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Effects of Carnosine on Volatile Generation from Maillard Reaction of Ribose and Cysteine

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Carnosine occurs naturally in meat and meat products in significant quantity, and it possesses strong antioxidant activity that inhibits lipid oxidation and enhances shelf life. In this study, the effects of carnosine on thermal flavor generation were investigated using the model system of cysteine and ribose, which was heated to the roasting temperature of 180 °C for 2 h at pH 5 and pH 8.5. The results indicated that carnosine affected volatile formation in a complex manner. Volatiles identified from the liquid phase of the reaction systems of ribose and cysteine showed that the sulfur-containing compounds such as thiophenes, thiazoles, and polysulfides were the most abundant compounds. The addition of carnosine into the reaction mixtures in general caused a reduction in contents of thiophenes and some important meaty flavor compounds such as 2-methyl-3-furanthiol, 2-furfurylthiol, and their associated dimers. On the other hand, it facilitated the generation of several important nitrogen-containing volatiles such as pyrazine, methylpyrazine, 2,6-dimethylpyrazine, and other alkyl pyrazines and thiazoles, which are known to elicit roasty and nutty flavor notes. The results suggested that carnosine acts as a nitrogenous source to facilitate the formation of nitrogen-containing compounds, possibly by degradation to form ammonia.

KEYWORDS: Maillard reaction; carnosine; ribose; cysteine; flavor generation

INTRODUCTION

Carnosine (β-alanyl-L-histidine) (**Figure 1**), an imidazole dipeptide, is one of the most abundant nitrogenous compounds present in the nonprotein fraction of vertebrate skeletal muscle with concentrations ranging from 1 to 50 mM (*1*, *2*). Specifically, its concentrations were reported to be 50, 150, and 276 mg/100 g tissue in muscles from chicken leg, beef leg, and swine shoulder, respectively (*1*). Carnosine has a pK_a value of 6.8 and can function as an effective buffer over the physiological pH range, thus maintaining an intra-muscular acid−base balance during exercise, which might be important for high-speed running of skeletal animals and athletic performance (*2*). Other putative functions attributed to carnosine include regulation of glycogenolysis, neutrotransmission, myosin activation, and lens cataract prevention (*3*). However, the most prominent biological activity resides in its role as an antioxidant, which has been demonstrated by biological studies (*1*, *3*) and in various food systems (*4*−*7*). Carnosine (1.5%) is more efficient than α-tocopherol and BHT (butylhydroxytoluene) (0.02%) in preventing color changes and rancidity of salted ground pork in frozen storage for up to 6 months, and in the inhibition of lipid oxidation in cooked salted ground pork stored for 7 days at 4 °C (*6*, *7*). Lee et al. (*8*) showed that carnosine inhibited metmyoglobin formation in raw beef during storage. In model system oxidation of liposome, carnosine depressed the rancidity caused by iron/ascorbate-induced phospholipid oxidation (*9*). Interestingly, recent studies showed that carnosine can delay aging in cultured human fibroblasts (*10*) and dietary supplementation of carnosine to animals clearly improved the external appearance, suggesting its potential as an anti-senescence drug (*11*).

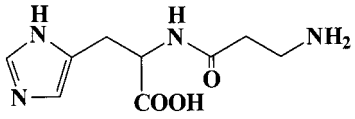

Figure 1. Chemical structure of carnosine.

The ability of carnosine to improve quality is partially attributed to its antioxidant activity. It is likely that carnosine can interact with primary products of peroxidation and with some volatile compounds that cause organoleptic deterioration (*9*). Because of its natural occurrence in meats and its antioxidant activity, carnosine offers great potential as a food additive to stabilize lipids and enhance shelf life in processed meats (*12*). On the other hand, carnosine in meats potentially has some influence on meat flavor thermal generation by causing changes in the overall volatile profile (*9*). However, information on this process is very limited. The objective of this study was to investigate the effects of carnosine on volatile generation using a Maillard reaction model system of D-ribose and L-cysteine.

