

Characterization of Volatile Compounds from the Reaction of 3-Hydroxy-2-butanone and Ammonium Sulfide Model System

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The reactions between 3-hydroxy-2-butanone and ammonium sulfide at 25, 50, 75, 100, 125, and 150 °C were studied. Four well-known flavor compounds, 2,4,5-trimethyloxazole, 2,4,5-trimethyl-3-oxazoline, 2,4,5-trimethylthiazole, and 2,4,5-trimethyl-3-thiazoline, were identified. Another four interesting intermediate compounds, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline, 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-oxazoline, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-thiazoline, and 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-thiazoline, were also identified by GC–EIMS and GC–CIMS. All these intermediate compounds were formed at 25 °C. On the other hand, tetramethylpyrazine was the major product with a reaction temperature higher than 100 °C.

Keywords: 3-Hydroxy-2-butanone; ammonium sulfide; 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline; 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-oxazoline; 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-thiazoline; 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-thiazoline

INTRODUCTION

Oxazoles, oxazolines, thiazoles, and thiazolines are four important heterocyclic aroma compounds containing nitrogen and oxygen or sulfur atoms. Maga (1981) has reviewed the occurrence of oxazoles and oxazolines in a variety of processed food systems. They possess potent sensory quality at a very low concentration described as green, sweet, and nutty aroma and have been identified in coffee (Stofflesma et al., 1968; Vitzthum et al., 1975), soy sauce (Nunomura et al., 1978), wheat (Harding et al., 1978), and cooked beef (Mussinan et al., 1976).

The occurrence of thiazoles and thiazolines has also been intensively reviewed (Maga, 1975; Ho and Jin, 1985). They are generally described as green, nutty, and vegetable-like aroma and have been found in heated foods such as baked potato (Coleman et al., 1981), roasted peanuts (Ho et al., 1983a), peanut butter (Joo and Ho, 1997), cocoa butter (Ho et al., 1983b), and fried chicken (Tang et al., 1983).

It has been proposed that thiazoles may be formed in processed foods by interaction of α -dicarbonyl compounds, aldehydes, ammonia, and hydrogen sulfide (Mussinan et al., 1976). In fact, thiazoles and thiazolines have been identified from the reaction of 2,3-pentanedione, acetaldehyde, ammonia, and hydrogen sulfide (Takken et al., 1976). Thiazoles have also been found in various model systems involving either degradation of glucose in the presence of fragmentation of cysteine or cystine (Shu et al., 1885a,b) or reaction of these with reducing sugars (Kato et al., 1973) or furaneol (Shu and Ho, 1988). In recent studies on the reaction of 3-hydroxy-2-butanone with ammonium acetate at low tem-

perature, an interesting compound, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline along with 2,4,5-trimethyl-3-oxazole and 2,4,5-trimethyl-3-oxazoline, was isolated and identified (Shu and Lawrence, 1995; Fu and Ho, 1997). Our objective in the present study was to isolate and identify the oxazoles, oxazolines, thiazoles, and thiazolines from the reaction of 3-hydroxy-2-butanone and the ammonium sulfide model system.

MATERIALS AND METHODS

Materials. 3-Hydroxy-2-butanone (acetoin) and ammonium sulfide (20 wt % solution in water) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Methylene chloride was obtained from Fisher Scientific Co. (Pittsburgh, PA). *n*-Tridecane was from Alltech Assoc., Inc. (Deerfield, IL).

Preparation of Reaction Mixture. A total of 0.88 g (0.01 mol) of 3-hydroxy-2-butanone and 6.8 mL (0.02 mol) of ammonium sulfide were mixed into 25 mL of distilled water with the pH value adjusted to 5.5 using 6 and 1 N HCl. The mixture was transferred into a 0.3-L Hoke SS-DOT sample cylinder, and the cylinder was sealed and heated at 25, 50, and 75 °C for 2 h. One milliliter of 1000 ppm *n*-tridecane was added into the reaction mixture as internal standard. Then the reaction mixture was extracted with a total of 50 mL of methylene chloride. The combined extract was dried over anhydrous sodium sulfate and concentrated to a final volume of 1 mL under a gentle stream of nitrogen gas. One microliter of extract was injected into the GC.

