

# Effect of Urea on Volatile Generation from Maillard Reaction of Cysteine and Ribose

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Urea occurs naturally in many food products, and its presence affects food quality. However, little is known about its impact on flavor generation in food production. In this study, the urea contents in beef, pork, and chicken were determined. The effects of urea and pH on thermal flavor generation were investigated using the model system of cysteine with ribose, which was heated to the roasting temperature of 180 °C for 2 h at pH 5 and pH 8.5. The results revealed relatively large amounts of urea in these meats and demonstrated that pH affects aroma generation. Volatiles identified from the reaction system of ribose and cysteine showed that sulfur-containing compounds such as thiophenes, thiazoles, and thiophenethiols were the most abundant compounds. The addition of urea into the reaction mixture caused the disappearance or reduction in content of some sulfur-containing compounds but resulted in the generation of several important nitrogen-containing volatiles, like pyrazine, methylpyrazine, 2,5- (and 2,6-)dimethylpyrazine and other alkylpyrazines, which are known to elicit roasty, nutty flavor notes. A plausible explanation for this phenomenon is that ammonia can be released from urea upon heating and the formed ammonia competes with hydrogen sulfide to react with Maillard reaction precursors to produce nitrogen-containing compounds such as alkylpyrazines.

**Keywords:** *Maillard reaction; urea; ribose; cysteine; flavor generation*

## INTRODUCTION

Many types of chemical reactions are responsible for meat flavor formation, and over 1000 volatile compounds have been reported in beef, pork, lamb, and chicken. Sulfur-containing heterocyclic aroma compounds are known to play an important role in contributing meaty flavor to roasted and cooked meats. Cysteine is an important precursor for the formation of these sulfur compounds and has been extensively used in the manufacturing of reaction flavors. During the cooking of meat, hydrogen sulfide is liberated from the cysteine residues of the meat proteins or peptides and participates in subsequent thermal reactions to generate a number of sulfur-containing volatile compounds.

The Maillard model system involving ribose and cysteine has been used widely to study generation of meaty flavors (Hofmann and Schieberle, 1995; Mottram and Whitfield, 1995a,b; Werkhoff et al., 1990). Over 180 compounds have been identified from these reaction systems, and the key odorants elicit an overall roasty, meatlike odor (Hofmann and Schieberle, 1995). These compounds can be mainly categorized as thiols, thiophenes, thiazoles, and polysulfides. The odor quality of some specific volatile compounds from the aqueous reaction mixture of ribose and cysteine were identified after Aroma Extract Dilution Analysis (AEDA) conducted by Schieberle and Hofmann (1995). For example, 2-furfurylthiol and 5-methyl-2-furfurylthiol were described

as roast- and coffee-like while 2-methyl-3-furanthiol was described as meatlike. It has been reported that the overall odor profiles of such reaction flavor and the volatiles formed are affected by a number of factors such as sugar moiety, temperature, reaction time, and the presence or absence of water as well as the pH of the reaction solution (Lane and Nursten, 1983; Mottram and Whitfield, 1995b). For example, in the dry-heated mixture of ribose and cysteine, 2-furfurylthiol followed by 2-acetyl-2-thiazoline were characterized as the most odor-active compounds (Schieberle and Hofmann, 1998); while in aqueous reaction system, 2-furfurylthiol, 2-methyl-3-furanthiol, 2-thenyl mercaptan, and ethyl mercaptan were the major contributors to the overall roasty, meatlike odor.

Urea is formed in the liver, passed via bloodstream to the kidneys, and excreted into the urine. High levels of urea are found in blood and tissues. It was reported that fishes such as sharks, dogfish, rays, and skates contain high levels of urea (Cheuk and Finne, 1984). Small amounts of urea are formed in many kinds of fermented foods and beverages (Matsudo and Sasaki, 1995). The presence of urea in food products affects food quality. For instance, urea is an essential precursor of ethyl carbamate, a well-known carcinogen in alcoholic beverages (Kodama and Suzuki, 1995). In fish, urea can be converted to ammonia, which affects fish freshness and has been suggested as a quality index (Cheuk and Finne, 1984). However, little is known about the role of urea in flavor generation. The objective of this study was to investigate the effects of urea on volatile generation using Maillard reaction model of D-ribose and L-cysteine.