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EXPERIMENTAL PROCEDURES

Materials. D-Ribose, tridecane, and sodium sulfate were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Carnosine and L-cysteine were obtained from Sigma Chemical Co. (St. Louis, MO). Methylene chloride used was of HPLC grade from Fisher Scientific (Fair Lawn, NJ).

Thermal Reactions. A mixture of 0.01 mol of L-cysteine and D-ribose, with or without equal mol of carnosine, was dissolved in 100 mL of distilled water. The solutions were adjusted to pH 5.0 or 8.5 prior to thermal reaction in 150-mL Hoke stainless steel cylinders (Hoke Inc., Clifton, NJ). The solutions were then heated at 180 °C in an oven for 2 h. The reaction was immediately stopped by cooling the cylinders under a stream of cold water.

Liquid/Liquid Extraction of Volatile/Semivolatile Compounds. After the brown reaction mixture was cooled, it was mixed with 0.5 mL of internal standard (tridecane, 1 mg/mL) and extracted with methylene chloride (50 mL × 3 times). The extract was dried with anhydrous sodium sulfate and concentrated under a nitrogen flow to 10 mL in a flask. It was then transferred to a Kuderna-Danish concentrator and further concentrated to 1−1.5 mL.

GC/Mass Spectrometry. The concentrated isolates from different reaction mixtures were analyzed by GC/mass spectrometry (GC/MS), using a Hewlett-Packard 6890 GC equipped with a fused silica capillary column (86.3 m × 0.25 mm i.d.; 1 μm thickness, RTX-5, Restek) coupled to a Hewlett-Packard 5973 series mass selective detector. Mass spectra were obtained by electron ionization at 70 eV and a source temperature of 250 °C.

Identification of the Volatile Compounds. Identification of the volatile compounds was based on GC/MS analysis. The compounds were identified by comparing the mass spectral data with those of authentic compounds available in either the Wiley 275 library or previous publications (*13*−*16*).

Quantification of the Volatile Compounds. Quantification of the volatile/semivolatile compounds from the liquid phases was based on using tridecane as an internal standard.

RESULTS AND DISCUSSION

Studies investigating changes in water-soluble meat components in relation to meat flavor during cooking revealed that the most significant loss among carbohydrates and amino acids occurred for ribose and cysteine. This indicated the importance of ribose and cysteine as meat flavor precursors (*17*). Strecker degradation or thermal decomposition of cysteine at roasting temperatures generates mercaptoacetaldehyde, acetaldehyde, cysteineamine, ethane-1,2-diol, H_2S, and NH_3, all of which react with sugar degradation products and with each other to form heterocyclic compounds. Maillard reaction model system involving ribose and cysteine has been used widely to study the generation of meaty flavors (*13*−*16*). Over 180 compounds have been identified from such reaction system and the key odorants elicit an overall roasty, meatlike odor (*15*). The odor qualities of specific volatile compounds from aqueous reaction mixtures of ribose and cysteine have been identified (*15*, *18*). For example, 2-furfurylthiol and 5-methyl-2-furfurylthiol were described as roasty, coffee-like; 2-methyl-3-furanthiol was meatlike; and 2-methyl-3-thiophenethiol was meatlike and sulfury (*18*).

This study investigated the effect of carnosine on meat flavor generation using a Maillard reaction model system of ribose and cysteine. The reactions were conducted thermally at 180 °C with starting pHs of both 5 and 8.5. As reported by other researchers, the pH changed about 1 to 2 units after 2 h of thermal reactions (**Table 1**). Because pH plays a very important role in flavor compound formation (*19*), the effects of this pH shift actually reflected the effects of pH on volatile production in this study, which is consistent with previous findings (*19*, *20*). The volatile/semivolatile compounds were analyzed in liquid phases.