Preparation of the Solution for the Storage Study. For the storage study, a series mixture was prepared with 0.01 mol of acetoin (0.88 g) and 0.02 mol of ammonium sulfide (6.8 mL) with the addition of H₂O to a total volume of 25 mL. The pH values of all of the solutions were adjusted to 5.5 with 1 and 6 N HCl solution, and then the tubes were sealed and stored in a controlled environment incubator shaker (New Brunswick Scientific Co., New Brunswick, NJ) with constant shaking at 25 °C until further use. At the end of the storage period, internal standard *n*-tridecane was added and then extracted with methylene chloride three times (20, 20, and 10 mL). The combined extract was dried over anhydrous sodium

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Table 1. Volatile Compounds Identified in the Reaction of 3-Hydroxy-2-butanone/Ammonium Sulfide at 75 °C

no.	compound
1	2,4,5-trimethyl-3-oxazoline
2	2,4,5-trimethyloxazole
3	3-mercaptobutane
4	4,5-dimethylthiazole
5	2,4,5-trimethylthiazole
6	2,4,5-trimethyl-3-thiazoline
7	tetramethylpyrazine
8	2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline
9	2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline
10	2-(1-mercaptoethyl)-2,4,5-trimethyl-3-oxazoline
11	2-(1-mercaptoethyl)-2,4,5-trimethyl-3-oxazoline
12	2-(1-hydroxyethyl)-2,4,5-trimethyl-3-thiazoline
13	2-(1-hydroxyethyl)-2,4,5-trimethyl-3-thiazoline
14	2-(1-mercaptoethyl)-2,4,5-trimethyl-3-thiazoline
15	2-(1-mercaptoethyl)-2,4,5-trimethyl-3-thiazoline

sulfate and concentrated to 1 mL under a gentle stream of N₂. One microliter of extract was injected into the GC.

Quantitation and Characterization of Volatile Compounds. *Gas Chromatography.* GC analysis was accomplished by using a Varian 3400 gas chromatograph. A fused silica gel capillary column (30 m × 0.25-mm i.d., film thickness 0.25 μm, DB-1701; J&W Scientific, Folsom, CA) was used to analyze the volatile compounds. The operating conditions were as follows: injector and detector temperatures, 250 and 270 °C, respectively; helium carrier flow rate, 1 mL/min; GC temperature program, 40–260 °C at 3 °C/min followed by an isothermal hold at 260 °C for 10 min.

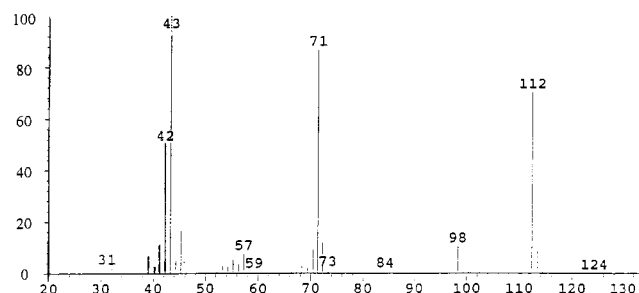
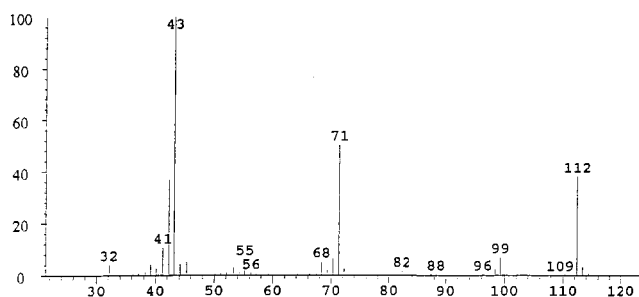
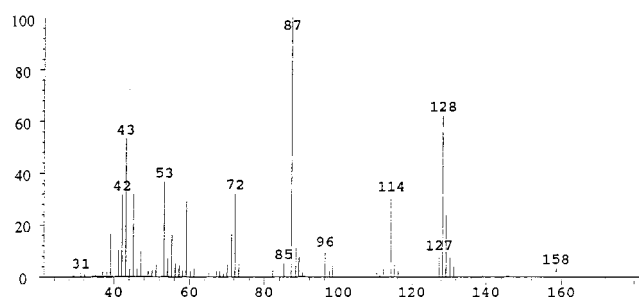
Gas Chromatography–Mass Spectrometry Analysis. EI mass spectra were obtained using a Hewlett-Packard 5790 gas chromatograph coupled with a Hewlett-Packard 5970A MSD detector with electron ionization at 70 eV and an ion source temperature of 250 °C. The operating conditions were the same as those used in the GC analysis described above. The data were recorded and analyzed using Hewlett-Packard MS Chem-Station data with NIST/EPA/MSDC mass spectral database.

