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Effect of Lipid Composition on Meat-like Model Systems Containing Cysteine, Ribose, and Polyunsaturated Fatty Acids

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This paper compares the volatile constituents of model systems containing the important meat aroma precursors cysteine and ribose, with and without either methyl linoleate, an *n*−6 fatty acid, or methyl α-linolenate, an *n*−3 acid, both of which are present in meat. Many of the volatile compounds formed from the reaction between cysteine and ribose were not formed, or formed in lower amounts, when lipid was present. This may be due to the reaction between hydrogen sulfide, formed from the breakdown of cysteine, and lipid degradation products. In addition, cysteine and ribose modified lipid oxidation pathways, so that alcohols and alkylfurans were formed rather than saturated and unsaturated aldehydes. Several volatile compounds, which have been found at elevated levels in cooked meat from animals fed supplements high in *n*−3 acids, were formed when methyl α-linolenate reacted with cysteine and ribose. The possible effects of increasing the *n*−3 content of meat upon flavor formation during cooking are discussed.

KEYWORDS: Aroma volatiles; polyunsaturated fatty acids; meat; linoleic acid; α-linolenic acid; cysteine; ribose

INTRODUCTION

The fatty acid composition of animal muscle can be altered by varying the lipid composition of the animal's diet (*1*), and such changes can affect the flavor characteristics of the meat when it is cooked (*2*−*5*). For example, relatively high levels of α-linolenic acid (C18:3*n*−3) appear to be partially responsible for the characteristic pastoral flavor of meat from ruminants fed on grass (*6*).

Elmore et al. (*3*, *4*) studied the compositions of the aroma volatiles of cooked beef and lamb muscle from animals fed dietary supplements high in *n*−3 fatty acids, which caused an increase in the ratio of *n*−3 to *n*−6 acids in the muscle lipids of both species. Many of the compounds found at higher levels in the meat from the animals fed the supplements were known products of *n*−3 fatty acid oxidation, for example, 2-ethylfuran, 2-(2-pentenyl)furan, and (*E*,*E*)-2,4-heptadienal, or compounds formed by their reaction with products of the Maillard reaction, for example, 2-ethyl-(2*H*)-thiapyran (*7*) and 2-hexyl-4,5-dimethyl-3-thiazoline (*8*). Other compounds formed from the oxidation of *n*−9 and *n*−6 acids were also present at elevated levels in the supplement-fed animals, for example, hexanal, (*E*)-2-nonenal, and 1-octanol. This effect was possibly due to the high level of oxidation initiated by the less stable *n*−3 polyunsaturated fatty acids (PUFAs), resulting in greater free radical attack on the mono- and diunsaturated fatty acids (*3*).

Mottram and Edwards (*9*) suggested that structural phospholipids were important in the development of cooked meat flavor. Whitfield et al. (*10*) showed that the reaction between cysteine and ribose, which has long been recognized as being important in the production of meat flavor (*11*, *12*), can be modified in buffered solutions by the addition of phosphatidylcholine (lecithin), an important phospholipid. Levels of many of the cyclic sulfur-containing compounds present in the cysteine/ribose mixtures were reduced when lecithin was present. In addition, several different heterocyclic sulfur-containing compounds were present in the lecithin-containing reaction mixture, indicating the participation of lecithin breakdown products in the Maillard reaction. Levels of lipid-derived alkylfurans were also higher in the cysteine/ribose/lecithin reaction mixture than in the lecithin blank, showing that products from the Maillard reaction influenced the formation of the products of lipid oxidation.

Farmer et al. (*12*) reported that a similar cysteine/ribose/lecithin reaction mixture possessed a much more pronounced "meaty, beefy" odor than a reaction mixture containing cysteine and ribose only. Farmer and Mottram (*13*, *14*) compared the volatile compounds that were obtained when beef triacylglyc-

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erols, phosphatidylethanolamine (cephalin), lecithin, and beef phospholipids were reacted individually, with and without both cysteine and ribose in buffered solutions. Compounds formed via the Maillard reaction, which changed in amount when lipid was present, were affected to a lesser degree by the beef triacylglycerols than any of the three phospholipids. The reaction between cephalin, ribose, and cysteine gave the most desirable meaty aroma of the eight reaction mixtures. This work also showed that only phospholipids, not triacylglycerols, contributed to the generation of typical meaty aroma.