Table 1. Initial and Final pH, and Final Appearance, of the Thermal Reaction Mixtures of Ribose and Cysteine With and Without Carnosine

model system	initial pH	final pH	final appearance
ribose + cysteine	5	4.01	dark brown
	8.5	4.96	dark, clear
ribose + cysteine + carnosine	5	4.18	brown
	8.5	6.92	dark brown

About 60 compounds were positively identified from the reaction of ribose and cysteine with or without carnosine. As shown in **Table 2**, these compounds can be classified as carbonyls, furans, thiophenes, thiazoles, pyrazines, disulfides, and other sulfur-containing compounds. Carnosine appeared to have complex effects on volatile formation in this model system.

The effects of carnosine on the formation of thiophenes were inhibitory in general. In the presence of carnosine, most of the thiophenes were reduced in contents, with some falling below the detection limit. However, the formation of 2-ethylthiophene, 2-methyl-5-ethylthiophene, and 2-acetyl-5-methylthiophene appeared to increase with the addition of carnosine. Most of these thiophenes were previously identified in meats. 2-Ethylthiophene and 2-methyl-5-ethylthiophene were found in trace amounts in grilled pork (*21*), and 2-formyl-5-methylthiophene was previously found to have an earthy-roasty aroma (*22*). The concentrations of two thiophenethiols (2-thiophenethiol and 2-methyl-3-thiophenethiol) have also been greatly reduced by the addition of carnosine at both pHs. 2-Thiophenethiol was reported to have antioxidant activity and 2-methyl-3-thiophenethiol has been described as egg-like, fatty, onion and leek-like, or as having a roasted and cooked meat character depending on the concentrations used in different studies (*23*, *29*). The data showed that thiophenone formation was not affected much by carnosine.

Some sulfur-containing compounds, including a couple of well-known meat impact compounds, were also affected by carnosine. A large amount of 2-furfurylthiol was formed at pH 5, and less than half of that was formed at pH 8.5. Carnosine depressed the formation of 2-furfurylthiol at pH 5, and depressed it to an undetectable level at pH 8.5. Its dimer, bis(2-furfuryl)-disulfide, was identified only at pH 5 in the absence of carnosine. 2-Furfurylthiol has been described as roasty and coffee-like, and is a characteristic compound with the highest flavor dilution factor from the reaction between ribose and cysteine (*18*). Its formation is probably from the reaction of H_2S with furfural derived from ribose (*24*). Because the formation of furfural is largely favored at acidic condition, much more furfurylthiol was generated at pH 5. Similarly, the very important meaty flavor compound 2-methyl-3-furanthiol was produced in a relatively large amount at pH 5 and much less at pH 8.5. The presence of carnosine reduced its concentration to undetectable levels at both pHs. Its oxidized dimer, bis(2-methyl-3-furyl)disulfide, was only detected at pH 5 in the absence of carnosine. The data suggest that the inhibition of monomer is the reason for the absence of the dimer. 2-Methyl-3-furanthiol and its dimer are two characteristic meaty flavor compounds with high potency. Gasser and Grosch (*25*, *26*) reported that the odor thresholds of these two compounds were 0.0025−0.01 ng/L (air) and 0.0007−0.0028 ng/L (air), respectively.

Many polysulfur heterocyclic compounds have been found in Maillard model systems (*27*). In this study, a couple of polysulfides were found in large amounts; with 3,5-dimethyl-

Table 2. Volatile Compounds Generated from the Liquid Phases of the Thermal Reactions of Ribose and Cysteine, With and Without Carnosine, at pH 5 or pH 8.5

