam distillation under reduced pressure. *J. Agric. Food Chem.* **2003**, *51*, 3079–3082.
- (4) Kumazawa, K.; Masuda, H. Investigation of the change in the flavor of a coffee drink during heat processing. *J. Agric. Food Chem.* **2003**, *51*, 2674–2678.
- (5) Kumazawa, K.; Masuda, H.; Nishimura, O.; Hiraishi, S. Change in flavor of coffee drink during heating. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1998**, *45*, 108–113.
- (6) Mayer, F.; Czerny, M.; Grosch, W. Sensory study of the character impact aroma compounds of a coffee beverage. *Eur. Food Res. Technol.* **2000**, *211*, 272–276.
- (7) Hofmann, T.; Czerny, M.; Calligaris, S.; Schieberle, P. Model studies on the influence of coffee melanoidins on flavor volatiles of coffee beverages. *J. Agric. Food Chem.* **2001**, *49*, 2382–2386.
- (8) Hofmann, T.; Schieberle, P. Chemical interactions between odor-active thiols and melanoidins involved in the aroma staling of coffee beverages. *J. Agric. Food Chem.* **2002**, *50*, 319–326.
- (9) Yamada, M.; Kahara, Z. *A method for producing stable coffee drinks*; Japan Kokai Tokyo Koho, 95,184,546. July 25, 1995.
- (10) Blank, I.; Sen, A.; Grosch, W. Potent odorants of the roasted powder and brew of Arabica coffee. *Z. Lebensm.-Unters. Forsch.* **1992**, *195*, 239–245.
- (11) Tominaga, T.; Dubourdieu, D. Identification of cysteinylated aroma precursors of certain volatile thiols in passion fruit juice. *J. Agric. Food Chem.* **2000**, *48*, 2874–2876.
- (12) Mata-Segreda, J. F. Spontaneous hydrolysis of ethyl formate: isobaric activation parameters. *Int. J. Chem. Kinet.* **2000**, *32*, 67–71.
- (13) Robinson, B. A.; Tester, J. W. Kinetics of alkaline hydrolysis of organic esters and amides in neutrally-buffered solution. *Int. J. Chem. Kinet.* **1990**, *22*, 431–448.
- (14) Holland, J. M.; Miller, J. G. Hydrolysis of alkyl acetates in a phosphate-buffered aqueous medium. *J. Phys. Chem.* **1961**, *65*, 463–466.

Received for review June 29, 2003. Revised manuscript received October 4, 2003. Accepted October 5, 2003.

JF030458V