Effect of the Polyunsaturated Fatty Acid Composition of Beef Muscle on the Profile of Aroma Volatiles

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The effect of *n*−3 polyunsaturated fatty acids (PUFAs) in beef muscle on the composition of the aroma volatiles produced during cooking was measured. The meat was obtained from groups of steers fed different supplementary fats: (i) a palm-oil-based control; (ii) bruised whole linseed, which increased muscle levels of α-linolenic (C18:3 *n*−3) and eicosapentaenoic acid (EPA, C20:5 *n*−3); (iii) fish oil, which increased EPA and docosahexaenoic acid (C22:6 *n*−3); (iv) equal quantities of linseed and fish oil. Higher levels of lipid oxidation products were found in the aroma extracts of all of the steaks with increased PUFA content, after cooking. In particular, *n*-alkanals, 2-alkenals, 1-alkanols, and alkylfurans were increased up to 4-fold. Most of these compounds were derived from the autoxidation of the more abundant mono- and di-unsaturated fatty acids during cooking, and such autoxidation appeared to be promoted by increased levels of PUFAs.

Keywords: *Aroma volatiles; beef; polyunsaturated fatty acids; lipid oxidation*

INTRODUCTION

The main sources of volatiles in cooked meat are the thermal degradation of lipid and the Maillard reaction, which occurs between amino acids and sugars (Mottram, 1994). Heat-induced oxidation of fatty acids, particularly unsaturated fatty acids, produces degradation products, such as aliphatic aldehydes, ketones, and alcohols, which may have intrinsic flavors. These degradation products may react further with Maillard products to give other compounds that may contribute to flavor (Mottram and Edwards, 1983; Elmore et al., 1997).

Altering the fatty acid composition of beef muscle can affect its flavor characteristics (Ford et al., 1976). For example, the relative levels of linoleic acid (C18:2 *n*−6) and α-linolenic acid (C18:3 *n*−3) in grain and forage are largely responsible for the differences in volatile composition, and hence the flavor, of beef finished on these diets (Larick et al., 1987; Larick and Turner, 1990).

The nutritional value of *n*−3 polyunsaturated fatty acids (PUFAs) in the human diet is well recognized, and increased consumption of these fatty acids, particularly eicosapentaenoic acid (EPA, C20:5 *n*−3) and docosahexaenoic acid (DHA, C22:6 *n*−3), has been recommended (Department of Health, 1994). To help meet these recommendations we have produced beef with increased levels of *n*−3 PUFAs through feeding dietary supplements of linseed and fish oil (Scollan et al., 1999). These supplements doubled the concentrations of dietary *n*−3 PUFA in the longissimus lumborum muscle, compared with steers fed a palm-oil-based supplement. The C_{20} and C_{22} PUFAs are deposited in the phospholipids of ruminants (Ashes et al., 1992), which are important sources of lipid-derived flavor compounds during cooking (Mottram and Edwards, 1983; Mottram, 1996). Because of the low oxidative stability of these fatty acids, it seems likely that changes in their concentration, although small, would result in alterations to the composition of the aroma volatiles produced during cooking. This paper examines the aroma profiles of cooked steaks, in relation to their fatty acid composition, and discusses the implications for the eating quality of meat.

MATERIALS AND METHODS

Sample Preparation. Four groups of Charolais steers matched for initial live weight (436 ± 3.4 kg) were individually fed ad libitum diets, adjusted to provide 60% of dry matter from grass silage and 40% from concentrates based on barley and sugar beet. The concentrate contained one of four fat supplements: (1) Megalac, a palm-oil-based milk substitute (Velac Ltd., Royston, U.K.), used as control; (2) whole linseed, lightly bruised; (3) Fish oil, South American herring oil, containing 30% PUFA (Issac Spencer Ltd., Fleetwood, U.K.); (4) Linseed and fish oil mixture (1:1 oil weight basis). Approximately 45% of the dietary fat intake was provided by the supplements. All diets had a similar vitamin E content (α-tocopherol acetate, 345 mg/kg concentrate) to prevent oxidation of PUFAs in tissues. Steers were slaughtered conventionally, with captive bolt stunning, after an average of 120 days on test. After chilling at 4 °C for 48 h postmortem, the loin was boned out, vacuum-packed, and conditioned at 1 °C for 10 days. Steaks consisting of a complete cross-section of longissimus lumborum muscle, 15 mm thick, were cut posterior to the ninth rib position, vacuum-packed, blast frozen, and stored at −20 °C for 3 to 6 months before cooking and determination of flavor volatiles.