RT	compound	amount (mg/g ribose)			
		RC 5[a]	RCC 5[b]	RC 8.5[c]	RCC 8.5[d]
	thiophene				
7.64	thiophene	0.032	0.025	0.017	0.015
10.05	2-methylthiophene	0.590	0.496	0.527	0.190
14.03	2-ethylthiophene	0.016	0.025	0.017	0.024
19.73	2-methyl-5-ethylthiophene	0.011	0.022	0.012	0.017
23.54	thiophene-3-carboxaldehyde	0.161	0.028	0.045	0.020
27.37	2-thiophenemethanol	0.175	0.144	0.146	
29.12	5-methyl-2-thiophenecarboxaldehyde	0.017		0.019	
30.35	2-formyl-2,3-dihydrothiophene	0.402	0.718	0.351	0.323
35.65	3-methyl-2-thiophenecarboxaldehyde	0.010		0.010	
39.53	2-acetyl-5-methylthiophene	0.038	0.091	0.020	0.060
34.52	2-(2-thienyl)propanal	0.020	0.021	0.028	0.027
35.28	1-(2-thienyl)propanone			0.061	0.045
33.34	2-formyl-5-methylthiophene	0.031		0.059	
42.30	thieno[2,3-b]thiophene	0.099	0.045	0.127	0.022
46.70	2-methyl-thieno[2,3-b]thiophene	0.034		0.054	
47.46	dihydrothienothiophene			0.072	0.027
53.67	5-methylthieno[2,3-d]thiophene	0.026	0.008	0.035	0.010
55.18	methyldihydrothienothiophene			0.024	0.006
41.14	dimethyldihydrothienothiophene			0.037	0.020
	thiophenethiol				
21.20	2-thiophenethiol	0.138	0.037	0.105	
25.77	2-methyl-3-thiophenethiol	0.078	0.031	0.312	0.130
	thiophenone				
20.30	dihydro-3(2H)-thiophenone	0.021	0.014	0.047	0.051
21.95	dihydro-2-methyl-3(2H)-thiophenone	0.379	0.456	0.585	0.540
	other sulfur-containing compounds				
11.36	ethanethioic acid, S-methyl ester	0.087	0.036	0.071	0.065
14.30	2-methyl-3-furanthiol	0.083		0.040	
14.77	3-mercapto-2-pentanone	0.110	0.038	0.072	
15.06	2-mercapto-3-pentanone	0.075		0.049	
15.86	2-furfurylthiol	0.895	0.256	0.318	
34.55	3,5-dimethyl-1,2,4-trithiolane (isomer)	0.221	0.339	0.385	0.119
35.06	3,5-dimethyl-1,2,4-trithiolane (isomer)	0.229	0.344	0.440	0.113
42.11	3-methyl-1,2,4-trithiane			0.173	0.142
51.35	3,6-dimethyl-1,2,4,5-tetrathiacyclohexane	0.050	0.041	0.132	0.023
60.43	*bis*(2-methyl-3-furyl)disulfide	0.015			
66.44	*bis*(2-furfuryl)disulfide	0.057	0.033	0.030	
	pyrazine				
9.57	pyrazine			0.059	0.115
12.23	methylpyrazine			0.210	0.346
16.11	2,6-dimethylpyrazine			0.031	0.098
16.43	ethylpyrazine			0.043	0.089
17.37	2,3-dimethylpyrazine			0.038	0.084
24.76	2-ethyl-3/6-methylpyrazine			0.009	0.018
25.06	trimethylpyrazine				0.013
43.64	5H-5-methyl-6,7-dihydrocyclopentapyrazine			0.020	0.042
	thiazole				
9.85	thiazole			0.078	0.091
11.67	2-methylthiazole	0.016	0.044	0.010	0.027
14.61	5-methylthiazole	0.022	0.035	0.020	0.027
22.67	5-ethyl-2-methylthiazole	0.009	0.040		
26.14	2-acetylthiazole	0.027	0.024	0.320	0.463
26.75	5-ethyl-2,4-dimethylthiazole	0.030	0.040	0.025	0.028
32.37	2-propionylthiazole			0.018	0.045
	miscellaneous				
7.84	2-pentanone	0.173	0.377	0.164	0.271
8.11	3-pentanone	0.025	0.211	0.049	0.094
8.58	methyl-2-cyclopenten-1-one		0.009		0.025
10.99	2,4-pentanedione	0.084	0.085	0.042	
12.94	furfural	0.495	0.286		
13.62	furanmethanol			0.035	0.013

[a] Ribose/cysteine reaction system at pH 5. [b] Ribose/cysteine/carnosine reaction system at pH 5. [c] Ribose/cysteine reaction system at pH 8.5. [d] Ribose/cysteine/carnosine reaction system at pH 8.5.

