

Formation of Sulfur-Containing Flavor Compounds from Reactions of Furaneol and Cysteine, Glutathione, Hydrogen Sulfide, and Alanine/Hydrogen Sulfide

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Four reactions were carried out to compare the sulfur-containing compounds formed via Maillard reaction/Strecker degradation of cysteine with Furaneol and via the participation of hydrogen sulfide in the thermal degradation of Furaneol. GC–MS analysis showed that certain sulfur-containing compounds, such as 2,5-dimethylthiophene, 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone, and 3,5-dimethyl-1,2,4-trithiolane were found in four reactions, while thiirane and 2-methylthiophene were only found in the Strecker degradation of cysteine and Furaneol. Furthermore, this study showed that the more sulfur-containing compounds were formed in the participation of hydrogen sulfide than in the Maillard reaction/Strecker degradation of glutathione and even cysteine, indicating that the availability of hydrogen sulfide in the reaction may be the limiting factor in the amount and the type of sulfur-containing compounds formed in the reactions. Cysteine and glutathione are used in the reaction because of the different states in which cysteine exists. The amino group of the cysteine residue in glutathione is peptide bonded and cannot participate in the Strecker degradation with a dicarbonyl compound. The amino group in the free cysteine molecule, however, is accessible to dicarbonyl compound and the Strecker degradation is possible. Therefore, the reaction mechanisms involved in the reaction between cysteine and Furaneol would be different from those in the reaction between glutathione and Furaneol.

Keywords: Sulfur-containing flavor compounds; cysteine; glutathione; Strecker degradation; 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone; hydrogen sulfide

INTRODUCTION

Sulfur-containing volatile compounds is a major food aroma class found in vegetables, cooked meat, and other processed foods (Gasser and Grosch, 1988; Farmer and Mottram, 1990; Block *et al.*, 1992). While the sulfur-containing volatiles in vegetables such as the *Allium* genus are formed by enzymatic actions (Granroth 1970; Boelens *et al.*, 1971; Lawson *et al.*, 1991; Block, 1992; Block *et al.*, 1992), sulfur-containing flavors found in meat products are normally formed through thermal processing (Shahidi *et al.*, 1986; Tressl, 1989; Vercellotti *et al.*, 1989; Huang *et al.*, 1989; Zhang and Ho, 1991; Whitfield, 1992). It has been accepted that the sulfur-containing amino acids, cysteine and cystine, are indispensable components for generation of a meat-like aroma through thermal processing. They participate in the Maillard reaction and Strecker degradation to form those sulfur-containing compounds. In addition to the Maillard reaction and Strecker degradation, it is also believed that upon heating cysteine and cystine evolve hydrogen sulfide, one of the first compounds identified in early studies attempting to characterize the volatile compounds of cooked red meats (Osborne, 1928; Crocker, 1948) and poultry (Bouthilet, 1951a,b; Pippen and Erying, 1957). Glutathione, a tripeptide, can also rapidly evolve H₂S. Glutathione produces H₂S at the beginning of the cooking, while the cysteine in muscle protein serves as the precursor of this aroma chemical in meat on prolonged heating (Ohloff *et al.*, 1985). Since all proteinaceous foods probably emanate H₂S upon

heating, any effect on food flavor must be attributed to the concentration of H₂S, the reaction of H₂S with other compounds, or both.

In this study, we report that the formation of the volatile compounds generated from the reactions of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (or Furaneol) with cysteine, glutathione, alanine/sodium sulfide, and sodium sulfide.

Furaneol was selected to be one of the reactants because it exists in many foods. Furaneol can be found in pineapple (Rodin *et al.*, 1965), strawberries (Re *et al.*, 1973), beef broth (Tonsbeek *et al.*, 1968), and roasted almonds (Takei and Yamanishi 1974). It was reported that Furaneol is one of the thermal degradation products of fructose (Ohloff, 1970). It has been characterized in various model systems such as the degradation of fructose (Shaw *et al.*, 1968), pyrolysis of D-glucose (Fagerson, 1969; Heyns *et al.*, 1966; Johnson *et al.*, 1969), pyrolysis of 1-deoxy-1-piperidino-D-fructose (Mills *et al.*, 1969), roasting of alanine and rhamnose (Shaw and Berry, 1977), and reaction between rhamnose and piperidine acetate (Hodge and Fisher, 1963).

