Formation of Sulfur Aroma Compounds in Reaction Mixtures Containing Cysteine and Three Different Forms of Ribose

Donald S. Mottram* and Ian C. C. Nobrega[†]

School of Food Biosciences, The University of Reading,
Whiteknights, Reading RG6 6AP, United Kingdom

The headspace volatiles produced from buffered and unbuffered cysteine model systems, containing inosine 5′-monophosphate, ribose 5-phosphate, or ribose, were examined by GC-MS. Sulfur compounds dominated the volatiles of all systems and included mercaptoketones, furanthiols, and disulfides. The inosine monophosphate systems produced much lower quantities of volatiles than ribose phosphate or ribose systems. In the systems buffered with phosphate or phthalate buffers, both ribose and ribose phosphate systems gave similar quantities of sulfur volatiles. However, in the absence of buffer, the ribose system was relatively unreactive, especially for volatiles formed via the 2,3-enolization route in the Maillard reaction, where 4-hydroxy-5-methyl-3(2*H*)-furanone is a key intermediate. A number of keto−enol tautomerisms, which are known to be acid−base-catalyzed, occur in the 2,3-enolization route. This may explain the catalysis of the ribose systems by the buffers. In the ribose phosphate systems, however, Maillard mechanisms probably played a less important role, because ribose 5-phosphate readily dephosphorylated to give 4-hydroxy-5-methyl-3(2*H*)-furanone on heating and thus provided an easier route to aroma compounds than the Maillard reaction.

KEYWORDS: Aroma volatiles; Maillard reaction; meat flavor; thiols; disulfides, phosphate buffer

INTRODUCTION

The aroma of cooked meat is provided by a complex mixture of volatile compounds produced during the cooking (*1*, *2*). Among these volatiles, sulfur-containing compounds are considered to be particularly important. During cooking, a major route to these compounds is the Maillard reaction between reducing sugars and the amino acid cysteine. One of the most important sugars present in meat is ribose. The main sources of ribose in meat are inosine 5′-monophosphate and smaller quantities of ribose 5-phosphate and free ribose. It is well-known that inosine 5′-monophosphate is formed in muscle postslaughter from the enzymatic dephosphorylation and deamination of adenosine triphosphate, the ribonucleotide that is essential to muscle function in the live animal (*3*). Further enzymatic breakdown of the inosine monophosphate may lead to ribose and ribose 5-phosphate, although most of the ribose in meat remains bound within the nucleotide. The reaction of ribose with cysteine, in heated aqueous model systems, has been shown to give meatlike aromas (*4*, *5*). This reaction is widely used in the preparation of "reaction product" flavorings with meatlike aroma characteristics.

Inosine 5′-monophosphate is well recognized as a flavor potentiator and is associated with the taste sensation known as "umami" (*6*, *7*). However, it may also provide a source of ribose

for Maillard reactions occurring during the cooking of meat. In a recent investigation of the volatiles produced from aqueous model systems containing cysteine and inosine 5′-monophosphate at different pH values, many sulfur compounds were identified, including thiols, disulfides, and mercaptoketones arising from the reaction of the pentose and cysteine (*8*). Although the potential for ribose and cysteine to generate meatlike aromas has been widely studied in aqueous model systems, the relative contributions of inosine 5′-monophosphate, ribose 5-phosphate, and ribose have not been investigated. Because ribose may be present in any of these forms in meat, such a comparison would improve the understanding of aroma formation in cooked meat.

One problem associated with the use of aqueous model systems to study these reactions is the fact that buffers (used to control the model system pH) can influence the rate of the Maillard reaction (*9*, *10*) and thus the formation of volatiles. An alternative option is to study these reaction mixtures using meat itself as a buffer system (*11*). However, the volatiles produced from the meat may interfere with the identification and quantification of the reaction mixture volatiles. To minimize these problems, these reactions have been carried out in the present study in both buffered and unbuffered aqueous systems.

This investigation compares the sulfur-containing volatile compounds produced in the reactions of three ribose-containing compounds (inosine 5′-monophosphate, ribose 5-phosphate, or free ribose) with cysteine, using unbuffered (initial pH 5.6) and buffered (phosphate, pH 5.6; phthalate, pH 5.6; and phosphate, pH 4.2) model systems.

* Author to whom correspondence should be addressed (telephone +44 118 931 6519; fax +44 118 931 0080; e-mail D.S.Mottram@ reading.ac.uk).
[†] Present address: Universidade Federal da Paraíba, Campus IV, Departamento de Tecnologia Rural, Bananeiras-PB, 58220-000 Brazil.

Sulfur Aroma Compounds from Cysteine/Ribose Reaction Systems

J. Agric. Food Chem., Vol. 50, No. 14, 2002 **4081**

EXPERIMENTAL PROCEDURES

Materials. Inosine 5′-monophosphate (disodium salt), D-ribose 5-phosphate (disodium salt, grade III, from yeast), D-ribose, L-cysteine, disodium pyrophosphate, tetrasodium pyrophosphate, potassium hydrogen phthalate, and 1,2-dichlorobenzene (internal standard) were purchased from Sigma-Aldrich Co. Ltd., Dorset, U.K. Authentic samples of reference compounds were either purchased from a range of laboratory chemical suppliers or obtained as gifts from flavor laboratories.

Preparation of Phosphate and Phthalate Buffers. Phosphate buffers (0.2 M) were prepared in glass-distilled water by mixing appropriate proportions of disodium and tetrasodium pyrophosphate solutions (0.2 M). A phthalate buffer (pH 5.6) was prepared from a potassium hydrogen phthalate solution (0.3 M) by adjusting the pH with sodium hydroxide (5 M). Phthalate buffer was prepared at 0.3 M because this gave better pH control when compared to 0.2 M.

Reaction Mixtures. Round-bottom thick-wall Pyrex ampules of 10 mL volume (S. Murray and Co., Old Woking, Surrey, U.K.) were used as reaction vessels. Reactions between cysteine and the ribose derivatives (inosine 5′-monophosphate, ribose 5-phosphate, or free ribose) were carried out in phosphate buffer, phthalate buffer, and water (unbuffered) at an initial pH of 5.6. The pH chosen relates to the pH in normal meat (5.6–5.8). The reactions were also carried out in the phosphate buffer at pH 4.2.