1,2,4-trithiolane and 3-methyl-1,2,4-trithiane as the most abundant compounds, similar to what was reported by Mottram and Whitefield (*13*, *14*) in their studies involving cysteine, ribose, and phospholipids. Because of its low threshold and high potency, 3-methyl-1,2,4-trithiane has been used as a meat-flavoring agent (*28*) and has been identified from pork (*29*). Its

formation was significant at pH 8.5, with carnosine inhibition at both pHs. The syn- and anti-3,5-dimethyl-1,2,4-trithiolanes are important meaty flavor components and were characterized as meaty, boiled beef at low concentration, and onion-like and sulfurous at high concentration (*30*). They have also been identified in various cooked foods (*31*). They are the major volatile products in the reaction of glucose with cysteine (*32*). These polysulfur compounds can be formed from the condensation of acetaldehydes, H_2S, and mercaptoacetaldehyde, all of which are thermal breakdown products of cysteine (*13*). Thermal degradation of cysteine alone can generate trithiolanes and dithiazines (*33*). Therefore, neither the sugar type nor the presence of carnosine is a determinant in their formation. It was reported that heterocyclic compounds with 1, 2, or 3 sulfur atoms in five- and six-membered rings, such as thiophenes, trithiolanes, and dithiazines, are much more prevalent in boiled meat than in roast meat (*27*).

The thiazoles identified in this study indicated that both carnosine and pH could influence their formation. The formation of unsubstituted thiazole was favored under basic conditions more than in an acidic environment, whereas 2-acetylthiazole was more prominent at pH 8.5. Carnosine may have increased the levels of 2-methylthiazole, 5-methylthiazole, and 2-propionylthiazole. Thiazole compounds have been found in various food systems, and they contribute to a wide variety of characteristic aromas to foods. Monosubstituted alkylthiazoles, such as 2-methylthiazole and 2-isopropylthiazole, possess green, vegetable aromas, whereas compounds such as 4,5-dimethylthiazole, 2,4-dimethyl-5-ethylthiazole, and 2,4,5-trimethylthiazole have been described as nutty, roasted, and meaty (*34*). Alkyl-substituted thiazoles, in general, are considered to be more potent than pyrazines (*35*). Like pyrazines, their formation also requires heating at elevated temperatures. Therefore, they are often detected in fried, roasted, or grilled foods such as cooked meats, coffee, roasted peanuts, and potato chips. For instance, 4,5-dimethylthiazole, trimethylthiazole, 2-acetylthiazole, and 5-ethyl-2,4-dimethylthiazole were commonly found in grilled meats (*36*).

This study identified eight pyrazines, including pyrazine, methylpyrazine, ethylpyrazine, dimethylpyrazines, and dihydrocyclopentapyrazine. Methylpyrazine was the most abundant. These pyrazines have been widely used in imitating cooked meat flavors to impart roasted or toasted notes (*37*). The data showed that thermal reaction of ribose and cysteine generated the interesting bicyclic 6,7-dihydro-5H-cyclopentapyrazine, which was found in roasted, grilled, fried, and pressure-cooked meat (*17*). Pyrazine formation appeared to be much more affected by pH than by carnosine, because their formation only occurred at pH 8.5. However, carnosine influenced the formation of pyrazine to some extent. The results showed that the amounts of pyrazine, methylpyrazine, dimethylpyrazine, and ethylpyrazine were higher in the presence of carnosine.