Fatty Acid Analysis. Samples of muscle tissue from all eight steers (longissimus lumborum muscle), taken 48 h postmortem, were blended separately in a small food processor. The fatty acids were extracted, methylated, and analyzed by gas chromatography, as described by Whittington et al. (1986). Duplicate 1-g samples were hydrolyzed in 6 mL of 5 M potassium hydroxide in methanol/water (1:1) at 60 °C for 2 h.

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After dilution with 12 mL of water, nonsaponifiables were removed by 3 extractions with 5 mL of petroleum spirit (bp 40−60 °C). After acidification with 0.5 mL of sulfuric acid, the fatty acids were methylated with a solution of diazomethane in diethyl ether and analyzed by gas chromatography, using a 60 m × 0.25 mm i.d. CPSil-88 for FAME column (60 m × 0.25 mm i.d., 0.2 μm film thickness, Chrompack, London). All analyses were performed using a Carlo Erba 6000 gas chromatograph. A split injection, 70:1, was used, with the injector at 215 °C. After 15 min at 180 °C, the column temperature was raised at 1.5 °C/min to 220 °C and held at that temperature until C22:6 *n*−3 eluted. The carrier gas was helium at a flow rate of 1.0 mL/min. GC peaks were identified using standards where available (Sigma Chemical Co. Ltd., Poole, U.K.). Fatty acids were quantified using heneicosanoic acid methyl ester, added prior to saponification, as an internal standard. Column response and linearity were checked using a monoenoic fatty acid mixture, GLC 50 (Supelco, Poole, U.K.), and on-column injection.

Analysis of Volatiles from Cooked Meat. Steaks from five animals from each feeding regime were trimmed of subcutaneous fat, and samples of each steak (80 g) were placed in separate 100 mL bottles fitted with airtight, PTFE-lined screw tops. The samples were cooked at 140 °C in an autoclave for 30 min, after which they were allowed to cool and held at 4 °C overnight. The following day the steaks were chopped and samples immediately taken for volatile analysis.

Aroma volatiles were collected on Tenax TA. Nitrogen, at 40 mL/min, flowed over the sample (60 g), which was held at 60 °C for 1 h, sweeping volatiles onto a glass-lined, stainless steel trap (105 mm × 3 mm i.d.) containing 85 mg of Tenax TA (Scientific Glass Engineering Ltd., Milton Keynes, U.K.). A standard (100 ng of 1,2-dichlorobenzene in 1 μL of hexane) was added to the trap at the end of the collection and excess solvent, and any water retained on the trap, were removed by purging the trap with nitrogen at 40 mL/min for 5 min.

All analyses were performed on a Hewlett-Packard 5972 mass spectrometer fitted with a HP5890 Series II gas chromatograph and a G1034 Chemstation. A CHIS injection port (Scientific Glass Engineering Ltd.) was used to thermally desorb the volatiles from the Tenax trap onto the front of a BPX5 fused silica capillary column (50 m × 0.32 mm i.d., 0.5 μm film thickness; Scientific Glass Engineering Ltd.). During the desorption period of 5 min, the oven was held at 0 °C. After desorption, the oven was heated at 40 °C/min to 40 °C and held for 2 min before heating at 4 °C/min to 280 °C. Helium at 8 psi 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 beef aroma components.

The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current of 50 μA. The mass spectrometer scanned from *m*/*z* 29 to *m*/*z* 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 and then comparing LRI values with either those of authentic standards or with published values.