Separate solutions of cysteine (0.1 M) and the ribose-containing compound (0.1 M) were prepared using water or the appropriate buffer. Before they were made up to volume, the pH was checked and adjusted to 5.6 or 4.2 with sodium hydroxide or hydrochloric acid solutions, if necessary. Equal quantities of the solutions were mixed, and 6.0 mL of the mixture (i.e., 0.3 mmol of each reactant) was transferred to the reaction ampules. The ampules were flame-sealed and then heated in a CERTOclav autoclave (Kelomat, Traun, Austria) for 30 min at 140 °C under a pressure of 0.28 MPa (2.8 bar). The pH values of the reaction mixtures were measured before and after heating. Each reaction was carried out in triplicate.

To examine the effect of the heat treatment on ribose 5-phosphate and free ribose alone, the cysteine aliquots were substituted by distilled water or phosphate buffer (pH 5.6).

Headspace Collection of Volatiles. After cooling, each reaction mixture was transferred to a 250 mL conical flask containing 20 mL of the appropriate buffer (pH 5.6) or distilled water (unbuffered reactions). The flask was fitted with a Drechsel head, and a glass-lined stainless steel tube (155 mm × 3 mm i.d.), containing 85 mg of Tenax-TA (Scientific Glass Engineering, Ringwood, Australia), was attached by a stainless steel reducing union to the head outlet. During the collection of the volatile components, the dilute reaction solution was maintained at 60 °C in a water bath while the Tenax trap was kept at room temperature. The volatiles were swept from the flask onto the adsorbent in the trap using a flow of oxygen-free nitrogen (40 mL/min) for 1.5 h. At the end of this time, the flask was removed, an internal standard (1,2-dichlorobenzene, 130 ng in 1 μL of diethyl ether) was added to the front end of the trap, and residual solvent and any moisture retained on the trap were removed by purging with nitrogen at 40 mL/min for 5 min.

Solvent Extraction of Volatiles from Heated Sugar Solutions. The heated sugar solution blanks (6 mL) were extracted with 6 mL of dichloromethane. The solvent fraction was passed through a small funnel packed with glass fiber. Fifty microliters of a dichlorobenzene solution (720 μg/mL in diethyl ether) was added to the solvent extract, which was then concentrated to 3 mL with a flow of nitrogen. One microliter of each extract was injected by a split/splitless injection system into the gas chromatograph–mass spectrometer (GC-MS), and the components in the extract were quantified using the internal standard.

GC-MS. All analyses were performed on a Hewlett-Packard 5972 mass spectrometer, fitted with an HP5890 series II gas chromatograph and a G1034C Chemstation. A CHIS injection port (Scientific Glass Engineering) held at 250 °C was used to thermally desorb the volatiles from the Tenax trap onto the front of a 50 m × 0.32 mm i.d., 0.5 μm film thickness, BPX5 fused-silica capillary column (Scientific Glass Engineering). During the desorption period of 5 min, the oven was held at 0 °C using the GC subambient facility. After desorption, the oven was heated to 50 °C over 1 min, at which it was held for 2 min before heating at 4 °C/min to 280 °C. Helium at 55 kPa was used as the carrier gas, resulting in a flow of 1.75 mL/min at 40 °C. A series of *n*-alkanes (C_6–C_{22}) was analyzed, under the same conditions, to obtain linear retention index (LRI) values for the components. Analyses of some samples were also carried out on a 50 m × 0.32 mm i.d., 0.5 μm film thickness, BP20 column (Scientific Glass Engineering) to provide additional LRI data. Gas chromatographic conditions were the same as for the BPX5 column except that the maximum oven temperature was 250 °C.

An HP 7673 autosampler was used to introduce 1 μL of the solvent extracts onto the GC column via a split/splitless injection port heated at 280 °C. The oven temperature was held at 50 °C for 2 min and then programmed at 4 °C/min to 280 °C (BPX5 column) or 250 °C (BP20 column).

The interface of the GC to the MS was maintained at 280 °C, and the MS was operated in the electron-impact mode with an ionization energy of 70 eV and a scan rate of 1.9 scans/s over the mass range of 29–400 amu. Components were identified by comparison of their mass spectra and LRI with those from authentic compounds analyzed in our laboratory or by comparison with spectra contained in the NIST/EPA/NIH mass spectral database or in other publications.

Approximate quantities of the volatiles in the concentrated headspace from each sample were estimated by comparison of their peak areas, in the total ion current chromatogram, with that of the 1,2-dichlorobenzene internal standard using a response factor of 1. This allowed comparison of the relative contributions the volatiles made to the headspaces of the different systems but did not provide absolute concentrations in the aqueous solutions. The mean coefficient of variance (CV) for quantities of individual components was 22% and, with the exception of some compounds that were present in relatively small amounts, no compound showed a CV >40%.

RESULTS AND DISCUSSION

On heating, the pH values of the unbuffered reaction mixtures containing cysteine and ribose 5-phosphate or free ribose dropped from the initial pH of 5.6 to pH 4.2 and 3.9, respectively. The pH of the inosine monophosphate system was unchanged after heating. Phosphate (0.2 M pyrophosphate) and phthalate buffers (0.3 M) maintained the pH within 0.3 pH unit through the heating process.

The formation of aroma volatiles in Maillard systems has been demonstrated to be strongly influenced by changes in pH (*11–13*). The pH of the unbuffered reaction systems dropped during the progress of the reaction, making it difficult to determine how pH influenced the volatile reaction products. Although buffers maintained a constant pH through the reaction, phosphate buffers have been suggested to catalyze the Maillard reaction (*10*). Thus, in the present study, the influence of pH and buffer has been assessed by comparing unbuffered systems with phosphate-buffered systems at pH 5.6 and 4.2 (the initial and final pH values of unbuffered cysteine/ribose reaction systems) and phthalate-buffered systems at 5.6.