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## EXPERIMENTAL PROCEDURES

**Materials.** Urea, D-ribose, acetoin (3-hydroxy-2-butanone), tridecane, and sodium sulfate were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). L-Cysteine and diacetyl (2,3-butanedione) were bought from Sigma Chemical Co. (St. Louis, MO). Beef, pork, and chicken meat were purchased from a local supermarket. The urea determination kit was bought from Boehringer Mannheim Co. (Indianapolis, IN). Methylene chloride used was of HPLC grade from Fisher Scientific (Fair Lawn, NJ).

**Thermal Reactions.** A total of 0.01 mol of L-cysteine and D-ribose, with or without an equal mol of urea, was dissolved in 100 mL of distilled water. The solutions were adjusted to pH 5.0 or pH 8.5 prior to thermal reactions in 150-mL Hoke stainless steel cylinders (Hoke Inc., Clifton, NJ) that were heated at 180 °C in an oven for 2 h. The reactions were immediately stopped by cooling under a stream of cold water. The thermal reactions between acetoin/diacetyl and urea were conducted at the same pattern and conditions.

**Isolation of the Volatile Compounds.** After being cooled, the brown reaction mixture was mixed with 0.5 mL of internal standard (tridecane, 1 mg/mL) and extracted using methylene chloride (50 mL  $\times$  3 times). The extract was dehydrated by anhydrous sodium sulfate, concentrated under a nitrogen flow to 10 mL in a flask, and then transferred to a Kuderna–Danish concentrator and further concentrated to 1–1.5 mL.

**Gas Chromatographic Analysis.** The volatile compounds isolated from the thermal reaction systems were analyzed by a Varian 3400 gas chromatograph (GC) equipped with a fused silica capillary column (60 m  $\times$  0.25 mm i.d.; 1  $\mu$ m thickness, DB-1, J&W Scientific Inc., Folsom, CA) and a flame ionization detector. For each sample, 1  $\mu$ L was injected into the GC with a split ratio 25:1. The GC was run with an injector temperature of 270 °C, a detector temperature of 300 °C, and a helium carrier flow rate of 1 mL/min. The column temperature was programmed as follows: from 40 °C to 260 °C at 2 °C/min increasing rate.

**GC/Mass Spectrometry Analysis.** The concentrated isolates from different reaction mixtures were analyzed by GC/mass spectrometry (GC/MS), using a Hewlett-Packard 6890 GC coupled to a Hewlett-Packard 5973 mass selective detector. The fused silica capillary column (60 m  $\times$  0.25 mm i.d.; 1  $\mu$ m thickness, DB-1) was purchased from Supelco. The injection was carried out at 250 °C in a splitless mode. The carrier gas flow was 1 mL/min. The temperature program was the same as that for GC analysis. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 280 °C.

**Identification of the Volatiles.** The identification of the volatile compounds was based on GC/MS analysis. The compound from the isolate was identified by comparing the mass spectral data with those of authentic compounds available in the Wiley 138 library or previous publications (Hofmann and Schieberle, 1995; Mottram and Whitfield, 1995a,b; Mulders, 1973; Werkhoff et al., 1990).

**Urea Determination.** The determination of urea content in meat was conducted based on the method of Cheuk and Finne (1984) and Boehringer Mannheim Co. (Indianapolis, IN). For sample preparation, about 5 g of the homogenized meat sample was mixed with approximately 20 mL of perchloric acid (1 mol/L) and homogenized for 2 min in a homogenizer tube. The content was transferred into a beaker with about 40 mL water, and the pH of the solution was adjusted to 7.0 with potassium hydroxide (10 and 2 mol/L). Then the content was transferred into a 100-mL volumetric flask, and water was added to make the final volume 100 mL. The supernatant was used for urea determination.