In the reaction between ribose and cysteine, the sulfur-containing amino acid can undergo Strecker degradation to produce ammonia, which can react with other flavor intermediates to produce nitrogen-containing heterocyclic compounds such as pyrazines. To understand whether carnosine would act as a nitrogenous source in the reactions involving ribose, cysteine, and carnosine, thermal reactions between ribose and carnosine without cysteine at both pH 5 and pH 8.5 were conducted under the same conditions as other thermal reactions. The volatile compounds identified from the liquid phases are listed in **Table 3**. Hydrocarbons, ketones, and furanoids were the major compounds detected. Derived from ribose, furfural is the most predominant compound generated. Importantly, several pyrazines were found in the liquid solutions at pH 8.5. This clearly indicated that carnosine provided a nitrogen source for the formation of pyrazines. However, exactly how carnosine acts as a nitrogen donor remains unclear at this point. Although another experiment of heating carnosine alone under the same thermal conditions did not produce meaningful volatile compounds (data not shown), it was not necessary to indicate that ammonia was not generated. Nonetheless, a recent article reported that carnosine can react with low-molecular-weight compounds that bear carbonyl groups (aldehydes and ketones) (*10*). It is possible that carnosine reacted with carbonyls derived from ribose and this aided the degradation of carnosine.

Table 3. Volatile Compounds Identified from the Liquid Phases of Reactions between Ribose and Carnosine at Both pH 5 and pH 8.5

compounds	amount (mg/g ribose) pH 5	pH 8.5
hydrocarbons and ketones		
cyclohexene	0.486	
1-hydroxy-2-butanone		0.043
3-hydroxy-2-butanone	0.112	0.188
1,2-cyclopentanedione	0.085	
2/3-methyl-2-cyclopenten-1-one		0.014
2-hydroxy-3-methyl-2-cyclopenten-1-one		0.089
3-ethyl-2-hydroxy-2-cyclopenten-1-one		0.039
3,-dihydroxyacetophenone		0.188
3,4-dihydroxyacetophenone	0.055	
2,5-hexanedione	0.048	
1-(acetyloxy)-2-propanone	0.576	
1-ethyl-2-methylbenzene	0.045	
furanoids		
furfural	33.203	0.188
2(5H)-furanone	0.118	0.125
1-(2-furanyl)–1-propanone	0.057	
1-(2-furanyl)–2-propanone		0.088
2,2′-methylenebis-furan	0.042	
2-acetyl-5-methylfuran		0.005
2-furanmethanol		0.278
difurylmethane	0.075	
hydroxy dimethyl furanone		0.179
1-(2-furanyl)-butan-3-one	0.356	0.034
4-(2-furanyl)-3-buten-2-one	0.051	0.042
1-(2-furanyl)-1,3-butanedione	0.021	
2-acetylfuran	0.236	0.064
1-(2-furanyl)-propan-2-one		0.079
pyrazines and pyridines		
pyrazine		0.025
methylpyrazine		0.153
2,3-dimethylpyrazine		0.01
2,5 or 2,6-dimethylpyrazine		0.188
ethylpyrazine		0.008
trimethylpyrazine		0.046
2-(*n*-propyl)-pyrazine		0.004
2-ethyl-5-methylpyrazine		0.004
2-ethyl-6-methylpyrazine		0.009
2-acetylpyridine		0.006
2-methylpyridine		0.008
2,4-dimethylpyridine		0.024

In conclusion, the results of this study showed complex effects of carnosine on volatile formation, which was further complicated by the pH changes. Carnosine reduced the amounts of some well-known meaty flavor impact compounds and at the same time increased the formation of some nitrogen-containing compounds. The mechanisms underlying these observations are uncertain at this point. The pH stabilizing action exhibited by carnosine could partially be the reason, given the fact that nitrogen-containing compounds are favored at higher pH. On the other hand, it is likely that carnosine acts as nitrogen donor, as suggested by the data from the reactions involving ribose

and carnosine only. However, exactly how carnosine provided the nitrogen source remains to be elucidated.

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