Approximate quantities of the volatiles were estimated by comparing their peak areas with those of the 1,2-dichlorobenzene internal standard, obtained from the total ion chromatograms, using a response factor of 1.

Statistical Analysis. Analysis of variance (ANOVA) was carried out on the quantitative data for each compound identified in the GC-MS analyses of volatiles and also on the data for each fatty acid. For those compounds exhibiting significant difference in the ANOVA, Fisher's least significant difference test was applied to determine which sample means differed significantly ($p < 0.05$).

RESULTS AND DISCUSSION

The percentages of the major PUFAs in the complete diet, silage plus concentrates, are shown in Table 1.

Table 1. Polyunsaturated Fatty Acid (PUFA) Composition of the Four Feeds

	% PUFA in fat total dietary lipid (w/w)			
fatty acid	control	linseed	fish oil	fish oil + linseed
C18:2 *n*−6 linoleic	18.1	17.9	16.5	17.1
C18:3 *n*−3 α-linolenic	27.3	47.8	27.1	39.6
C20:5 *n*−3 eicosapentaenoic	—	0.2	4.5	2.3
C22:6 *n*−3 docosahexaenoic	—	0.1	2.8	1.5

Whereas linoleic acid levels were similar across all feeds, the linseed supplement increased the concentration of α-linolenic acid, and the fish oil increased EPA and DHA. The mixture of fish oil and linseed gave intermediate levels of all these fatty acids in the diet. Fat source did not influence feed intake or growth rate. Full details of the animals, feeds, production characteristics, and carcass characteristics of the steers used in this work have been published elsewhere (Scollan et al., 1999).

The fatty acid compositions of the total lipid of longissimus lumborum muscle from the four treatment groups are given in Table 2. In general, these results are similar to those reported by other workers and ourselves (Marmer and Maxwell, 1984; West and Chrystall, 1989; Enser et al., 1996). There was no significant difference in the total fatty acid content due to dietary treatment, nor in the content of the major saturated fatty acids, palmitic (C16:0) and stearic (C18:0). The content of linoleic acid (C18:2 *n*−6) was similar in muscles from animals on all feeds, as expected from its similar feed content. Relative to the control diet, dietary linseed doubled the content of C18:3 *n*−3 and increased C20:5 *n*−3 by 45%. Fish oil doubled the muscle content of C20:5 *n*−3 and C22:6 *n*−3. The combined linseed/fish oil treatment raised the C18:3 *n*−3 to a position intermediate between fish oil alone and linseed alone and raised C22:6 *n*−3 to a level as high as the fish oil alone. However, C22:5 *n*−3 was unaffected by the diet. Arachidonic acid (C20:4 *n*−6) levels were decreased, particularly in the animals fed fish oil or the mixed fat.

In beef muscle C18:2 *n*−6 and C18:3 *n*−3 are present in the triacylglycerols, as well as in the polar lipid (Marmer et al., 1984), whereas the C_{20} and C_{22} PUFAs are present only in the polar lipids (Ashes et al., 1992). The phospholipids are considered to be prime targets for lipid oxidation reactions because, in addition to their high degree of unsaturation, they are exposed to proteins and other catalysts of lipid oxidation as components of membranes in contact with the cytosol (Boylston et al., 1996). Under cooking conditions similar to those used in this present work, Fogerty et al. (1990) showed that fatty acids and fatty aldehydes were liberated from the phospholipids of various meats. The greatest losses occurred in beef, where 32% of fatty acids, 62% of PUFAs, and over 90% of fatty aldehydes were hydrolyzed from the phosphatidylcholines and phosphatidylethanolamines. However, only slight liberation of fatty acids occurred from intramuscular triacylglycerol. Because free fatty acids are much more readily oxidized than the fatty acid moieties of intact lipids, the ready hydrolysis of phospholipids may partly explain why they play a much more important role in meat flavor formation than do triacylglycerols (Mottram and Edwards, 1983). However, the high degree of unsaturation in the phospholipid is the most likely explanation for this effect (Farmer and Mottram, 1992).