CI mass spectra were performed on a Finnigan ITS-40 Magnum ion trap mass spectrometer coupled with a Varian 3400 gas chromatograph, and reactant gas (methane) was used. A fused silica gel capillary column (30 m × 0.25-mm i.d., film thickness 0.25 μm, DB-5; J&W Scientific, Folsom, CA) was used. The operating conditions were as follows: injector temperature, 260 °C; transfer line temperature, 260 °C; helium carrier flow rate, 1 mL/min; GC temperature program, 60–260 °C at 6 °C/min followed by an isothermal hold at 260 °C for 12 min.

RESULTS AND DISCUSSION

Volatile Compounds Identified in the 3-Hydroxy-2-butanone (Acetoin)/Ammonium Sulfide Model System. Fifteen compounds were tentatively identified by GC–EIMS at six different temperatures (25, 50, 75, 100, 125, and 150 °C) as shown in Table 1. They are 2,4,5-trimethyl-3-oxazoline (**1**), 2,4,5-trimethyloxazole (**2**), 3-mercaptobutane (**3**), 4,5-dimethylthiazole (**4**), 2,4,5-trimethylthiazole (**5**), 2,4,5-trimethyl-3-thiazoline (**6**), tetramethylpyrazine (**7**), 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline (**8**, **9**), 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-oxazoline (**10**, **11**), 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-thiazoline (**12**, **13**), and 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-thiazoline (**14**, **15**).

Compounds **8** and **9** have a molecular weight of 157 as determined by GC–CIMS. They have the same GC–EIMS spectra as shown in Figure 1. This mass spectrum matches well with the spectral data published previously by Shu and Lawrence (1995) and Fu and Ho (1997). They were, therefore, identified as syn and anti isomers of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline. These two peaks were also observed by Shu and

**Figure 1.** EI mass spectrum of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline.**Figure 2.** EI mass spectrum of 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-oxazoline.**Figure 3.** EI mass spectrum of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-thiazoline.

Lawrence (1995) as isomers in their study in which they were described as mild, yeasty, nutty, and bread-crust-like aroma.

The mass spectrum of compounds **10** and **11** is extremely similar with the previous spectra of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline as shown in Figure 2 except they have a molecular weight of 173 as determined by GC–CIMS. They are proposed to be the syn and anti isomers of 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-oxazoline.