Aromas of Heated Reaction Systems. After heating, the aromas of the three unbuffered systems differed. The odor attributes were described by three assessors who smelled the undiluted reaction mixtures. Whereas the cysteine/ribose phosphate mixture generated strong "rubbery" and "corned beef" notes, cysteine/ribose produced milder "meaty" and "savory" aromas. In the inosine monophosphate system, however, odors such as "rotten egg", "unpleasant", and "sulfury" were generated. Differences in color intensity were also observed, with the cysteine/ribose phosphate mixture turning to dark yellow and the ribose and inosine monophosphate systems to a medium to pale yellow. However, when the reactions containing ribose 5-phosphate or ribose were prepared in phosphate (pH 5.6 and

4.2) or phthalate buffer (pH 5.6), no clear distinctions in odor or color between them were observed. Both reactions generated a dark yellow color and strong "rubbery", "corned beef", and "sulfury" odors. For the reactions involving inosine 5′-monophosphate, little change was observed when they were prepared in buffers at pH 5.6 compared with the unbuffered system. At pH 4.2 (phosphate buffer), however, more "meaty" odors emerged, whereas the unpleasant "rotten egg" note was no longer detected.

Volatile Compounds from the Reaction Systems. The approximate quantities of 66 sulfur-containing volatiles, found in the headspace of unbuffered and buffered model systems containing cysteine and ribose 5-phosphate, ribose, or inosine 5′-monophosphate, are shown in **Table 1**, together with the major non-sulfur compounds. Wherever possible, the identities were confirmed by comparison of mass spectra and LRI with those for authentic compounds. When authentic standards were not available, tentative identifications were made by comparison with literature mass spectra and retention data.

The volatiles formed in these reactions were dominated by sulfur-containing compounds with thiols present in highest quantities. These included mercaptoketones, furanthiols, and thiophenethiols. Symmetrical and unsymmetrical disulfides derived from these thiols were also detected, and they included oxoalkyl, furyl, and thienyl disulfides. Other compounds found included thiophenones, dithiolanones, and thiophenes. The major non-sulfur volatiles were 2-furfural and 2,4-pentanedione. It was readily apparent that the inosine monophosphate systems produced much lower quantities of volatiles, and many of the compounds found in the ribose and ribose phosphate systems were present in only trace amounts in the inosine monophosphate reaction mixtures.

Three furans and thiophenes with an −SH group in the 3-position were found in the heated reaction mixtures. It has been known for some time that such compounds, and related disulfides, possess strong meatlike aromas and exceptionally low odor threshold values (*21−24*). Thiols and disulfides containing 2-furylmethyl moieties were also present in the reaction mixtures. These have sulfury, onion-like characteristics, and similar aromas have been reported for the mercaptoketones (*25*). These results correlate well with odor attributes of the heated reaction mixtures assessed in the present study.

The sulfur-containing volatiles are likely to be formed from the reactions of hydrogen sulfide with carbonyl compounds. Hydrogen sulfide can derive from the hydrolysis of cysteine or from the Strecker degradation of cysteine in the presence of dicarbonyl compounds. Other aroma intermediates that can be formed from cysteine include mercaptoacetaldehyde, acetaldehyde, and ammonia. Carbonyl and dicarbonyl compounds derive mainly from the breakdown of ribose via the Maillard reaction. Two important intermediates in Maillard reactions involving pentoses are 4-hydroxy-5-methyl-3(2*H*)-furanone and 2-furfural. The initial step of the Maillard reaction produces an Amadori compound, which is deaminated and dehydrated via either 1,2- or 2,3-enolization (**Figure 1**). The 1,2-enolization route gives 2-furfural, via 3-deoxypentosone, whereas 2,3-enolization results in 1-deoxypentosone and 4-hydroxy-5-methyl-3(2*H*)-furanone. The last two compounds are considered to be key aroma intermediates and can dehydrate to other important intermediates, such as dicarbonyl compounds (*15*).

Most of the mercaptoketones, furanthiols, thiophenethiols, disulfides, thiophenones, dithiolanones, and thiophenes reported in the present paper have been shown to be produced in the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone with hydrogen sulfide or cysteine (*15, 26*). Mechanisms for the formation of these compounds were proposed.

Relative Reactivities of Ribose and Ribose 5-Phosphate. Interesting differences in the formation of volatiles were found between cysteine/ribose 5-phosphate and cysteine/ribose in the unbuffered systems. The ribose phosphate system appeared to be more reactive, producing much larger quantities of most volatile compounds (**Table 1**). This was particularly noticeable with the major class of volatiles, the mercaptoketones and their oxidation products (oxoalkyl disulfides). The difference in the total quantities of furanthiols, as shown in **Table 1**, does not appear to be as great. However, relatively large quantities of 2-furanmethanethiol in the ribose system are largely responsible for the class total in this system. The dominant volatile in the ribose system was the non-sulfur compound 2-furfural, which was present at a level ∼4 times higher than in the ribose phosphate system. Reaction of this compound with hydrogen sulfide, from cysteine breakdown, is the probable route to 2-furanmethanethiol (*27*).

A possible explanation for the increased reactivity of ribose 5-phosphate in comparison to ribose may be that different mechanisms for its breakdown and reaction with cysteine occur. It has been reported (*28*) that, in aqueous solution, ribose 5-phosphate is relatively easily dephosphorylated and dehydrated to yield 4-hydroxy-5-methyl-3(2*H*)-furanone and 1-deoxypentosone (**Figure 2**). This furanone can readily form thiol-substituted furans and thiophenes by reaction with hydrogen sulfide, produced in the degradation of cysteine (*15, 29*). Reaction of the α-dicarbonyls, 2,3-butanedione, and 2,3-pentanedione with hydrogen sulfide can yield the mercaptoketones, which are dominant products of the reaction. Mechanisms for the formation of these diones and mercaptoketones from the 1-deoxypentosone intermediate have been suggested (*15*).

The dephosphorylation of ribose 5-phosphate, as shown in **Figure 2**, may provide an easier route to 4-hydroxy-5-methyl-3(2*H*)-furanone, 1-deoxypentosone, and the dione intermediates than the Maillard pathway via Amadori intermediates, which is required for the free ribose system. Hence, sulfur compounds from reactions of these intermediates with cysteine or hydrogen sulfide were more readily produced from ribose 5-phosphate. 2-Furfural is formed via 3-deoxypentosone, which is produced from Amadori intermediates in the Maillard reaction. This is not produced by the dephosphorylation of ribose 5-phosphate and, therefore, the formation of 2-furfural would not be expected to be enhanced in the ribose phosphate system compared with the ribose system.