**Ammonia Determination in Urea Solution.** The effect of heat treatment on ammonia release from urea was investigated by determining ammonia concentration in urea solution. Solutions containing urea (50 mL of 100 mM) inside bottles were heated at different temperatures inside an oven for 2 h before measurement, which was conducted by using an ammonia electrode (model 95-12) coupled with an Orion

**Table 1. Urea Concentration in Meats**

	meat type		
	beef	chicken	pork
urea content <sup>a</sup>	41.153 $\pm$ 0.011	7.250 $\pm$ 0.006	25.634 $\pm$ 0.011

<sup>a</sup> The value is the average of three samples ( $n = 3$ ) (mg/100 g of meat).

ion analyzer (model EA 940). The values are averages of triplicates.

## RESULTS AND DISCUSSION

Urea contents in beef, pork, and chicken were determined, and the data are listed in Table 1. The results indicated that these meats possess high levels of urea, but the content in beef is the highest and chicken meat contains the least amount of urea among these meats. This might be due to the higher blood content in beef than in chicken. Urea biosynthesis occurs almost exclusively in the liver, and it is passed via the bloodstream to the kidneys and excreted into the urine. As a result of circulation, urea is naturally contained in muscle tissues. The occurrence of urea affects the quality of food products. For instance, during iced or refrigerated storage urease-positive microorganisms will rapidly convert urea to ammonia, which gives an unpleasant odor and affects fish freshness (Cheuk and Finne, 1984). Removal of urea is necessary to prevent such a defect. In fact, Pastoriza and Sampedro (1991) emphasized the significant loss of urea from ray muscle as a result of thermal treatment, which improved its sensory characteristics noticeably. However, information concerning the influence of urea on flavor generation is virtually unavailable. It is worthwhile to investigate the role of urea in volatile production during meat cooking due to its relative high content.

The Maillard reaction between ribose and cysteine was chosen as an experimental model for this study. The reaction mixtures of ribose and cysteine at both pH 5 and pH 8.5 were heated at 180 °C for 2 h. Expectedly, sulfur-containing compounds were the major volatiles identified in both pH conditions (Table 2). The most abundant odorant was 2-furfurylthiol, which is roasty and coffee-like. This was in conformity with a previous study by Parliament and Stahl (1994), who investigated the effect of pH, time, and temperature on 2-furfurylthiol formation and showed that at higher temperatures 2-furfurylthiol was the major compound generated. Our data also revealed large amounts of 2-methylthiophene, 2-methyltetrahydrothiophen-3-one, and 3,5-dimethyl-1,2,4-trithiolane. Among them, thiophenes are known to be substantial contributors to overall meaty flavor, and many thiophenes were isolated from meat-based products (Nonaka et al., 1967; Wilson et al., 1973; Werkhoff et al., 1993). It appears that pH has an effect on the overall odor of the thermally treated reaction mixture of ribose and cysteine. Besides the difference regarding the concentration of sulfur-containing compounds between pH 5 and pH 8.5, a distinguishing aspect resided in the generation of pyrazines. At pH 5, there was no pyrazines generated while interestingly at pH 8.5 several nitrogen-containing compounds including pyrazine, methylpyrazine, and ethylpyrazine were produced, giving the reaction mixture additional roasted notes besides the sulfury, meatlike odors. This result was in alignment with the finding by Hofmann and Schieberle (1995). In their study, sensory evaluation