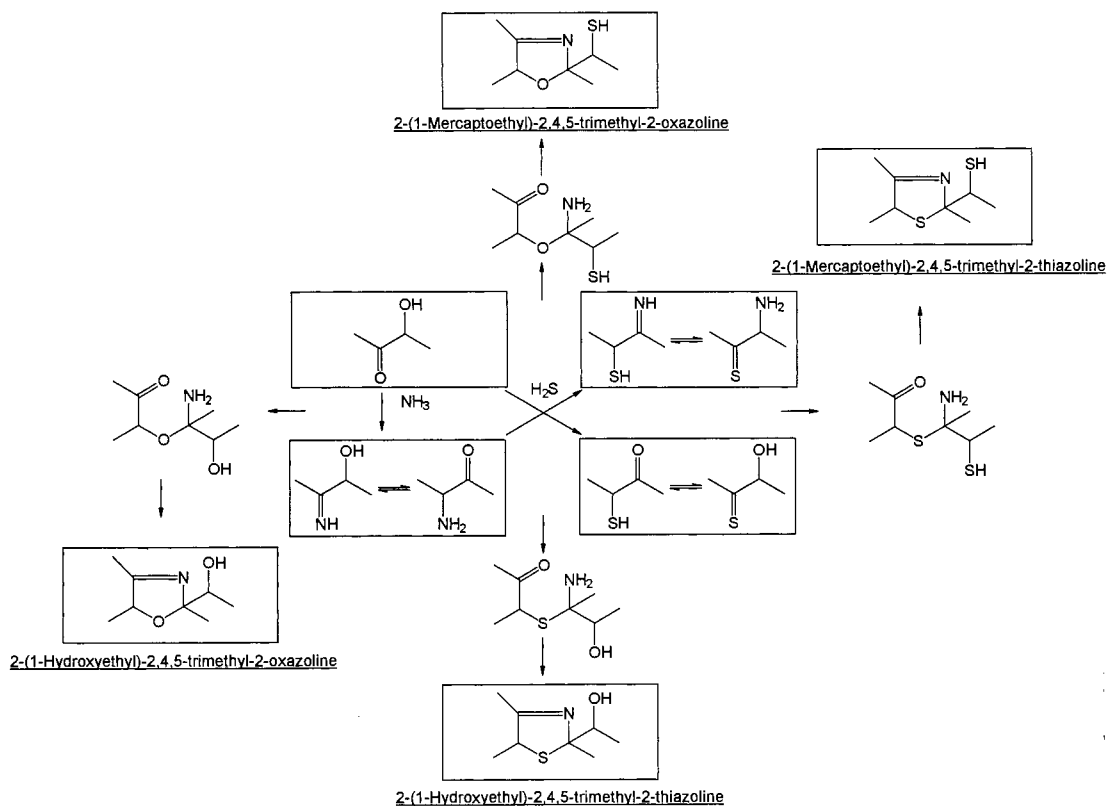
Compounds **12** and **13** also have a molecular weight of 173 as determined by GC–CIMS. However, their GC–EIMS spectra shown in Figure 3 suggested that they were sulfur analogues of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline. These two compounds were proposed to be the syn and anti isomers of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-thiazoline.

Compounds **14** and **15** have a molecular weight of 189 as determined by GC–CIMS. Their GC–EIMS spectra shown in Figure 4 suggested that they were sulfur analogues of compounds **10** and **11**. They were proposed to be the syn and anti isomers of 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-thiazoline.

Formation of 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline, 2-(1-Mercaptoethyl)-2,4,5-trimethyl-3-oxazoline, 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-thiazoline, and 2-(1-Mercaptoethyl)-2,4,5-trimethyl-3-thiazoline. Six temperatures (25, 50, 75, 100, 125,

Table 2. Quantitation of Volatile Compounds Generated from 3-Hydroxy-2-butanone/Ammonium Sulfide Model System

compound	quantity (mg/g of acetoin)					
	25 °C	50 °C	75 °C	100 °C	125 °C	150 °C
2,4,5-trimethyl-3-oxazoline	0.284	0.101	0.175	0.346	0.300	0.019
2,4,5-trimethyloxazole	0.106	0.113	0.111	0.142	0.516	1.846
3-mercaptobutane	0.698	0.327	0.249	0.172	1.002	1.329
4,5-dimethylthiazole	0.000	0.000	0.000	0.000	0.070	0.077
2,4,5-trimethylthiazole	0.093	0.086	0.186	0.596	2.447	3.765
2,4,5-trimethyl-3-thiazoline	0.000	0.018	0.127	0.325	1.503	1.686
tetramethylpyrazine	0.000	0.080	1.180	9.776	58.540	85.909
2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline	22.441	21.394	10.474	3.258	0.264	0.700
2-(1-mercaptoethyl)-2,4,5-trimethyl-3-oxazoline	0.491	0.710	0.144	0.154	0.034	0.048
2-(1-hydroxyethyl)-2,4,5-trimethyl-3-thiazoline	0.174	1.230	2.356	1.270	1.532	0.769
2-(1-mercaptoethyl)-2,4,5-trimethyl-3-thiazoline	0.300	1.849	3.302	3.052	2.161	1.809

Scheme 1. Formation of Oxazolines and Thiazolines in the Model Reaction of 3-Hydroxy-2-butanone and Ammonium Sulfide System

and 150 °C) were investigated in this model system. Quantitation of volatile compounds is summarized in Table 2. The data show that at and below 75 °C, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline was the most dominant product, whereas tetramethylpyrazine was the major product at or higher than 100 °C. These results agreed with the study of Fu and Ho (1997) in which it was observed that at a temperature below 25 °C, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline was the major product, while at a temperature higher than 85 °C, tetramethylpyrazine was formed predominantly. In the present study, due to the presence of hydrogen sulfide, the formation of tetramethylpyrazine was reduced at high temperatures. On the other hand, oxazoles, thiazoles, and thiazolines were found to be more stable despite the dramatic decrease in oxazoline production.