Effect of Buffer. The very marked differences in quantities of volatiles between the ribose and ribose 5-phosphate systems observed in the reactions carried out in unbuffered solutions were not found when the reactions were carried out in buffered solution (**Table 1**). In the presence of either phosphate or phthalate buffer at pH 5.6, the levels of volatiles from the ribose systems were generally much higher than in the unbuffered system, whereas the differences between the ribose phosphate systems were generally small. Furthermore, when the ribose reaction system was buffered at 4.2, which corresponds to the final pH attained by the unbuffered system, enhancement of volatiles was also observed. The dominating classes of volatiles in all of the systems were mercaptoketones and furan- and thiophenethiols. As discussed above, these compounds can be derived from the Amadori intermediate of the Maillard reaction via the 1,2-enolization route, which is favored by higher pH. The marked increase in these compounds in the buffered ribose systems, at both pH 5.6 and 4.2, compared with the unbuffered

Table 1. Approximate Quantities[a] of Volatiles Identified in the Headspace of Heated Cysteine Model Systems Containing Ribose 5-Phosphate (Rib-PO$_4$), Ribose, or Inosine 5′-Monophosphate (IMP)[b]

compound [m/z (rel intensity)]	unbuffered			phosphate buffer, pH 5.6			phthalate buffer, pH 5.6			phosphate buffer, pH 4.2			MS ref
	Rib-PO$_4$	ribose	IMP	Rib-PO$_4$	ribose	IMP	Rib-PO$_4$	ribose	IMP	Rib-PO$_4$	ribose	IMP	
initial pH	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	4.2	4.2	4.2	
final pH	4.2	3.9	5.7	5.5	5.5	5.7	5.4	5.3	5.4	3.9	3.9	4.5	
3-mercapto-2-butanone	2670	29	10	8860	9820	43	5570	3520	70	3890	2090	500	c
3-mercapto-2-pentanone	9220	850	tr	2490	4020	23	2370	1540	40	9260	6600	700	c
2-mercapto-3-pentanone	1870	40	7	2620	3280	37	1640	921	53	2200	1330	533	c
total mercaptoketones	*13760*	*919*	*17*	*13970*	*17120*	*103*	*9580*	*5981*	*163*	*15350*	*10020*	*1733*	
2-methyl-3-furanthiol	2940	1010	97	2050	2230	67	2960	2620	143	10230	8250	1867	c
2-furanmethanethiol	3310	2610	13	496	1750	23	840	3540	47	2360	3160	5000	c
3-thiophenethiol	2400	37	50	716	476	123	1100	353	213	3770	848	367	14
2-methyl-3-thiophenethiol	699	55	20	1260	730	23	1180	663	40	1390	1050	400	c
2-thiophenemethanethiol	104	16	–	124	36	tr	171	67	tr	128	66	21	c
total furan- and thiophenethiols	*9453*	*3728*	*180*	*4646*	*5222*	*236*	*6251*	*7243*	*443*	*17878*	*13374*	*7655*	
bis(1-methyl-2-oxopropyl) disulfide[d]	20	–	–	262	131	–	458	62	–	tr	tr	tr	c
3-(1-methyl-2-oxopropyldithio)pentan-2-one[d]	212	tr	–	181	10	–	190	95	–	9	tr	tr	15
2-(1-methyl-2-oxopropyldithio)pentan-3-one[d]	16	tr	–	202	200	–	233	66	–	tr	tr	tr	15
bis(1-ethyl-2-oxopropyl) disulfide[d]	333	34	–	42	29	–	49	23	–	24	12	tr	c
3-(1-methyl-2-oxobutyldithio)pentan-2-one[d]	111	7	–	60	53	–	47	32	–	4	9	tr	c
bis(1-methyl-2-oxobutyl) disulfide[d]	–	–	–	40	33	–	12	tr	–	–	–	tr	c
total oxoalkyl disulfides	*692*	*41*	*–*	*787*	*456*	*–*	*989*	*278*	*–*	*37*	*21*	*tr*	
1-[2-methyl-(3-furyldithio)]propan-2-one	41	13	–	–	–	–	–	–	–	12	27	3	15
2-[2-methyl-(3-furyldithio)]butan-3-one	71	5	tr	146	149	tr	580	–	–	43	42	17	c
bis(2-methyl-3-furyl) disulfide	77	56	7	33	87	tr	240	55	tr	167	319	63	c
3-[(2-methyl-(3-furyldithio)]pentan-2-one	281	38	–	86	35	–	180	117	–	157	201	30	c
2-[(2-methyl-(3-furyldithio)]pentan-3-one	48	8	tr	+	74	–	152	83	–	34	37	13	c
2-(2-furylmethyldithio)butan-3-one	33	3	–	18	112	–	53	353	–	3	3	tr	c
2-methyl-3-(2-furylmethyldithio)furan	47	47	–	+	+	–	18	148	–	14	32	tr	c
3-(2-furylmethyldithio)pentan-2-one	215	63	–	tr	35	–	37	102	–	11	22	tr	c
2-(2-furylmethyldithio)pentan-3-one	+	+	–	13	52	–	12	87	–	3	3	tr	c
bis(2-furylmethyl) disulfide	43	80	–	–	20	–	–	68	–	tr	7	tr	c
total furyl disulfides	*856*	*313*	*7*	*296*	*564*	*–*	*1272*	*1013*	*–*	*444*	*693*	*126*	
2-(3-thienyldithio)butan-3-one	+	–	–	94	11	tr	61	tr	tr	11	tr	–	15
2-methyl-3-(3-thienyldithio)furan	80	tr	7	38	14	tr	54	45	7	63	17	13	15
2-methyl-3-[2-methyl-(3-thienyldithio)]furan	49	11	tr	63	23	tr	88	45	tr	24	31	17	16
2-(3-thienyldithio)pentan-3-one	46	tr	–	27	8	10	14	tr	–	8	tr	tr	15
3-(2-furylmethyldithio)thiophene	+	7	–	–	–	–	–	–	–	–	–	–	17
bis(3-thienyl) disulfide [115, **230** (65), 71 (42), 166 (38), 45 (28), 69 (13), 197 (12), 116 (11)]	49	tr	3	–	–	20	–	–	tr	8	tr	tr	
2-methyl-3-(3-thienyldithio)thiophene [129, **244** (63), 45 (34), 71 (21), 115 (17), 85 (18), 180 (14), 69 (13)]	25	tr	tr	13	tr	10	tr	tr	tr	8	tr	tr	
bis(2-methyl-3-thienyl) disulfide	9	tr	tr	21	tr	–	tr	tr	–	tr	tr	3	15
total thienyl disulfides	*258*	*18*	*10*	*256*	*56*	*40*	*217*	*90*	*7*	*122*	*48*	*33*	
2-methylthiophene	1220	300	70	744	550	33	1180	537	73	1470	828	833	c
4,5-dihydro-2-methylthiophene	–	–	–	123	199	–	403	246	–	–	–	+	18
2-ethylthiophene	–	–	–	51	144	3	180	50	3	–	–	–	c
2,3-dimethylthiophene	62	tr	tr	96	201	tr	261	71	tr	–	–	–	c
2-formylthiophene	146	68	tr	133	258	tr	239	239	tr	238	310	20	c
5-methyl-2-formylthiophene	55	3	3	27	17	13	78	18	3	tr	tr	tr	c
3-methyl-2-formylthiophene	190	8	7	226	359	10	500	462	7	110	173	17	c
2-acetyl-3-methylthiophene	46	–	–	54	–	–	60	tr	–	79	6	tr	c
2-propanoylthiophene	17	–	–	110	tr	tr	152	tr	tr	24	32	tr	c
3-ethyl-2-formylthiophene	60	8	tr	69	112	tr	118	132	tr	111	266	20	5
dimethylformylthiophene	291	20	–	63	26	tr	137	44	tr	132	129	20	5
total thiophenes	*2087*	*407*	*80*	*1696*	*1866*	*59*	*3308*	*1799*	*86*	*2164*	*1744*	*910*	
(E or Z)-3,5-dimethyl-1,2-dithiolan-4-one	457	9	27	224	116	20	361	119	30	1224	113	297	5
(E or Z)-3,5-dimethyl-1,2-dithiolan-4-one	490	7	20	131	72	13	240	70	23	1019	209	237	5
3-ethyl-1,2-dithiolan-4-one	21	–	–	36	22	–	44	20	–	24	24	7	5
3-methyl-1,2-dithian-4-one	190	6	3	128	307	10	222	453	10	290	458	47	19
total dithianones and dithiolanones	*1158*	*22*	*50*	*519*	*517*	*43*	*867*	*662*	*63*	*2557*	*804*	*588*	
4,5-dihydro-3(2H)-thiophenone	28	tr	3	76	147	17	108	90	10	33	23	7	c
4,5-dihydro-5-methyl-3(2H)-thiophenone	117	17	tr	+	+	+	+	+	+	251	51	20	c
4,5-dihydro-2-methyl-3(2H)-thiophenone	244	26	10	3040	1890	23	2200	1020	33	868	536	87	c
dihydro-2,(4 or 5)-dimethyl-3(2H)-thiophenone	tr	–	–	189	159	tr	253	104	tr	17	22	tr	5
dihydro-2,(4 or 5)-dimethyl-3(2H)-thiophenone	tr	–	–	306	260	10	302	123	tr	22	26	tr	5
dihydro-2 or 5-ethyl-3(2H)-thiophenone	–	–	–	138	90	tr	201	81	tr	–	–	tr	5
ethyl-3(2H)-thiophenone	–	–	–	40	15	–	52	12	–	–	–	–	5
total thiophenones	*389*	*43*	*13*	*3789*	*2561*	*50*	*3116*	*1430*	*43*	*1191*	*658*	*114*	