This paper reports the results of studies that aimed to explain the differences in the aroma volatiles of the cooked meat from animals fed dietary PUFA supplements. Linoleic acid is the most common $n-6$ fatty acid in the muscle of beef and lamb, whether fed on grass or concentrates, but the $n-3$ fatty acid α-linolenic acid increases in the muscle of grass-fed ruminants (*15*, *16*). The products formed when the methyl esters of these two compounds were separately heated with cysteine and ribose have been examined to show how dietary changes could modify the composition of cooked meat aroma volatiles.

EXPERIMENTAL PROCEDURES

Materials. L-Cysteine, D-(−)-ribose, citric acid, sodium citrate dihydrate, anhydrous disodium pyrophosphate, and tetrasodium pyrophosphate decahydrate were purchased from Sigma Chemical Co., Poole, U.K. Methyl linoleate, methyl α-linolenate, 2-propenal, (*E*)-2-butenal, and 1,4-dithiane-2,5-diol were purchased from Aldrich Chemical Co., Gillingham, U.K. (*E*)-2-Pentenal was purchased from Lancaster Synthesis, Morecambe, U.K. Pentane (Analar grade) was purchased from Merck Ltd., Poole, U.K.

Pyrophosphate buffer (pH 5.5) was prepared by adding tetrasodium pyrophosphate decahydrate (0.2 M) to sodium dihydrogen pyrophosphate (0.2 M) in deionized water. Citrate buffer (pH 5.5) was prepared from citric acid (0.05 M, 30 mL) and sodium citrate (0.05 M, 70 mL).

Preparation of Reaction Mixtures. Five reaction mixtures were made up in pyrophosphate buffer (pH 5.5), each mixture containing 0.5 mmol of each reagent in 20 mL of buffer. The five mixtures were as follows: (1) cysteine and ribose; (2) cysteine, ribose, and methyl linoleate; (3) cysteine, ribose, and methyl α-linolenate; (4) methyl linoleate; (5) methyl α-linolenate. Each reaction mixture was heated at 140 °C for 30 min in a 100 mL Duran borosilicate glass reagent bottle (Merck Ltd.), fitted with an airtight, PTFE-lined screw top, and was then allowed to cool.

Solid Phase Microextraction (SPME). Headspace volatiles from the reaction mixtures were collected at room temperature for 30 min onto two SPME fibers simultaneously (*17*). The fibers were a 50/30 μm divinylbenzene/Carboxen on poly(dimethylsiloxane) (PDMS) Stableflex fiber and a 75 μm Carboxen/PDMS fiber (Supelco, Poole, U.K.). Both fibers were conditioned before use by heating them in a gas chromatograph injection port at 250 °C for 30 min. For each SPME analysis, the screw top used during the heating of the reaction mixtures was replaced with a similar top containing two drilled holes. Four replicates of each sample were analyzed. The use of two fibers gives a gas chromatogram containing peaks from both low-boiling and higher-boiling volatiles. After extraction, the SPME devices were removed from the sample bottle and inserted into the injection port of the GC-MS system.

Gas Chromatography—Mass Spectrometry (GC-MS). All analyses were performed on a Hewlett-Packard 5972 mass spectrometer, coupled to a 5890 series II gas chromatograph and a G1034C Chemstation. The volatile compounds on each SPME fiber were desorbed for 3 min in a split/splitless injection port, held at 250 °C, onto a fused silica retention gap (5 m × 0.25 mm i.d.; Varian Chrompack, Middleburg, The Netherlands). The retention gap was attached to a CP-Sil 8 CB low-bleed/MS fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness; Varian Chrompack). The injection port was operated in splitless mode, with the splitter opening after 6 min. Immediately before the desorption of the fibers, 0.1 μL of an internal standard (100 ng of 1,2-dichlorobenzene in 1 μL of methanol) was injected into the gas chromatograph. The 75 μm Carboxen/PDMS fiber was always desorbed after the divinylbenzene/Carboxen on the PDMS fiber.

During desorption the oven was held at 40 °C. The retention gap contained five small loops, which were cooled in solid carbon dioxide, contained within a 250 mL glass beaker. After desorption, the carbon dioxide was removed. The oven was maintained at 40 °C for a further 2 min, and then the temperature was raised at 4 °C/min to 280 °C. Helium at 16 psi was used as the carrier gas, resulting in a flow of 1.0 mL/min at 40 °C. *n*-Alkanes (C_5-C_{25}) were run under the same conditions to obtain linear retention index (LRI) values for the components.