As a dihydrofuranone, the oxygen atom in Furaneol is readily exchangeable with the sulfur atom in hydrogen sulfide to form thiophene. Van den Ouweland and Peer (1975) reacted hydrogen sulfide and 5-methyl-4-hydroxy-3(2*H*)-furanone in an aqueous solution at 100 °C and obtained 16 compounds, 11 of which were mercapto derivatives from furan/furanone or from thiophene/thiophenone. In their study, Shu and Ho (1988) reacted cysteine and Furaneol at 160 °C and reported several thiophene/thiophenone compounds.

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MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma Chemical Co. unless otherwise specified. The reactions were carried out in a 500 mL Parr bomb (Parr Instrument Co., Moline, IL). Equimolar (0.1 M) Furaneol and cysteine, glutathione, sodium sulfide, and alanine or sodium sulfide were mixed and dissolved in 100 mL of 0.1 M sodium phosphate buffer solution at pH 7.0 and placed in a preheated reaction vessel. The bombs were then heated in a glycerol bath at 130 °C. After a 3 h reaction, the bomb was cooled to room temperature using running cool tap water. The reaction mass was stored in a refrigerator until the isolation.

To isolate the volatiles, the reaction mass was transferred to a 1000 mL distillation flask. Distilled water (100 mL) was then used to rinse the Parr bomb and was added to the distillation flask. The volatile compounds were simultaneously steam-distilled and extracted into 100 mL of methylene chloride using a Liken-Nickerson apparatus. The distillates were dried over anhydrous sodium sulfate and concentrated using a Kuderna-Danish apparatus to ~4 mL then slowly concentrated further under a stream of nitrogen to 200 μ L.

The flavor compounds were analyzed using a Hewlett-Packard 5890 gas chromatograph (GC) with a flame ionization detector (FID). Analysis was carried out on a 50 m \times 0.32 mm i.d. bonded methyl silicone (OV-1) column with a 0.52 μ m film thickness using a temperature program controlled by a Hewlett-Packard DOS Chemstation system from an initial temperature of 40 °C to a final temperature of 225 °C at a rate of 2 °C/min. The final temperature was held for 30 min before the next analysis. The carrier gas used is helium at a linear velocity of 27.0 cm/s as determined with pentane. The injection volume is 1 μ L with split flow at 400 mL/min. The data were acquired and processed using Hewlett-Packard Chemstation DOS software. Eighteen ethyl esters from ethyl formate to ethyl octadecanoate were used to calculate the retention index (I_E).

The identification was done by comparing both the sample compound's mass spectrum and the retention index (I_E) information with the mass spectra library and the retention index data bank in International Flavors and Fragrances, obtained using the authentic compounds.

Since residual Furaneol can be isolated and detected in the extract, the quantification of the volatile compounds in the extract was done by using Furaneol as the reference. The response factor of Furaneol was determined with a Furaneol standard (1500 ppm in methylene chloride) using the GC method to be $8.74 \times 10^7 \text{ mg}^{-1}$. Assuming the relative response factors of the other volatiles are the same as Furaneol, the amount of the volatile compounds can be estimated by computing the GC area against that of the Furaneol using the following equation:

$$\text{mg/mol} = \frac{\text{area of compound} \times \text{dilution factor}}{\text{molarity of sample} \times \text{response factor of Furaneol}}$$

RESULTS AND DISCUSSION

The reaction of cysteine with 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone has been known to include thermal degradation of the reactants and interactions among the degradation products and the reactants, such as (i) cysteine degradation to dehydroalanine, aldehydes, hydrogen sulfide, and ammonia, (ii) Furaneol degradation to dicarbonyls, ketones, and alcohols, (iii) Strecker degradation of cysteine and dicarbonyls, and (iv) other interactions among the degradation products (Shu and Ho, 1988).

By replacing cysteine with alanine and sodium sulfide, an analogue mixture to the thermal degradation product of cysteine, or with sodium sulfide, the pathway and the precursor of certain flavor compounds could be elucidated. Glutathione, on the other hand, is different from cysteine. The cysteine residue is in the middle of

the glutathione molecule. The amino group of the cysteine residue is tied up in the peptide bond so that no Strecker degradation between the cysteine residue and the dicarbonyl compound can occur. Therefore, the differences between the reaction of glutathione/Furaneol and cysteine/Furaneol could provide additional information on the mechanism of the formation of sulfur-containing compounds. First, sulfur-containing compounds from the reactions between glutathione and Furaneol via the thermal generation of hydrogen sulfide should be the same as those obtained from cysteine and Furaneol via the same mechanism. Second, the difference in the volatiles formed among the reactions could be due to different reactants. In the Strecker degradation, for example, the amino compound is cysteine for the reaction between cysteine and Furaneol, whereas the amino compound is the glutamate residue of glutathione in the reaction between glutathione and Furaneol. Subsequently, the Strecker degradation products would be different. So would be the final flavor compounds. Third, the amount of intermediate would be different among the reactions due to the different reactivity between cysteine and glutathione. That is, it was expected that more flavor compounds would be formed from cysteine than from glutathione.