ribose system, suggests that catalysis by the buffer occurred, which was greater than any effect of pH. It was interesting to note that quantities of 2-furfural decreased in the presence of buffer. 2-Furylmethanethiol, which is formed from 2-furfural, also showed little or no increase in the presence of buffer. These compounds are formed via the 1,2-enolization of the Amadori compound, and the apparent lack of a buffer effect on their formation suggests that the role of phosphate and phthalate

Table 1. (Continued)

compound [m/z (rel intensity)]	unbuffered			phosphate buffer, pH 5.6			phthalate buffer, pH 5.6			phosphate buffer, pH 4.2			MS ref
	Rib-PO$_4$	ribose	IMP	Rib-PO$_4$	ribose	IMP	Rib-PO$_4$	ribose	IMP	Rib-PO$_4$	ribose	IMP	
initial pH	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	5.6	4.2	4.2	4.2	
final pH	4.2	3.9	5.7	5.5	5.5	5.7	5.4	5.3	5.4	3.9	3.9	4.5	
2,3-dihydro-6-methylthiothieno[2,3-c]furan	tr	–	–	224	1320	–	406	840	–	tr	43	–	c
thieno[2,3-b]thiophene	290	8	10	+	43	23	147	47	23	275	37	3	20
thieno[3,2-b]thiophene	7	tr	tr	23	24	7	39	60	tr	–	–	–	20
a dihydrothienothiophene	68	tr	–	887	1710	7	1310	1490	3	137	96	7	5
a methyldihydrothienothiophene	5	tr	–	139	628	–	243	660	–	tr	tr	tr	5
a methyldihydrothienothiophene	73	3	–	156	110	–	400	143	–	33	26	tr	5
a methyldihydrothienothiophene	153	5	–	140	103	–	334	128	–	68	58	tr	5
a dimethyldihydrothienothiophene	30	tr	–	152	254	–	497	460	–	7	16	tr	5
total bicyclic compounds	*626*	*16*	*10*	*1721*	*4192*	*37*	*3376*	*3828*	*26*	*520*	*276*	*10*	
(E)-3,5-dimethyl-1,2,4-trithiolane	–	–	7	tr	tr	23	+	tr	tr	–	–	–	c
(Z)-3,5-dimethyl-1,2,4-trithiolane	–	–	tr	tr	tr	20	+	tr	tr	–	–	–	c
3-methyl-1,2,4-trithiane	–	–	7	27	8	97	38	6	7	–	–	–	c
1,2,4,5-tetrathiane	–	–	63	–	–	210	–	–	67	–	–	–	
total trithiolanes, trithianes, and tetrathianes	*–*	*–*	*77*	*27*	*8*	*350*	*38*	*6*	*74*	*–*	*–*	*–*	
2-pentanone	124	12	27	801	388	40	1040	469	67	352	123	117	c
3-pentanone	26	–	13	457	388	33	703	279	43	82	22	37	c
2,3-pentanedione	+	+	–	+	+	+	+	+	+	+	+	+	c
3-hydroxy-2-butanone	tr	–	–	453	98	–	1060	tr	–	+	+	–	c
2,4-pentanedione	2530	10	3	912	102	13	1170	33	13	5640	482	367	c
methylpyrazine	–	–	–	81	267	–	tr	294	–	tr	tr	–	c
2-furfural	1460	4990	tr	–	tr	tr	–	260	tr	1260	2290	27	c
total non-sulfur compounds	*4140*	*5012*	*43*	*2704*	*1243*	*86*	*3973*	*1335*	*123*	*7334*	*2917*	*665*	