Table 2. Fatty Acid Composition of Longissimus Lumborum Muscle from Steers Fed on Diets Containing Differing Fat Sources

fatty acid	quantity (mg/100 g of muscle)[a]				P^b
	control	linseed	fish oil	linseed/fish oil	
C12:0 lauric	3.2	3.8	3.7	4.3	NS
C14:0 myristic	121	152	173	169	NS
C16:0 palmitic	1029	1089	1305	1171	NS
C18:0 stearic	528	581	543	490	NS
C18:1 trans	63[c]	147[d]	184[d]	173[d]	<0.01
C18:1 *n*−9 oleic	1209	1471	1260	1225	NS
C18:2 *n*−6 linoleic	81	78	66	64	NS
C18:3 *n*−3 α-linolenic	22[c]	43[d]	26[c]	30[c]	<0.01
C20:3 *n*−6	7.8[e]	5.9[d]	4.9[c]	4.2[c]	<0.001
C20:4 *n*−6 arachidonic	23[e]	21[de]	14[c]	17[cd]	<0.001
C20:5 *n*−3 eicosapentaenoic	11[c]	16[d]	23[e]	15[cd]	<0.001
C22:5 *n*−3 docosapentaenoic	20	21	24	21	NS
C22:6 *n*−3 docosahexaenoic	2.2[c]	2.4[c]	4.6[d]	4.9[d]	<0.001
total fatty acids (mg/100 g muscle)	3529	4222	4292	3973	NS

[a] Mean for eight animals (minor components not reported). Means in the same row with different superscripts are significantly different ($P < 0.05$). [b] Probability that there is a difference between means; NS, no significant difference between means ($P > 0.05$).

The approximate quantities of the most abundant volatile compounds present in the headspace of the cooked beef from the four different feeding regimes are shown in Table 3. Compounds are grouped according to their functionality. For many volatiles it is possible to deduce whether they are derived from the Maillard reaction or from the thermal degradation of lipid. Volatile compounds formed via the Maillard reaction include heterocyclic nitrogen and sulfur compounds, such as pyrazines, thiophenes, and thiazoles, as well as furanones and furfurals. Certain nonheterocyclic compounds are also Maillard-derived. These include Strecker aldehydes (2- and 3-methylbutanal, benzeneacetaldehyde), alkanediones, and hydroxyketones. Aliphatic and furan disulfides are also formed via the Maillard reaction. Lipid-derived volatiles comprise aliphatic aldehydes, alcohols, hydrocarbons, and ketones, all with straight alkyl chains containing five or more carbon atoms. Alkylfurans are also lipid-derived. All these volatiles are formed by thermal oxidation of the fatty acid chains of triacylglycerols and phospholipids (Forss, 1972; Mottram, 1996).

The compounds formed solely from the Maillard reaction, such as pyrazines, furanones, and furfurals, did not show significant differences among the cooked beef samples from the four treatments. In contrast, examination of the lipid-derived volatiles showed significant effects of animal diet. The cooked beef samples that contained increased levels of PUFAs all showed higher concentrations of lipid oxidation products. The differences were particularly noticeable with the aliphatic aldehydes, both saturated and unsaturated.

Aldehydes were quantitatively the most dominant class of volatiles in the cooked beef. The most abundant volatiles were 2- and 3-methylbutanal, which are products of the Strecker degradation of the amino acids isoleucine and leucine, and their concentrations were unaffected by the nature of the experimental diet. However, the concentrations of saturated and monounsaturated straight-chain aliphatic aldehydes, derived from lipid, increased greatly in beef with higher PUFA content.