**Table 2. Volatile Compounds Generated from Thermal Reaction of Ribose and Cysteine, with/without Urea at pH 5 or pH 8.5**

compounds	amount (mg/g of ribose)				compounds	amount (mg/g of ribose)			
	RC5 <sup>a</sup>	RCU5 <sup>b</sup>	RC8.5 <sup>c</sup>	RCU8.5 <sup>d</sup>		RC5 <sup>a</sup>	RCU5 <sup>b</sup>	RC8.5 <sup>c</sup>	RCU8.5 <sup>d</sup>
					Pyrazines				
pyrazine		0.25	0.06	0.33	2-ethyl-5-methylpyrazine				0.04
methylpyrazine		3.18	0.35	3.80	2-ethyl-6-methylpyrazine		0.10		0.13
ethylpyrazine		0.17	0.04	0.22	trimethylpyrazine		0.09		0.18
2,3-dimethylpyrazine		0.11	0.03	0.18	2-ethyl-3,5-dimethylpyrazine		0.03		0.05
2,5- (and 2,6-) dimethylpyrazine		0.47		0.91	3-ethyl-2,5-dimethylpyrazine				0.09
vinylpyrazine				0.02	1-methylpyrrolo[1,2- <i>a</i> ]-pyrazine		0.04		
2-ethyl-3-methylpyrazine		0.04	0.03	0.03					
					Pyridines				
1 <i>H</i> -pyrrolo[2,3- <i>b</i> ]pyridine				0.21	1 <i>H</i> -pyrrolo[2,3- <i>c</i> ]pyridine		0.11		0.17
					Pyrrole				
1-(2-furfuryl)pyrrole	0.14				1 <i>H</i> -pyrrole		0.09		0.06
<i>N</i> -formylpyrrole		0.03							
					Thiazoles				
thiazole	0.07		0.10		2,4-dimethylthiazole				0.03
2-acetylthiazole		0.29	0.15	0.77	2,4,5-trimethylthiazole			0.05	
4-methylthiazole		0.07		0.03	5-ethyl-2,4-dimethylthiazole	0.07			
					Thiophenes				
thiophene	0.10				3-acetylthiophene		0.27		0.21
2-methyl-4,5-dihydrothiophene	0.05	0.02			2- or 3-thiophene carboxaldehyde			0.05	
2-methylthiophene	0.52	0.03	0.15		2-thiophenethiol	0.25		0.13	
3-methylthiophene	0.27		0.08	0.01	3-thiophenethiol	0.03			
2-formyl-5-methylthiophene			0.05		2-thiophenemethanethiol		0.09		
2-formyl-2,3-dihydrothiophene	0.16	0.02	0.05		2- or 3-thiophenemethanol	0.07			
2-acetyl-3-methylthiophene	0.09	0.06			2-thiophenecarboxylic acid	0.08		0.05	
thieno[3,2- <i>b</i> ]thiophene	0.14		0.11		3-thiophenecarboxylic acid	0.33			
2-acetylthiophene		0.17		0.19					
					Thiophenones				
2-methyltetrahydrothiophen-3-one	0.45		0.23	0.15	dihydro-2-methyl-3(2 <i>H</i> )-		0.09	0.19	0.07
5-methyl-2(5 <i>H</i> )-thiophenone	0.11		0.15		thiophenone				
dihydro-3(2 <i>H</i> )-thiophenone			0.05	0.03	tetrahydrothiophen-3-one	0.04		0.03	
					Other Sulfur-Containing Compounds				
2-furfurylthiol	1.55		0.22		3,5-dimethyl-1,2,4-trithiolane	0.72	0.21	0.18	0.29
methylthiofuran	0.35		0.04		2 or 3-mercaptopropanoic acid	0.91			
1,2-ethanedithiol			0.03		3-mercaptopropionic acid	0.23			
bis(2-furfuryl)disulfide	0.32				<i>S</i> -methyl ester ethanethioic acid	0.23		0.23	
1,3,5-trithiolane				0.05					
					Miscellaneous				
tetrahydro-4 <i>H</i> -thiopyran-4-one	0.11	0.03	0.11	0.03	2-methylfuran	0.09			
2 <i>H</i> -thiopyran-3(4 <i>H</i> )-one			0.15		2(5 <i>H</i> )-furanone	0.13		0.11	
3-hydroxy-2-butanone	0.05		0.03		dihydro-2(3 <i>H</i> )-furanone			0.02	
2-furfural	0.09				2-pentanone	0.09		0.05	
2-acetylpyrrole		0.20			3-pentanone	0.04			
2-furfuryl alcohol	0.09		0.09						