The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current of 35 μA. The ion source was maintained at 170 °C. The mass spectrometer scanned from *m*/*z* 29 to 400 at 1.9 scans/s. Compounds were identified by first comparing their mass spectra with those contained in the NIST/EPA/NIH Mass Spectral Database or in previously published literature, followed by comparing their LRI values with those of either authentic standards or published values.

Quantities of the volatiles were approximated by comparison of their peak areas with that of the 1,2-dichlorobenzene internal standard, obtained from the total ion chromatograms, using a response factor of 1.

Preparation of 3-Formylthiophenes. Equimolar mixtures of mercaptoacetaldehyde, a cysteine breakdown product, and a monounsaturated aldehyde, that is, 2-propenal, (*E*)-2-butenal, or (*E*)-2-pentenal, were heated at 140 °C for 30 min in citrate buffer (pH 5.5) (*18*). Two sets of these mixtures were prepared, one set containing an equimolar amount of ribose. The reaction products were extracted with 20 mL of pentane and analyzed by GC-MS.

RESULTS AND DISCUSSION

One hundred and seventeen compounds were found at levels >20 ng/100 mL of sample in at least one of the extracts. Of these compounds, those that could not be identified and had not been found in any of the meat samples examined in our previous work were not considered further in this work. These compounds comprised from 0.9% of the total chromatographic peak area in the cysteine/ribose/linoleic acid mixture up to 3.4% in the cysteine/ribose reaction mixture.

The compounds were subdivided into four groups: (1) compounds formed from the reaction between cysteine and ribose; (2) compounds formed from lipid oxidation alone (excluding methyl esters); (3) compounds formed from the interaction of lipid with cysteine and ribose; (4) methyl esters formed by lipid oxidation. A few compounds could be formed by more than one pathway and were assigned to all of the relevant groups. Compounds present below 10 ng/100 mL of sample were labeled as trace. The detection limit for the compounds was 2 ng/100 mL.

2-Alkylthiophenes and 2-alkyl-(2*H*)-thiapyrans, formed in those model systems containing cysteine, ribose, and a methyl ester, have been discussed in detail elsewhere (*19*).

Compounds Formed from the Reaction between Cysteine and Ribose. Most of the compounds listed in **Table 1** have been previously reported as components of cysteine/ribose reaction mixtures or cysteine decomposition products. Some early-eluting compounds have not been reported previously, for example, 2-butanone, 2-methylfuran, and tetrahydrofuran, probably because of their coelution with added solvents.

PUFA methyl esters affected the compounds formed from the reaction between cysteine and ribose to a much greater extent than the addition of triacylglycerols or phospholipids reported previously (*13*). Many of the volatile compounds formed from

Table 1. Aroma Compounds Found in SPME Extracts of Reaction Mixtures Containing Cysteine, Ribose, and PUFA Methyl Esters, Which Derive from the Reaction between Cysteine and Ribose

	approximate quantity[a]				
compound	cysteine ribose	18:2*n*–6 cysteine ribose	18:3*n*–3 cysteine ribose	LRI[b]	method of identification[c]
2-butanone[d,e]	28 (9)	24 (7)	10 (4)	605	MS + LRI
2-methylfuran[e]	223 (86)	170 (49)	128 (40)	609	MS + LRI
tetrahydrofuran	16 (4)	32 (22)	22 (4)	632	MS + LRI
thiophene[d]	91 (30)	45 (18)	57 (22)	673	MS + LRI
1-hydroxy-2-propanone[e]	30 (5)	43 (15)	28 (10)	732	MS + LRI
thiazole	26 (10)	tr	12 (5)	739	MS + LRI
2,3-dihydrothiophene	36 (15)	10 (3)	tr	767	MS + LRI
2-methylthiophene	443 (132)	83 (20)	54 (14)	775	MS + LRI
3-mercapto-2-butanone	14 (7)	30 (24)	tr	821	MS + LRI
2-ethylthiophene	37 (11)	tr	145 (65)	870	MS + LRI
2-methyl-3-furanthiol	807 (508)	58 (71)	tr	872	MS + LRI
2,5-dimethylthiophene	377 (48)	48 (11)	21 (5)	875	MS + LRI
2,3-dimethylthiophene	30 (6)	tr	–	893	MS + LRI
2-furanmethanethiol	92 (54)	87 (32)	17 (5)	915	MS + LRI
2-ethyl-5-methylthiophene	16 (4)	–	20 (7)	967	MS + LRI
3-thiophenethiol[d]	149 (64)	10 (12)	–	985	ms
dihydro-2-methyl-3(2*H*)-thiophenone	50 (13)	12 (4)	tr	999	MS + LRI
2-formylthiophene	27 (9)	18 (7)	69 (21)	1011	MS + LRI
2-methyl-3-thiophenethiol	233 (116)	tr	tr	1070	MS + LRI
3-methyl-2-formylthiophene	41 (11)	24 (5)	20 (6)	1140	MS + LRI
a thienylethanal	49 (10)	12 (3)	–	1142	ms (*12*)
2,3-dihydro-6-methylthieno(2,3c)furan	457 (84)	29 (3)	26 (10)	1202	MS + LRI
thieno(2,3*b*)thiophene	43 (18)	–	tr	1225	ms (*27*)
thieno(3,2*b*)thiophene	22 (8)	–	–	1268	ms (*27*)
a dihydrothienothiophene	1006 (202)	47 (8)	56 (15)	1329	ms (*12*)
a dihydromethylthienothiophene	163 (30)	tr	–	1387	ms (*12*)
a dihydromethylthienothiophene	45 (15)	–	–	1429	ms (*12*)
a dihydromethylthienothiophene	49 (17)	–	–	1434	ms (*12*)
a dihydrodimethylthienothiophene	76 (41)	–	–	1488	ms (*12*)
bis(2-methyl-3-furanyl) disulfide	202 (213)	tr	–	1552	MS + LRI
(2-methyl-3-furanyl) (2-furfuryl) disulfide	39 (35)	tr	–	1655	MS + LRI
(2-methyl-3-furanyl) (3-thienyl) disulfide	52 (50)	tr	–	1716	ms (*28*)
(2-methyl-3-furanyl) (2-methyl-3-thienyl) disulfide	38 (40)	–	–	1760	ms (*29*)