Aldehydes are probably the most interesting of the lipid-derived volatiles, since they have low odor threshold values and may contribute to the flavor of the cooked beef samples. Amounts of aliphatic alcohols also reflected the levels of PUFAs in the cooked beef. It is particularly interesting to consider how these aldehydes and alcohols may be formed in the cooked meat containing increased PUFAs. The alkanals, 2-alkenals, and alkanols reported in Table 3 all contain alkyl chains with between 4 and 9 saturated carbon atoms. These cannot derive from *n*−3 PUFAs, which do not contain sufficiently long saturated alkyl chains. They derive from the autoxidation of oleic acid (C18:1 *n*−9) and linoleic acid (C18:2 *n*−6). Oleic is the most abundant unsaturated fatty acid in meat (over 35% of total fatty acids), while linoleic is the most abundant di-unsaturated fatty acid. It appears that PUFAs induce an increase in thermal degradation of oleic and linoleic acids. This is suggested by the higher levels of aldehydes, derived from these fatty acids, in the meat with increased PUFAs.

The oxidative degradation of fatty acids involves a free radical mechanism, and the rate of the reaction is determined by the initial step, which involves the formation of an alkyl radical from an unsaturated fatty acid (Frankel, 1980). Such radicals are formed much more readily from PUFAs, such as C20:4, C20:5, and C22:6, than from C18:1 and C18:2. Once such radicals are formed, propagation of the breakdown of other fatty acid molecules occurs via a chain reaction. We believe that in the high-PUFA meat, autoxidation of the lipid fatty acids was initiated much more readily by the presence of higher quantities of C18:3 *n*−3, C20:5 *n*−3, and C22:6 *n*−3. Furthermore, once the free radical reaction started, the subsequent chain reaction was less dependent on the nature of the unsaturated fatty acid; therefore, breakdown of the abundant oleic and linoleic acids occurred. Hence, larger quantities of alkanals, 2-alkenals, and other breakdown products of C18:1 and C18:2 were found in the cooked beef containing higher concentrations of PUFAs.

An interesting cyclic unsaturated aldehyde, 1-formyl-5-propylcyclopentene, was tentatively identified in all the samples, but at significantly higher levels in the meat with the higher PUFA content. Trace quantities of the ethyl homologue were also found, but the concentrations were too small to quantify. 5-Ethyl-1-formylcyclopentene has been reported in cooked chicken and cooked pork, and its identity was confirmed by synthesis (Werkhoff et al., 1993). The methyl and butyl homologues were also found in chicken.

Compounds that appeared to arise directly from the *n*−3 PUFAs included several dienes and trienes. These

Table 3. Aroma Compounds Found in Headspace Extracts of Pressure-Cooked Steaks from Animals Fed on Four Different Diets