[a] Approximate quantities in headspace (ng/mmol of sugar) given as means of triplicate analyses; tr, trace (<0.5 ng/mmol of sugar); –, below detection limit (~0.1 ng/mmol of sugar); +, present in small amounts and quantification confounded by adjacent peak. [b] Each model system consisted of 0.3 mmol of cysteine and 0.3 mmol of the ribose-containing compound in 6 mL of water or buffer. [c] Mass spectra and LRI agree with those of authentic samples analyzed under similar conditions in our laboratory. [d] Present as a pair of diastereoisomers.

buffers in catalyzing the Maillard reaction is associated only with the 2,3-enolization step in the breakdown of the Amadori intermediate.

It has been known for some time that buffers may catalyze the Maillard reaction. A generally accepted mechanism for phosphate-mediated catalysis has been proposed by Potman and van Wijk (*10*). This involves the dihydrogen phosphate ion acting as a base and abstracting a proton during rearrangements leading to the Amadori compound.
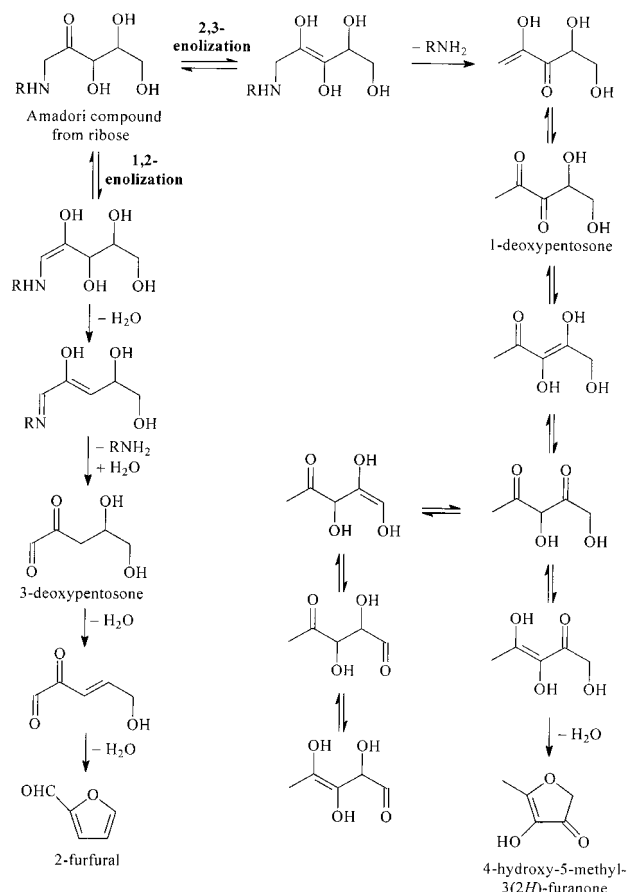
In the Maillard reaction, keto–enol tautomerisms are essential features of the rearrangement and degradation of the Amadori compound to deoxyosones (**Figure 1**). Such tautomerism involves the migration of a hydrogen atom between a carbon atom and the oxygen on the adjacent carbon. Because these steps are acid–base catalyzed, the presence of any buffer would be expected to enhance such steps in the Maillard reaction. In the buffered ribose/cysteine reactions, therefore, volatile compounds deriving from the 1-deoxypentosone route, where keto–enol tautomerisms are important steps, were produced in higher amounts. These included 2-methyl-3-furanthiol and several mercaptoketones. Whitfield and Mottram (*15*) recently proposed mechanisms for the formation of mercatopketones, in the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone with cysteine, that required hydrolysis to 1-deoxypentosone, followed by several keto–enol tautomerisms steps. Conversely, in the ribose/cysteine reactions, buffer had less effect on compounds such as 2-furanmethanethiol and 2-furfural, formed via 3-deoxypentosone, because keto–enol steps were less important in the mechanism leading to their formation. Furthermore, both the phosphate and phthalate buffers exhibited similar extents of catalysis, indicating that acid–base catalysis was the dominant mechanism and that the catalysis of the Maillard reaction is not associated with only phosphate buffers.

Detection of 4-Hydroxy-5-methyl-3(2*H*)-furanone in Reaction Systems. As discussed above, the much higher reactivity of ribose 5-phosphate in the unbuffered system is believed to result from the ready dephosphorylation of this sugar. However, the key intermediate in this mechanism is 4-hydroxy-5-methyl-3(2*H*)-furanone, and this compound could not be detected in the headspace of the heated reaction mixtures. The furanone is water-soluble, and this might explain why it was not detected in the analysis by headspace trapping on Tenax-TA. In view of the fact that this compound was very important for the proposed hypothesis, solvent extractions of the heated sugar solutions with a polar solvent (dichloromethane) were carried out. These showed that the amount of 4-hydroxy-5-methyl-3(2*H*)-furanone formed was considerable in the buffered and unbuffered solutions of ribose 5-phosphate (**Table 2**), whereas ribose gave a trace amount of the furanone at most.