[a] Estimated quantity (ng) in the headspace of 100 mL of reaction mixture, expressed as the peak area relative to that of 100 ng of 1,2-dichlorobenzene internal standard; means are from four replicate samples with standard deviations shown in parentheses; tr, <10 ng in the extract from 100 mL of reaction mixture; –, not detected, limit of detection = 2 ng in 100 mL of reaction mixture. [b] Linear retention index on a CP-Sil 8 CB low-bleed/MS column. [c] MS + LRI, mass spectrum and LRI agree with those of authentic compound; ms, mass spectrum agrees with spectrum in NIST/EPA/NIH Mass Spectral Database or with other literature spectrum. [d] Major component of heated cysteine. [e] Major component of heated ribose.

the reaction between cysteine and ribose were not formed or were formed in lower amounts when PUFA methyl esters were present in the reaction mixture. It is likely that the addition of methyl esters to cysteine/ribose mixtures also caused the formation of increased levels of nonvolatile material, which could not be extracted using headspace SPME.

Of the 33 compounds derived from cysteine and ribose, which are listed in **Table 1**, none increased when 18:2*n*−6 was added to the cysteine/ribose mixture, although two increased when 18:3*n*−3 was added. The two compounds that increased were 2-formylthiophene and 2-ethylthiophene. A third compound, 2-ethyl-5-methylthiophene, was unique in that it was present at similar levels in the cysteine/ribose mixture and the 18:3*n*−3/cysteine/ribose mixture but was absent from the 18:2*n*−6/cysteine/ribose mixture. It seems likely that all three of these compounds can be formed from the interaction of 18:3*n*−3 with cysteine and ribose, as well as from cysteine and ribose alone. Therefore, these compounds are included in both categories.

With the exceptions of these three compounds, the sulfur-containing compounds identified in the cysteine/ribose reaction system were present at reduced levels or were absent when lipid was present in the system. It seems likely that the changes in aroma volatiles caused by the addition of lipid to cysteine/ribose systems are caused by the reaction between hydrogen sulfide, formed from the breakdown of cysteine, and lipid degradation products, to give both volatile and nonvolatile material. The participation of hydrogen sulfide in such reactions reduces its availability to react with Maillard reaction-derived compounds.

Compounds Formed from Lipid Oxidation. Forty-five compounds formed from oxidation of methyl linoleate and methyl α-linolenate (excluding methyl esters), both with and without cysteine and ribose present, are listed in **Table 2**. Many of these compounds have been previously reported as products of the decomposition of 18:2*n*−6 and 18:3*n*−3 (*20*). The total quantity of volatile compounds formed from methyl α-linolenate oxidation was >5 times greater than the amount formed from methyl linoleate oxidation.