compound (*m*/*z* (rel intensity))	mean concentration in headspace (ng/100g)[a] control	linseed	fish oil	fish oil + linseed	P^d	method of identification[e]	LRI[f]
hydrocarbons							
heptane	52[a]	141[ab]	210[b]	185[b]	0.012	MS + LRI	700
octane	111[a]	311[b]	438[b]	396[b]	0.004	MS + LRI	800
an octatriene[g] 79, 77(63), **108**(44), 91(39), 39(23), 93(22), 78(16), 66(15), 41(14)	2[a]	5[a]	15[b]	7[a]	<0.001	ms	885
an octatriene[g] 79, 77(61), **108**(42), 91(40), 39(20), 93(20), 78(16), 66(14), 41(12)	5[a]	12[a]	34[b]	11[a]	0.001	ms	887
nonane	7[a]	9[ab]	17[c]	14[bc]	0.001	MS + LRI	900
a nonatriene[g] 107, **122**(36), 77(21), 39(11), 55(9), 108(8) 65(6)	3[a]	6[a]	18[b]	7[a]	<0.001	se	925
a nonadiene[g] 81, 79(71), **124**(65), 41(43), 53(37), 39(31), 55(28), 95(21), 68(17)	1[a]	4[a]	10[b]	5[a]	<0.001	se	942
a nonadiene[g] 81, 79(74), 124(60), 41(48), 53(38), 39(35), 55(32), 95(19), 67(18)	1[a]	6[ab]	17[c]	9[b]	<0.001	se	951
a nonadiene[g] 81, 79(76), **124**(66), 41(47), 53(39), 39(35), 55(31), 95(18), 67(18)	3[a]	9[b]	23[c]	10[b]	<0.001	se	954
saturated aldehydes							
3-methylbutanal	10817	11859	14307	11884	NS	MS + LRI	631
2-methylbutanal	12850	12290	14825	13050	NS	MS + LRI	689
pentanal	355[a]	999[b]	882[b]	875[ab]	0.032	MS + LRI	725
hexanal	545[a]	1304[b]	1284[b]	1381[b]	0.028	MS + LRI	818
heptanal	546[a]	2037[b]	1732[b]	2487[b]	0.007	MS + LRI	917
octanal	350	762	623	835	NS	MS + LRI	1015
nonanal	270	514	374	511	NS	MS + LRI	1115
unsaturated aldehydes							
(*E*)-2-methyl-2-butenal	47	55	85	55	NS	MS + LRI	763
(*E*)-2-heptenal	nd	14	8	11		MS + LRI	970
benzaldehyde	443	515	479	415	NS	MS + LRI	982
benzeneacetaldehyde	15	14	13	16	NS	MS + LRI	1061
(*E*)-2-octenal	3[a]	10[b]	7[ab]	11[b]	0.024	MS + LRI	1070
1-formyl-5-propylcyclopentene[g] 67, 95(40), 109(28), **138**(24), 39(22), 41(21), 79(17), 65(15), 66(15), 81(15)	3[a]	8[ab]	6[ab]	10[b]	0.03	se	1135
(*E*)-2-nonenal	21[a]	69[b]	44[ab]	78[b]	0.032	MS + LRI	1171
(*E*)-2-decenal	41[a]	99[ab]	58[ab]	108[b]	0.038	MS + LRI	1273
(*E*,*E*)-2,4-decadienal	4	6	4	7	NS	MS + LRI	1332
(*E*)-2-undecenal	26	59	35	60	NS	MS + LRI	1376
ketones							
2-pentanone	338	296	495	422	NS	MS + LRI	716
3-pentanone	124	120	169	153	NS	MS + LRI	724
2,3-pentanedione	256	395	297	251	NS	MS + LRI	730
3-hexanone	9[a]	16[ab]	21[b]	19[b]	0.011	MS + LRI	802
2-hexanone	11[a]	29[b]	35[b]	29[b]	0.009	MS + LRI	807
cyclopentanone	45	50	61	55	NS	MS + LRI	811
3-heptanone	14	11	15	20	NS	MS + LRI	896
2-heptanone	30[a]	41[a]	74[b]	39[a]	0.004	MS + LRI	902
2-methyl-2-cyclopenten-1-one	13	13	15	16	NS	MS + LRI	922
2-octanone	7[a]	11[ab]	15[b]	12[b]	0.008	MS + LRI	999
2-nonanone	6[a]	8[ab]	11[b]	8[ab]	0.022	MS + LRI	1098
2-decanone	5	6	6	5	NS	MS + LRI	1198
alcohols and hydroxyketones							
1-penten-3-ol	105	320	278	159	NS	MS + LRI	734
1-hydroxy-2-propanone	289	286	302	330	NS	MS + LRI	763
3-hydroxy-2-butanone	126	163	270	207	NS	MS + LRI	783
1-pentanol	154	285	261	236	NS	MS + LRI	800
3-hydroxy-2-pentanone[g]	48	59	58	83	NS	ms	838
2-hydroxy-3-pentanone[g]	30	36	35	38	NS	ms	844
1-hexanol	40[a]	138[b]	116[b]	157[b]	0.003	MS + LRI	888
1-heptanol	97[a]	265[b]	186[ab]	257[b]	0.021	MS + LRI	984
1-octen-3-ol	67	79	96	63	NS	MS + LRI	992
1-octanol	67[a]	145[b]	115[ab]	126[ab]	0.028	MS + LRI	1081
furans							
2-ethylfuran	382[a]	818[a]	2792[b]	1157[a]	0.001	MS + LRI	714
2,5-dimethylfuran	11[a]	13[a]	26[b]	18[ab]	0.017	MS + LRI	728
2,4-dimethylfuran[g]	11[a]	14[a]	33[b]	21[ab]	0.003	ms	735
2-propylfuran	9[a]	33[b]	66[b]	58[b]	0.001	MS + LRI	802
2-ethyl-5-methylfuran	4[a]	7[a]	21[b]	7[a]	0.002	MS + LRI	810
dihydro-2-methyl-3(2*H*)-furanone	569	558	507	579	NS	MS + LRI	830
2-furfural	196	200	168	138	NS	MS + LRI	853
2-furanmethanol	216	228	201	274	NS	MS + LRI	884
2-butylfuran	14[a]	38[b]	55[b]	48[b]	0.004	MS + LRI	898
2-acetylfuran	23	25	25	29	NS	MS + LRI	927
5-methyl-2-furanmethanol[g]	15	18	16	20	NS	ms[1]	970