Systems Containing Inosine 5′-Monophosphate. Although most of the sulfur-containing compounds, which were found in the ribose and ribose phosphate reaction systems, were also identified in one or more of the cysteine model systems containing inosine monophosphate, many were found only in very small quantities, only 38 being present in the headspace at concentrations >5 ng/mmol of inosine 5′-monophosphate (**Table 1**). Comparison of the quantities of volatiles from the inosine monophosphate systems with those containing ribose 5-phosphate and ribose showed that the inosine monophosphate systems were much less reactive. In the unbuffered inosine monophosphate systems and those buffered at pH 5.6, most compounds were found in the headspace at concentrations 10–100 times lower than in the corresponding ribose or ribose phosphate reactions, and for some compounds the differences were even greater. The only exceptions were the group of sulfur compounds comprising trithiolanes, trithianes, and tetrathianes, which were found in larger quantities in the inosine monophosphate systems at pH 5.6.
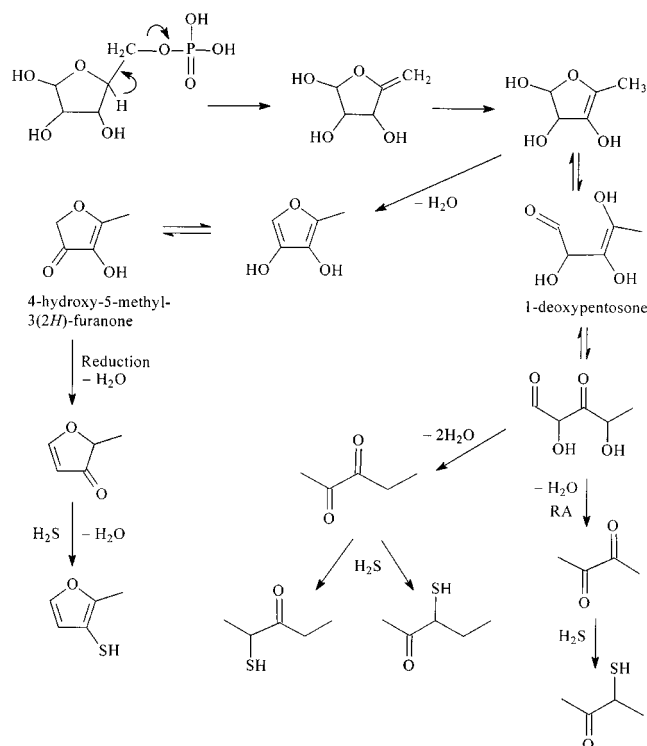
When the cysteine/inosine monophosphate reaction was carried out in phosphate buffer at pH 4.2, much higher amounts

Sulfur Aroma Compounds from Cysteine/Ribose Reaction Systems

J. Agric. Food Chem., Vol. 50, No. 14, 2002 **4085**

Figure 1. Formation of 4-hydroxy-5-methyl-3(2*H*)-furanone and 2-furfural from ribose via an Amadori compound in the Maillard reaction, showing tautomeric forms of 1-deoxypentosone.

Figure 2. Degradation of ribose 5-phosphate to give 4-hydroxy-5-methyl-3(2*H*)-furanone and 1-deoxypentosone and the subsequent reactions with hydrogen sulfide (RA = retroaldolization).

Table 2. Approximate Quantities[a] (Micrograms per Millimole of Sugar) of Major Compounds Identified in Dichloromethane Extracts of Heated Ribose 5-Phosphate or Ribose Solutions[b]

compound	unbuffered		phosphate buffer	
	Rib-PO$_4$	ribose	Rib-PO$_4$	ribose
final pH (initial pH 5.6)	4.4	3.8	5.7	5.7
2-furfural	263 (63)	103 (23)	tr	107 (3)
4-hydroxy-5-methyl-3(2*H*)-furanone	280 (27)	–	287 (47)	tr

[a] Quantities are the mean of triplicate analyses with the standard deviation in parentheses; tr, trace (<5 μg/mmol); –, not detected. [b] Each solution consisted of 0.3 mmol of ribose or ribose 5-phosphate in 6 mL of water or phosphate buffer.

of volatiles were formed in comparison to the reactions at pH 5.6 (**Table 1**). This was particularly notable for the thiols and disulfides. Interestingly, trithiolanes, trithianes, and tetrathianes, which were major components in the cysteine/inosine monophosphate reactions at pH 5.6, were not detected in the same systems at pH 4.2. These compounds are believed to be formed by the thermal degradation of cysteine in aqueous solution (*30*). Therefore, they do not require the presence of a sugar and the associated Maillard reactions for their formation. Their absence from the cysteine/inosine monophosphate reaction at low pH and their low concentrations in the ribose systems were probably due to competing reactions for intermediates of cysteine breakdown, reducing their availability for thiolane and trithiane formation.

Inosine 5′-monophosphate is a glycoside of hypoxanthine and ribose 5-phosphate. The *N*-glycoside link between ribose and the base is at the reducing group of the sugar and, therefore, Maillard-type reactions involving the ribose moiety will not occur until this link is hydrolyzed. It has been reported that the hydrolysis of inosine 5′-monophosphate is enhanced under acidic conditions (*31*). The present results demonstrate that inosine 5′-monophosphate is relatively stable in aqueous solution at a pH typical of that observed in meat (5.6) and that relatively little reaction occurs with cysteine. This is in contrast to ribose 5-phosphate and ribose, which undergo reactions with cysteine to give typical Maillard reaction products. At the lower pH of 4.2 some hydrolysis of inosine 5′-monophosphate occurred, which gave rise to increased concentrations of such products.

This investigation has shown that the reaction between cysteine and ribose in heated aqueous solution was strongly influenced by the nature of the ribose. When it is bound within inosine 5′-monophosphate, it is relatively unreactive compared with ribose 5-phosphate or free ribose, and hydrolysis of the glycoside appeared to be a prerequisite for Maillard-type reactions. In the absence of buffer, ribose 5-phosphate was much more reactive toward cysteine than free ribose, due to the ease with which it decomposes to 1-deoxypentosone. Both phosphate and phthalate buffers catalyzed the formation of mercaptoketones, furanthiols, thiophenethiols, and related compounds in the reaction between ribose and cysteine. The ribose dehydration product, 1-deoxypentosone, is a key intermediate in the formation of these compounds, and the buffer acts as an acid−base catalyst in the keto−enol tautomerism, which is associated with the formation and reactions of this intermediate. Ribose occurs in raw meat as the free sugar, as the sugar phosphate, and as inosine 5′-monophosphate. The relative amounts of these important flavor precursors may well be a determining factor in meat flavor quality.

Mottram and Nobrega

LITERATURE CITED

(1) Mottram, D. S. Meat. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 107−177.

(2) Mottram, D. S. Flavour formation in meat and meat products: a review. *Food Chem.* **1998**, *62*, 415−424.

(3) Lawrie, R. A. *Meat Science*, 6th ed.; Woodhead Publishing: Cambridge, U.K., 1998.