<sup>a</sup> Ribose/cysteine reaction system at pH 5. <sup>b</sup> Ribose/cysteine/urea reaction system at pH 5. <sup>c</sup> Ribose/cysteine reaction system at pH 8.5. <sup>d</sup> Ribose/cysteine/urea reaction system at pH 8.5. Data are the averages of duplicates.

revealed predominately sulfury, meatlike odors from cysteine and ribose reaction mixture at pH 5.0 while caramel, burnt notes were prominent at pH 7.0.

It was found that cysteine is thermally stable when heated alone under dry conditions; but in the presence of water, cysteine degrades and leads to the formation of a number of sulfur-containing compounds including some thiazoles, thienothiophenes, and cyclic polysulfides found in cysteine/ribose systems (Mottram and Whitfield, 1995a). Other compounds found in thermal degradation of cysteine are trithiolanes, trithianes, and tetrathianes (Zhang et al., 1988). On the other hand, the release of H<sub>2</sub>S from cysteine in the system also largely contributed to the formation of sulfur-containing compounds. When heated in aqueous phase, large amounts of hydrogen sulfide can be generated, and it participates in subsequent reactions to produce sulfur-containing compounds. At high temperatures, some amount of ammonia can also be generated, providing necessary substrates for pyrazine formation.

The addition of urea into the reaction system of ribose and cysteine enormously changed the flavor profile. Sulfur-containing compounds disappeared or were reduced to much lower levels. Thiophenes including 2-methylthiophene, thieno[3,2-*b*]thiophene, and thiophenethiols were not detected from the reaction mixtures with urea added at both pH values. However, a large amount of 3,5-dimethyl-1,2,4-trithiolane existed regardless of the presence of urea, seemingly not to be affected by the addition of urea. In addition, the formation of 2-acetylthiazole appeared to be favored at alkaline conditions with the presence of urea since more 2-acetylthiazole was generated at pH 8.5. On the other hand, a number of pyrazines that were not detectable in the system of ribose and cysteine were generated, incurring a toasted or roasted flavor. The major pyrazines formed include pyrazine, methylpyrazine, ethylpyrazine, 2,5- (and 2,6-)dimethylpyrazine, and other alkylpyrazines. Methylpyrazine accounted for about one-third of the volatiles generated, followed by

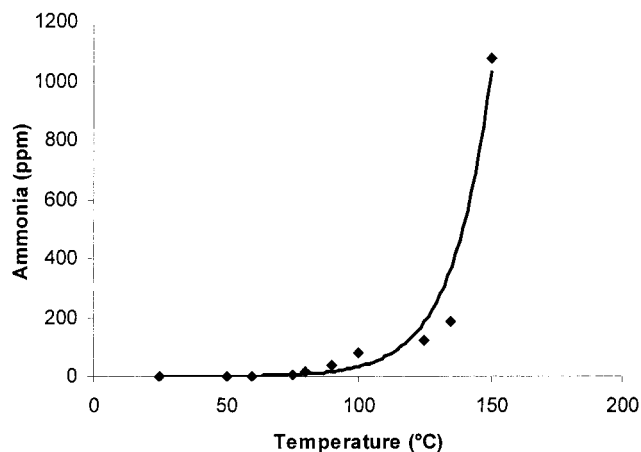
**Table 3. Volatiles Identified from Thermal Reaction Mixture of Urea and Diacetyl and Urea and Acetoin**

compounds	amount (mg/g of acetoin)			
	urea + diacetyl		urea + acetoin	
	pH 5	pH 8.5	pH 5	pH 8.5
4,5-dimethyl-1,3-benzenediol	32.79	23.19		
2,5-cyclohexadiene-1,4-dione	16.70	11.75		
trimethyl oxazole	5.45	2.74	0.11	0.31
tetramethylpyrazine			3.06	4.74
3-ethyl-2,5-dimethyl-1H-pyrrole			0.19	0.61