When cysteine and ribose were added to both 18:2*n*−6 and 18:3*n*−3, qualitative and quantitative changes occurred. In particular, several unsaturated aldehydes were present at much lower levels in the reaction mixtures containing methyl α-linolenate, cysteine, and ribose than in methyl α-linolenate alone. These included 2-propenal, (*E*)-2-butenal, (*E*)-2-pentenal, (*E*,*E*)-2,4-hexadienal, and (*E*,*E*)-2,4-heptadienal.

Farmer and Mottram (*14*) reported that when lecithin was heated with and without cysteine and ribose, lipid oxidation pathways were modified, so that alcohols and alkylfurans were formed, instead of saturated and unsaturated aldehydes. This effect was observed for some, but not all, aldehydes formed from both of the fatty acids. For example, when cysteine and

Table 2. Aroma Compounds Found in SPME Extracts of Reaction Mixtures Containing Cysteine, Ribose, and PUFA Methyl Esters, Which Derive from Lipid Oxidation

	approximate quantity[a]					
compound [*m*/*z* (relative intensity)]	18:2*n*–6	18:2*n*–6 cysteine ribose	18:3*n*–3	18:3*n*–3 cysteine ribose	LRI[b]	method of identification[c]
2-propenal	–	–	132 (66)	–	<500	MS + LRI
propanal	–	–	50 (19)	34 (8)	<500	MS + LRI
2-butanone	–	24 (7)	11 (4)	10 (4)	605	MS + LRI
2-methylfuran	–	170 (49)	30 (4)	128 (40)	609	MS + LRI
(*E*)-2-butenal	–	–	497 (83)	–	644	MS + LRI
1-penten-3-ol	–	–	50 (21)	48 (13)	689	MS + LRI
1-penten-3-one	–	tr	49 (11)	tr	691	MS + LRI
2-pentanone	–	tr	15 (5)	21 (10)	692	MS + LRI
2-ethylfuran	tr	tr	915 (263)	1912 (848)	705	MS + LRI
pentanal	72 (33)	11 (13)	45 (18)	–	704	MS + LRI
2-vinylfuran	–	tr	51 (13)	44 (26)	725	ms (*30*)
(*E*)-2-pentenal	–	–	437 (96)	tr	758	MS + LRI
(*E* and/or *Z*)-2-penten-1-ol	–	–	12 (6)	44 (11)	769	MS + LRI
1-pentanol	11 (5)	96 (51)	–	–	769	MS + LRI
2-ethyl-5-methylfuran	–	–	24 (6)	109 (59)	802	MS + LRI
hexanal	644 (302)	379 (430)	12 (5)	–	804	MS + LRI
(*E* and/or *Z*)-3-hexen-2-one	–	–	37 (12)	11 (5)	843	ms
(*E*)-2-hexenal	53 (22)	16 (29)	265 (65)	127 (114)	858	MS + LRI
2-(2-propenyl)furan	–	–	24 (3)	43 (17)	857	ms
1-hexanol	tr	47 (28)	–	–	872	MS + LRI
2-heptanone	29 (14)	40 (26)	15 (5)	tr	892	MS + LRI
2-butylfuran	tr	40 (30)	–	tr	893	MS + LRI
heptanal	21 (9)	21 (17)	–	–	906	MS + LRI
a 2,4-hexadienal	–	–	42 (22)	10 (3)	915	ms
(*E*,*E*)-2,4-hexadienal	tr	–	143 (36)	30 (19)	916	MS + LRI
unknown, MW 122 107, 122 (35), 77 (23), 39 (11), 108 (8), 55 (7), 65 (5)	–	–	51 (13)	60 (32)	921	se
a 2,5-nonadiene 81, 79 (78), 124 (61), 41 (50), 39 (41), 53 (41), 55 (32)	–	–	239 (72)	496 (107)	936	se
two 2,5-nonadienes 81, 79 (78), 124 (62), 41 (50), 39 (43), 53 (42), 55 (35)	–	–	425 (101)	511 (127)	946	se
a 2,5-nonadiene 81, 79 (78), 124 (66), 41 (51), 39 (43), 53 (41), 55 (35)	–	–	641 (167)	848 (196)	949	se
(*E*)-2-heptenal	334 (192)	487 (772)	38 (11)	tr	962	MS + LRI
benzaldehyde	tr	tr	185 (54)	34 (15)	971	MS + LRI
1-octen-3-ol	51 (23)	219 (175)	–	tr	983	MS + LRI
2,3-octanedione	21 (14)	–	–	–	985	ms + lri (*31*)
(*E* and/or *Z*)-5-octen-2-one	–	–	47 (18)	44 (16)	987	ms
2-pentylfuran	315 (161)	1736 (1264)	17 (2)	47 (30)	993	MS + LRI
(*E* and *Z*)-2-(2-pentenyl)furan	–	–	651 (185)	1828 (560)	1004	ms (*32*)
(*E*,*Z*)-2,4-heptadienal	tr	–	646 (201)	43 (24)	1004	ms + lri (*33*)
(*E*,*E*)-2,4-heptadienal	tr	tr	3083 (972)	297 (126)	1018	MS + LRI
5-ethyl-1-formylcyclopentene	31 (15)	24 (28)	–	–	1040	ms (*34*)
(*Z*)-2-(1-pentenyl)furan	–	tr	30 (7)	91 (62)	1058	ms
(*E*)-2-octenal	50 (33)	400 (531)	17 (6)	10 (6)	1063	MS + LRI
a 3,5-octadien-2-one	–	–	138 (43)	159 (45)	1099	ms
(*E* and/or *Z*)-3-nonen-2-one	tr	42 (47)	–	–	1144	MS + LRI
2-ethylbenzaldehyde	–	–	21 (6)	17 (4)	1200	se (*7*)
(*E*,*Z*)-2,4-decadienal	tr	20 (12)	–	–	1302	ms + lri (*33*)
(*E*,*E*)-2,4-decadienal	29 (8)	115 (65)	tr	tr	1327	MS + LRI