(4) Morton, I. D.; Akroyd, P.; May, C. G. Flavoring substances and their preparation. GB Patent 836,694, 1960.

(5) Farmer, L. J.; Mottram, D. S.; Whitfield, F. B. Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid. *J. Sci. Food Agric.* **1989**, *49*, 347−368.

(6) Maga, J. A. Flavor potentiators. *Crit. Rev. Food Sci. Nutr.* **1983**, *18*, 231−312.

(7) Sugita, Y. H. Recent developments in umami research. In *Developments in Food Flavours*; Birch, G. G., Lindley, M. G., Eds.; Elsevier Applied Science: London, U.K., 1986; pp 63−79.

(8) Madruga, M. S. Studies on some factors affecting meat flavour formation. Ph.D. Thesis, The University of Reading, U.K., 1994.

(9) Bobbio, F. O.; Bobbio, P. A.; Trevisan, L. M. V. Maillard reaction II: Catalytic effect of anions. *Lebensm. Wiss. Technol.* **1973**, *6*, 215−218.

(10) Potman, R. P.; van Wijk, T. A. Mechanistic studies of the Maillard reaction with emphasis on phosphate-mediated catalysis. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; American Chemical Society: Washington, DC, 1989; pp 182−195.

(11) Madruga, M. S.; Mottram, D. S. The effect of pH on the formation of Maillard-derived aroma volatiles using a cooked meat system. *J. Sci. Food Agric.* **1995**, *68*, 305−310.

(12) Nursten, H. E. Recent developments in studies of the Maillard reaction. *Food Chem.* **1980**, *6*, 263−277.

(13) Meynier, A.; Mottram, D. S. The effect of pH on the formation of volatile compounds in meat-related model systems. *Food Chem.* **1995**, *52*, 361−366.

(14) Werkhoff, P.; Brüning, J.; Emberger, R.; Güntert, M.; Hopp, R. Flavor chemistry of meat volatiles: new results on flavor components from beef, pork and chicken. In *Recent Developments in Flavor and Fragrance Chemistry*; Hopp, R., Mori, K., Eds.; VCH: Weinheim, Germany, 1993; pp 183−213.

(15) Whitfield, F. B.; Mottram, D. S. Investigation of the reaction between 4-hydroxy-5-methyl-3(2*H*)-furanone and cysteine or hydrogen sulfide at pH 4.5. *J. Agric. Food Chem.* **1999**, *47*, 1626−1634.

(16) Werkhoff, P.; Brüning, J.; Emberger, R.; Güntert, M.; Köpsel, M.; Kuhn, W.; Surburg, H. Isolation and characterization of volatile sulfur-containing meat flavor components in model systems. *J. Agric. Food Chem.* **1990**, *38*, 777−791.

(17) Zhang, Y.; Ho, C. T. Formation of meatlike aroma compounds from thermal reaction inosine 5′-monophosphate with cysteine and glutathione. *J. Agric. Food Chem.* **1991**, *39*, 1145−1148.

(18) Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavour and Fragrances: by Glass Capillary Gas Chromatography*; Academic Press: New York, 1980.

(19) Hartman, G. J.; Scheide, J. D.; Ho, C.-T. Volatile products formed from a flavor model system at high and low moisture levels. *Lebensm. Wiss. -Technol.* **1984**, *17*, 222−225.

(20) ten Noever de Brauw, M. C.; Bouwman, J.; Tas, A. C.; La Vos, G. F. *Compilation of Mass Spectra of Volatile Compounds in Food*; Institute for Nutrition and Food Research TNO: Zeist, The Netherlands, 1979.

(21) Buttery, R. G.; Haddon, W. F.; Seifert, R. M.; Turnbaugh, J. G. Thiamin odor and bis(2-methyl-3-furyl) disulfide. *J. Agric. Food Chem.* **1984**, *32*, 674−676.

(22) Evers, W. J.; Heinsohn, H. H.; Mayers, B. J.; Sanderson, A. Furans substituted at the three position with sulfur. In *Phenolic, Sulfur and Nitrogen Compounds in Food Flavors*; Charalambous, G., Katz, I., Eds.; American Chemical Society: Washington, DC, 1976; pp 184−193.

(23) MacLeod, G.; Ames, J. M. 2-Methyl-3-(methylthio)furan: a meaty character impact aroma compound identified from cooked beef. *Chem. Ind. (London)* **1986**, 175−176.

(24) Gasser, U.; Grosch, W. Identification of volatile flavour compounds with high aroma values from cooked beef. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 489−494.

(25) Mottram, D. S.; Madruga, M. S.; Whitfield, F. B. Some novel meatlike aroma compounds from the reactions of alkanediones with hydrogen sulfide and furanthiols. *J. Agric. Food Chem.* **1995**, *43*, 189−193.

(26) Whitfield, F. B.; Mottram, D. S. Heterocyclic volatiles formed by heating cysteine or hydrogen sulfide with 4-hydroxy-5-methyl-3(2*H*)-furanone at pH 6.5. *J. Agric. Food Chem.* **2001**, *49*, 816−822.

(27) Shibamoto, T. Formation of sulfur- and nitrogen-containing compounds from the reaction of furfural with hydrogen sulfide and ammonia. *J. Agric. Food Chem.* **1977**, *25*, 206−208.

(28) Peer, H. G.; van den Ouweland, G. A. M. Synthesis of 4-hydroxy-5-methyl-2,3-dihydro-3-furanone from D-ribose 5-phosphate. *Recl. Trav. Chim. Pays-Bas* **1968**, *87*, 1017−1020.

(29) van den Ouweland, G. A. M.; Peer, H. G. Components contributing to beef flavor. Volatile compounds produced by the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone and its thio analog with hydrogen sulfide. *J. Agric. Food Chem.* **1975**, *23*, 501−505.

(30) Shu, C.-K.; Hagedorn, M. L.; Mookherjee, B. D.; Ho, C.-T. Volatile components of the thermal degradation of cysteine in water. *J. Agric. Food Chem.* **1985**, *33*, 438−442.

(31) Matoba, T.; Kuchiba, M.; Kimura, M.; Hasegawa, K. Thermal degradation of flavor enhancers, inosine 5′-monophosphate and guanosine 5′-monophosphate in aqueous solution. *J. Food Sci.* **1988**, *53*, 1156−1159.