Table 1 lists the compounds identified in all four of the reactions. It can be seen that carbonyls are the major products in the glutathione reaction, while sulfur-containing compounds predominate in the volatiles of the reactions of alanine/sodium sulfide and sodium sulfide. The large number of sulfur-containing compounds formed in the reactions of Furaneol with alanine/sodium sulfide or sodium sulfide maybe due to the higher concentration of hydrogen sulfide as S^{2-} than what was generated from cysteine or glutathione.

The results also show that cysteine is more reactive than glutathione. In addition to the amount of sulfur-containing compounds, other heterocyclic compounds (i.e., pyrazines and thiazole/thiazolines) formed in the reaction of cysteine and Furaneol are more, in number and amount, than what were formed in the reaction of glutathione and Furaneol. It was also reported that the amount of volatiles obtained from glutathione was less than that obtained from cysteine in the reaction with glucose or 2,4-decadienal at pH 7.5 and 180 °C for 1 h (Zhang and Ho, 1989, 1991). It seems that cysteine, as a reactant, will participate in flavor reaction via the Maillard reaction and Strecker degradation more readily than via thermal degradation. Glutathione, however, will degrade to smaller intermediates such as hydrogen sulfide, which in turn participates in the flavor reaction. It was reported that the thermal degradation of glutathione is temperature dependent (Zheng and Ho, 1994). A higher reaction temperature will produce more intermediates and subsequently more volatile compounds. This can be confirmed by comparing the amount of volatiles obtained from this reaction to those obtained by Zhang and Ho (1989, 1991).

While some sulfur-containing compounds such as 2,5-dimethylthiophene, 2,5-dimethyl-4-hydroxy-3(2*H*)-thiophenone, 3,5-dimethyl-1,2,3-trithiolane, were identified in all four reactions, other sulfur-containing compounds are reaction specific. Two such compounds are thiirane and 2-methylthiophene. They were identified only in the reaction between cysteine and Furaneol. It is believed that the Strecker degradation of cysteine is responsible for the formation of these two compounds. During the Strecker degradation of cysteine, mercapto

Table 1. Volatile Compounds Identified from the Reaction of Furaneol with Cysteine (F/C), Glutathione (F/G), Alanine and Sodium Sulfide (F/A/S), and Sodium Sulfide (F/S)