[a] Estimated quantity (ng) in the headspace of 100 mL of reaction mixture, expressed as the peak area relative to that of 100 ng of 1,2-dichlorobenzene internal standard; means are from four replicate samples with standard deviations shown in parentheses; tr, <10 ng in the extract from 100 mL of reaction mixture; –, not detected, limit of detection = 2 ng in 100 mL of reaction mixture. [b] Linear retention index on a CP-Sil 8 CB low-bleed/MS column. [c] MS + LRI, mass spectrum and LRI agree with those of authentic compound; ms + lri, mass spectrum identified using NIST/EPA/NIH Mass Spectral Database and LRI agrees with literature value; ms, mass spectrum agrees with spectrum in NIST/EPA/NIH Mass Spectral Database or with other literature spectrum; se, tentative identification based on mass spectral properties and linear retention index.

ribose were heated with methyl linoleate, levels of pentanal decreased significantly, accompanied by a corresponding increase in 1-pentanol, compared with when methyl linoleate was heated alone (**Table 2**). Farmer (*21*) suggested that the antioxidant effect of Maillard reaction products might involve the modification of lipid oxidation pathways, with a reduction in the formation of unsaturated aldehydes, which have low odor thresholds, and an increase in levels of less odor-potent furans and alcohols.

Compounds Formed from the Interaction of Lipid with Cysteine and Ribose. Eleven compounds formed from the interaction of lipid with cysteine and ribose are listed in **Table**

Table 3. Aroma Compounds Found in SPME Extracts of Reaction Mixtures Containing Cysteine, Ribose, and PUFA Methyl Esters, Which Derive from Lipid–Maillard Interactions

compound [*m/z* (relative intensity)]	approximate quantity[a]			LRI[b]	method of identification[c]
	cysteine ribose	18:2*n*–6 cysteine ribose	18:3*n*–3 cysteine ribose		
2-ethylthiophene	37 (11)	tr	145 (65)	870	MS + LRI
2-methyl-(2*H*)-thiapyran	–	–	33 (12)	913	MS + LRI
2-ethylpyridine	–	–	22 (2)	919	MS + LRI
2-ethyl-5-methylthiophene	16 (4)	–	20 (7)	967	MS + LRI
3-formylthiophene	–	tr	76 (27)	1003	MS + LRI
2-formylthiophene	27 (9)	18 (7)	69 (21)	1011	MS + LRI
2-ethyl-(2*H*)-thiapyran	–	–	469 (185)	1020	MS + LRI
2-methyl-3-formylthiophene	–	–	52 (18)	1096	MS + LRI
2-pentylthiophene	–	26 (28)	–	1170	MS + LRI
2-ethyl-3-formylthiophene	–	–	83 (39)	1177	MS + LRI
unknown sulfur-containing compound MW 140 **140**, 139 (93), 111 (35), 59 (26), 125 (17), 97 (14), 45 (12), 51(12), 141(12)	–	tr	25 (6)	1210	

[a] Estimated quantity (ng) in the headspace of 100 mL of reaction mixture, expressed as the peak area relative to that of 100 ng of 1,2-dichlorobenzene internal standard; means are from four replicate samples with standard deviations shown in parentheses; tr, <10 ng in the extract from 100 mL of reaction mixture; –, not detected, limit of detection = 2 ng in 100 mL of reaction mixture. [b] Linear retention index on a CP-Sil 8 CB low-bleed/MS column. [c] MS + LRI, mass spectrum and LRI agree with those of authentic compound.