2,5- (and 2,6-)dimethylpyrazine and ethylpyrazine at both pH 5 and pH 8.5. The results indicated that pyrazine formation was more favored at pH 8.5. The alkyl pyrazines generally possess roasted nutlike notes (Ohloff and Flament, 1979). Pyrazines are widely distributed in foods, and their sensory properties are quite diverse (Maga, 1992). Several mechanisms have been proposed for the formation of various pyrazines (Vernin and Parkanyi, 1982). The nitrogen atoms in a pyrazine molecule can be incorporated into the compound by two possible pathways. Essentially, amino acids can react with dicarbonyls to form pyrazines, or free ammonia can react with  $\alpha$ -hydroxyketones to form pyrazines. Since urea contains two amine residues in its molecule, it is possible that under a high-temperature environment the amine residue can be cleaved from urea to form ammonia, which then reacts with sugar to generate pyrazines.

To provide a tentative explanation for this dramatic flavor change caused by urea addition, another set of experiments were conducted. A sample of 100 mM diacetyl and acetoin was reacted with an equal concentration of urea, separately, at the same heating and pH conditions. The volatiles identified from these reaction mixtures are listed in Table 3. Regardless of the pH, the reaction between urea and diacetyl generated no pyrazine while large amount of tetramethylpyrazine was produced in the mixture of urea and 3-hydroxy-2-butanone. It is well-known that ammonia can react with acetoin but not with diacetyl to produce tetramethylpyrazine. The result of this experiment indicated that under atmospheric conditions urea could degrade to  $\text{NH}_3$ , affected only by heat, regardless the presence of cysteine or ribose. To verify this explanation, we conducted an assay to determine the ammonia concentration in urea solution upon heating. The result shown in Figure 1 indicated that as temperature increased, the ammonia release from urea was facilitated with the significant increase at high temperatures above 100 °C. Therefore, addition of urea into the mixture of ribose and cysteine resulted in a large amount of ammonia, which can compete with  $\text{H}_2\text{S}$  to react with the precursors derived from ribose for the formation of heterocyclic flavor compounds. Consequently, several types of pyrazines were generated on one hand, and the production of sulfur-containing volatiles was reduced on the other hand.

The final pH, final appearance, and aroma description of the thermal reaction products of ribose and cysteine in the presence or absence of urea are summarized in Table 4. The color developments in the reaction systems indicate that the degradation or the interactions of reactants proceeded drastically. The pH of the reaction systems changed greatly except in the system containing ribose, cysteine, and urea at initial pH 8.5. In general, the ribose and cysteine systems without the

**Figure 1.** Ammonia concentration release from 10 mM urea solution upon heating.**Table 4. Final pH, Final Appearance, and Flavor Description of Thermal Reaction Mixtures of Ribose and Cysteine with/without Urea**

model system	initial pH	final pH	final appearance	aroma description
ribose + cysteine	5	3.86	dark brown	sulfury, meaty
	8.5	6.05	slightly brown	sulfury, meaty
ribose + cysteine + urea	5	7.43	dark brown	roasted meaty
	8.5	8.35	dark brown	roasted meaty

presence of urea possessed a sulfury, meaty flavor; while with the addition of urea, the aroma has a roasted, meaty, and nutty note.

In conclusion, this study revealed the presence of relatively high levels of urea in meats. Urea appeared to impose a significant influence on the volatile generation in the ribose/cysteine reaction system. Under the action of heat, urea releases ammonia that can compete with hydrogen sulfide for the available precursors for the formation of volatile compounds. By modifying the contents of urea in the reaction flavor system, it is possible to modify the flavor profiles.

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