Stability Study of 3-Hydroxy-2-butanone/Ammonium Sulfide Model System. In our 96-day storage study of the mixture of 3-hydroxy-2-butanone and ammonium sulfide. Both 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and tetramethylpyrazine were moni-

tored. 2-(1-Hydroxyethyl)-2,4,5-trimethyl-3-oxazoline decreased dramatically during the early stage of the storage, while tetramethylpyrazine increased over the entire period of storage time (Figure 5). This study also supported the mechanism proposed by Shu and Lawrence (1995) who observed that, during their 3-week storage study of the mixture of acetoin and diammonium hydrogen phosphate, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline was readily formed during the early stage of the reaction while tetramethylpyrazine was increased more gradually during the final 10–21-day period. The pathway for the formation of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline may be reversible, so that 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline initially formed is able to be converted back to α -hydroxyimine, which in turn was tautomerized to α -aminoketone, so that the formation of tetramethylpyrazine could be followed (Shu and Lawrence, 1995).

Proposed Reaction Scheme for Volatile Compounds Formed in the 3-Hydroxy-2-butanone/Ammonium Sulfide Model System. Scheme 1 summarizes the reaction for the formation of oxazolines and

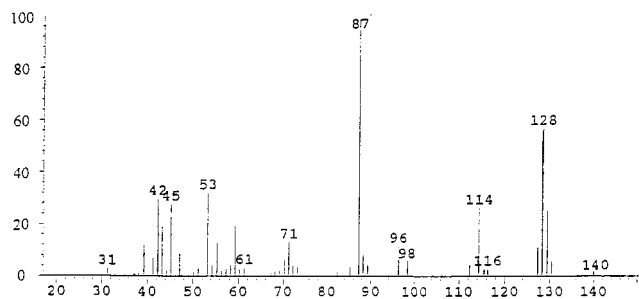


Figure 4. EI mass spectrum of 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-thiazoline.

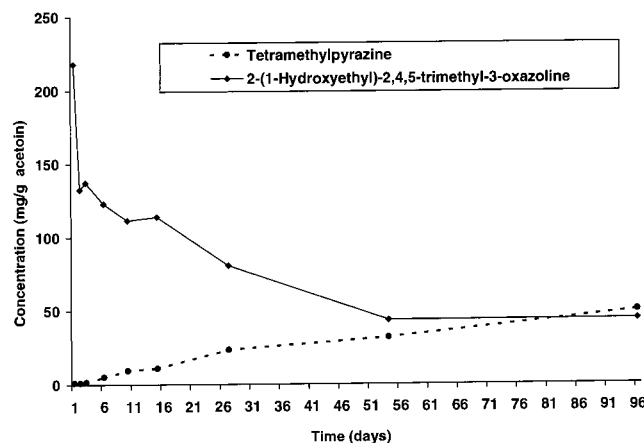


Figure 5. Concentration change of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and tetramethylpyrazine during the stability study.

thiazolines in the reaction of 3-hydroxy-2-butanone and ammonium sulfide model system. 3-Hydroxy-2-butanone reacts with ammonia and hydrogen sulfide generated from ammonium sulfide to form 2-amino-3-butanone, 3-mercapto-2-butanone, and 3-mercapto-2-aminobutane. These compounds react with each other to form four important intermediate compounds: 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline, 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-oxazoline, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-thiazoline, and 2-(1-mercaptoethyl)-2,4,5-trimethyl-3-thiazoline. Upon heating, these intermediate compounds may undergo further transformation into 2,4,5-trimethyloxazole, 2,4,5-trimethyl-3-oxazoline, 2,4,5-trimethylthiazole, and 2,4,5-trimethyl-3-thiazoline.

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Received for review May 20, 1998. Revised manuscript received October 16, 1998. Accepted October 27, 1998.

JF980536S