Table 4. Volatile Methyl Esters Found in SPME Extracts of Reaction Mixtures Containing Cysteine, Ribose, and PUFA Methyl Esters

compound	approximate quantity[a]				LRI[b]	method of identification[c]
	18:2*n*–6	18:2*n*–6 cysteine ribose	18:3*n*–3	18:3*n*–3 cysteine ribose		
methyl butanoate	–	17 (13)	tr	24 (11)	723	MS + LRI
methyl pentanoate	tr	54 (40)	23 (9)	130 (48)	825	MS + LRI
methyl hexanoate	20 (11)	340 (191)	100 (36)	292 (94)	926	MS + LRI
methyl heptanoate	27 (19)	333 (244)	580 (208)	1183 (384)	1026	MS + LRI
methyl 7-octenoate	tr	–	138 (43)	94 (18)	1117	ms
methyl octanoate	764 (357)	3189 (2260)	11063 (3344)	14684 (3479)	1132	MS + LRI
methyl 8-nonenoate	–	tr	50 (24)	63 (34)	1216	ms
methyl nonanoate	–	tr	59 (20)	68 (27)	1224	MS + LRI
methyl 9-decenoate	–	–	35 (16)	73 (32)	1316	ms (*35*)
methyl 9-oxononanoate	tr	–	38 (24)	124 (33)	1439	ms
methyl 8-(2-furyl)octanoate	–	17 (11)	–	108 (37)	1629	ms

[a] Estimated quantity (ng) in the headspace of 100 mL of reaction mixture, expressed as the peak area relative to that of 100 ng of 1,2-dichlorobenzene internal standard; means are from four replicate samples with standard deviations shown in parentheses; tr, <10 ng in the extract from 100 mL of reaction mixture; –, not detected, limit of detection = 2 ng in 100 mL of reaction mixture. [b] Linear retention index on a CP-Sil 8 CB low-bleed/MS column. [c] MS + LRI, mass spectrum and LRI agree with those of authentic compound; ms, mass spectrum agrees with spectrum in NIST/EPA/NIH Mass Spectral Database or with other literature spectrum.

3. Only one of these compounds, 2-pentylthiophene, was at its highest level when methyl linoleate was present in the reaction mixture. All of the 11 compounds, apart from 2-ethyl-3-formylthiophene, have been identified in cooked meat. In previous work, 2-ethylpyridine and 3-formylthiophene were at higher levels in cooked meat from lambs with raised PUFA levels relative to their amount in the meat from control animals (*4*). 2-Ethyl-(2*H*)-thiapyran and 2-ethylthiophene increased when the PUFA levels in beef were raised (*7*).

2-Ethylthiophene and 2-ethyl-5-methylthiophene could both be formed by the action of hydrogen sulfide on 2-ethylfuran and 2-ethyl-5-methylfuran or their precursors, (*E*,*E*)-2,4-hexadienal and (*E*,*E*)-2,4-heptadienal (*22*). 2-Ethylfuran, 2-ethyl-5-methylfuran, (*E*,*E*)-2,4-hexadienal, and (*E*,*E*)-2,4-heptadienal were all produced by the oxidation of methyl α-linolenate (**Table 2**). Levels of 2-ethylfuran and 2-ethyl-5-methylfuran were even higher in the methyl linolenate/cysteine/ribose reaction mixture. Zhang and Ho (*23*) showed that 2-alkylthiophenes and 2-alkyl-5-methylthiophenes were produced when 2,4-decadienal was heated with cysteine in aqueous solution.

2-Pentylpyridine was found in the cysteine/ribose/methyl linoleate reaction mixture at a level of 18 ng/100 mL of reaction mixture, that is, below the minimum level for inclusion in **Table 3**, and was absent from the other reaction mixtures. It has been reported in various reaction mixtures and cooked foods (*24*) and can be formed from 2,4-decadienal and the amino group of cysteine (*25*). 2-Ethylpyridine is likely to be formed in a similar way, from (*E*,*E*)-2,4-heptadienal. 2-Pentylthiophene and 2-ethyl-(2*H*)-thiapyran are likely be formed from the reaction of hydrogen sulfide with (*E*,*E*)-2,4-nonadienal and (*E*,*E*)-2,4-heptadienal, respectively (*7*).