compound identified	amount ^a (mg/mol)				compound identified	amount ^a (mg/mol)			
	F/C	F/G	F/A/S	F/S		F/C	F/G	F/A/S	F/S
	Aldehyde/Ketones								
(<i>E</i>)-3-penten-2-one	1.07	0.50			2-hydroxy-3-pentanone	1.82	0.68	0.78	
(<i>E</i>)-4-hexen-3-one		0.12			2-methylpropanal		0.04	<0.02	0.04
1-hydroxy-2-butanone		0.18			2-penten-3-one		2.36	0.17	
1-hydroxy-2-propanone		1.48			3,4-hexanedione	0.54	1.02		
2,3,4-trimethyl-2-cyclopentenone			2.12		3-hexanone	0.49	0.93		
2,3-butanedione	34.49	10.14	14.97	14.47	3-hydroxy-2-pentanone	0.30	0.74		0.17
2,3-dimethyl-2-cyclopentenone	0.16				3-methyl-2-butanone	0.10	0.11	0.06	0.16
2,3-hexanedione	0.17	0.20	0.35	0.36	3-methyl-3-hexen-2-one			0.51	1.30
2,3-pentanedione	0.19	0.27	0.10	0.20	3-pentanone	2.44	2.38	2.52	1.45
2,4-hexanedione		2.07	0.73	1.47	4-hexen-3-one	0.11			
2-butenal		0.49	<0.02		4-penten-2-one	1.19			
2-hexanone	0.17	0.44			acetaldehyde	0.35	5.42		
2-hydroxy-3-butanone	0.10	0.12			acetone		0.07		
	Furans								
2,4,5-trimethyl-3(2 <i>H</i>)-furanone	0.13	0.71		0.62	2,5-dimethyl-4-hydroxy-3(2 <i>H</i>)-furanone ^b	4.36	7.64	0.23	3.51
2,5-dimethyl-3(2 <i>H</i>)-furanone	0.14	0.28	0.07	0.04	4,5-dimethyl-2(3 <i>F</i>)-dihydrofuranone				1.24
	Pyrazines								
2-ethyl-5-methylpyrazine	0.40				2,5-dimethylpyrazine	0.37	0.14		
trimethylpyrazine	^c								
	Sulfides								
2,5-dimethyl-4-mercapto-3(2 <i>H</i>)-furanone	0.11		1.89		3-mercapto-2-pentanone			0.49	0.31
2-mercapto-3-butanone	0.21		4.63	22.10	3-methyl-2-butanethiol			5.44	15.58
2-mercapto-3-pentanone			0.39	0.46	4-(ethylthiol)butan-2-one				0.38
2-methyl-2-[(1-methylethyl)-thiol]propane				0.93	4-(methylthio)phenol				2.20
2-methyl-2-pentanethiol			0.11	0.10	dibutyl disulfide				1.88
2-methyl-3-(thioacetyl)furan				0.36	ethanethioic acid, <i>S</i> -methyl ester				3.42
3,5-dimethyl-1,2,4-trithiolane	1.31	1.88	2.47	3.74	thiirane	11.48			
	Thiazoles/Thiazolines								
2,4,5-trimethylthiazole	1.64	0.17			2-acetylthiazole		0.22		
2,4,5-trimethyl-3-thiazoline	0.67				2-thiazolyl ethyl ketone	1.07			
2,4-dimethylthiazole	2.03	2.48			4,5-dimethylisothiazole		0.24		
	Thiophenes								
2,3,4-trimethylthiophene				0.47	2-ethyl-5-methylthiophene			0.16	1.92
2,4-dimethylthiophene			0.11	0.17	2-ethyltetrahydrothiophen-3-one				4.60
2,5-dimethylthiophene	0.31	0.95	0.70	0.56	2-methylthiophene	0.25			
2,5-dimethyl-2-hydroxy-3(2 <i>H</i>)-thiophenone			3.25	6.28	2-methyltetrahydrothiophen-3-one	0.82			
2,5-dimethyl-4-hydroxy-3(2 <i>H</i>)-thiophenone	0.22	0.15	1.26	1.16	3-acetylthiophene			0.34	0.48
2-acetylthiophene			2.14	3.13	3-acetyl-2,5-dimethylthiophene			1.33	1.18
2-acetyl-3-methylthiophene			0.62	0.64	5-methyltetrahydrothiophen-3-one	0.33			
2-acetyl-5-methylthiophene			3.90		tetrahydrothiophen-3-one	0.09			
2-ethyltetrahydrothiophen-3-one			2.46		thiophene	2.60			

^a Calculations based on Furaneol. ^b Starting material. ^c Coeluted with 2-ethyl-5-methylpyrazine.

acetaldehyde is produced with the formation of α -amino ketone. The cyclization of mercaptoacetaldehyde leads to the formation of thiirane or the condensation with acetone to form 2-methylthiophene with the loss of water. Since no Strecker degradation of cysteine occurs in the other reactions, no mercaptoacetaldehyde is formed. Consequently, no thiirane or 2-methylthiophene was found. On the other hand, 2,5-dimethylthiophene is found in all four reactions. It is obvious that 2,5-dimethylthiophene is formed from the exchange of the oxygen atom in 2,5-dimethylfuran with the sulfur atom in hydrogen sulfide.

In 1995, Rizzi reported that mercaptans are from the substitution of the hydroxy group of allylic alcohols with the sulfhydryl group (Rizzi, 1995). Furaneol is unstable with heat and will undergo degradation to produce a variety of carbonyl compounds and hydroxy carbonyls such as 2-hydroxy-3-butanone and 2-hydroxy-3-pentanone as observed in this study. By comparing the sulfur-containing compounds observed in this study, it can be found that the majority of the thiols identified in all four reactions results from the reaction of hydrogen sulfide with aliphatic aldehydes or alcohols, the thermal degradation products of Furaneol. Sulfur-containing compounds other than thiols, such as thiophenes, are also identified in large quantity in the

reaction between Furaneol and sodium sulfide or sodium sulfide/alanine. Among the thiophene derivatives identified, 2,4-dimethylthiophene was reported to possess a fried onion odor (Arnold et al., 1969). 2-Acetylthiophene, 3-acetylthiophene, and 2-acetyl-5-methylthiophene were also identified in cooked beef and pork (Wilson et al., 1973; Mussinan and Walradt, 1974).

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