3-Formylthiophene was positively identified in the methyl linolenate/cysteine/ribose mixture. 2-Methyl-3-formylthiophene and 2-ethyl-3-formylthiophene were tentatively identified. Farmer and Whitfield (*18*) showed that mercaptoacetaldehyde, from the decomposition of cysteine, reacted with monounsaturated aldehydes to give 2-alkyl-3-formylthiophenes, including 2-ethyl-3-formylthiophene. Hence, 2-propenal, (*E*)-2-butenal, and (*E*)-2-pentenal, which were all found at high levels in heated methyl α-linolenate, could react with mercaptoacetaldehyde to form 3-formylthiophene, 2-methyl-3-formylthiophene, and 2-ethyl-3-formylthiophene, respectively. When 1,4-dithiane-2,5-diol (the dimer of mercaptoacetaldehyde) was heated with 2-enals, these formylthiophenes were a major product in the reaction mixture, but the dihydrothiophene homologues were always present at higher concentrations. However, when 1,4-dithiane-2,5-diol and the monounsaturated aldehyde were heated in the presence of an equimolar amount of ribose, yields of the thiophene were

much higher. For example, when ribose was absent, the amount of 3-formyl-2-methylthiophene formed was only 10% of the amount of dihydro-3-formyl-2-methylthiophene formed, whereas when ribose was present, the amounts of each formed were approximately the same. The electron impact mass spectrum for 2-methyl-3-formylthiophene, which has not been previously reported, is *m/z* (%) 125 (100), **126** ($M^{+\bullet}$, 95), 97 (59), 45 (20), 53 (16), 69 (12), 127 (12), 39 (8), 50 (7), 51(7). The mass spectra of 3-formylthiophene and 2-ethyl-3-formylthiophene are reported elsewhere (NIST/NIH/EPA Database, *18*). A pathway for the formation of 2-alkyl-3-formylthiophenes from mercaptoacetaldehyde and an alkenal has been proposed by Farmer and Whitfield (*18*).

One compound in **Table 3** could not be identified. A compound with a similar mass spectrum and retention time has also been found in cooked beef and lamb (*3*, *4*), although its concentration in cooked meat, like that of 2-methyl-3-formylthiophene, was not affected by the PUFA composition.

Methyl Esters. Volatile methyl esters were formed, due to the oxidative cleavage of the hydrocarbon chain, when methyl linoleate and methyl α-linolenate were heated. Increased levels of methyl esters were formed when cysteine and ribose were present (**Table 4**), showing that cysteine and ribose increased the rate of lipid oxidation. Greater amounts of methyl esters were formed from methyl α-linolenate than methyl linoleate, due to the more rapid oxidation of methyl α-linolenate. Methyl 8-(2-furyl)octanoate was absent in heated methyl α-linolenate but was present at 108 ng/100 mL in the cysteine/ribose/18:3*n*−3 reaction mixture. The presence of the furan ring again showed how lipid oxidation pathways are modified by the Maillard reaction.

Role of PUFAs in Meat Flavor. Increasing the *n*−3 content of meat may confer nutritional benefits to the consumer. However, if levels of *n*−3 PUFAs are raised too high, off-flavors may result. The results presented in this paper indicate that the formation of flavor during the cooking of the meat could be affected by *n*−3 PUFAs in four ways:

(1) Breakdown products of *n*−3 acids have a shorter chain length than those of the *n*−6 and *n*−9 acids they replace. They are more volatile and may have lower odor thresholds. They will also be present at relatively high concentrations compared with the breakdown products of less saturated *n*−6 and *n*−9 acids.

(2) Many of the breakdown products of *n*−3 acids are more reactive than those of *n*−6 acids, possessing proportionately more double bonds, as well as being shorter in chain length. These breakdown products will affect meat flavor by interacting with the Maillard reaction more readily than *n*−6 and *n*−9 breakdown products, reducing levels of meaty aroma compounds, such as sulfur-substituted thiophenes and furans (*26*).

(3) Compounds formed from the interaction between the breakdown products of *n*−3 acids and the Maillard reaction will also have characteristic aromas.

(4) As *n*−3 PUFAs are readily oxidized, they could initiate the free radical oxidation of more saturated acids, increasing levels of breakdown products from *n*−6 and *n*−9 acids, which will also alter the aroma profile of the cooked meat.

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