Table 3 (Continued)

compound (*m/z* (rel intensity))	mean concentration in headspace (ng/100g)[a] control	linseed	fish oil	fish oil + linseed	P^d	method of identification[e]	LRI[f]
furans (continued)							
5-methyl-2-furfural	14	15	14	16	NS	MS + LRI	979
2-pentylfuran	342	332	520	328	NS	MS + LRI	996
2-(2-pentenyl)furan (mixture of *E* and *Z*)[g]	100[a]	154[a]	482[b]	218[a]	<0.001	ms[2]	1006
1-(5-methyl-2-furyl)-2-propanone[g]	11	9	10	11	NS	ms[3]	1056
2-hexylfuran	10[a]	37[b]	34[b]	42[b]	0.014	MS + LRI	1095
2-heptylfuran	4[a]	6[ab]	6[ab]	7[b]	0.031	MS + LRI	1196
3-phenylfuran[g]	12	9	10	10	NS	ms	1238
2-octylfuran	5	6	7	7	NS	MS + LRI	1297
nitrogen-containing							
pyrazine	330	286	332	325	NS	MS + LRI	760
methylpyrazine	949	902	922	1052	NS	MS + LRI	844
trimethyloxazole	9	7	11	11	NS	MS + LRI	863
2,5(and/or 2,6)-dimethylpyrazine	476	464	483	578	NS	MS + LRI	932
ethylpyrazine	169	155	160	173	NS	MS + LRI	931
2,3-dimethylpyrazine	9	8	10	10	NS	MS + LRI	937
2-ethyl-5-methylpyrazine	111	94	107	121	NS	MS + LRI	1010
trimethylpyrazine	51	50	59	65	NS	MS + LRI	1015
2-ethyl-3-methylpyrazine	37	37	36	45	NS	MS + LRI	1016
2-methyl-5(and/or 6)-vinylpyrazine	12	12	11	13	NS	ms	1031
3-ethyl-2,5-dimethyl pyrazine	72	60	69	74	NS	MS + LRI	1086
sulfur-containing							
dimethyl disulfide	1635	1433	1736	1726	NS	MS + LRI	765
2,3-dihydrothiophene[g]	9[ab]	8[a]	13[ab]	14[b]	0.027	ms	780
2-methylthiophene	284[a]	237[a]	442[b]	359[ab]	0.014	MS + LRI	787
3-methylthiophene[g]	11	9	15	13	NS	MS + LRI	795
ethyl methyl disulfide	7	7	13	10	NS	ms + lri	846
4,5-dihydro-2-methylthiophene[g]	6[ab]	5[a]	8[b]	8[ab]	0.023	ms	853
2-ethylthiophene	5[a]	6[a]	15[b]	8[a]	<0.001	MS + LRI	874
2,5-dimethylthiophene	10	8	12	12	NS	MS + LRI	878
dihydro-3(2*H*)-thiophenone	42	41	45	53	NS	MS + LRI	976
dimethyl trisulfide	631	690	716	1051	NS	MS + LRI	982
dihydro-5-methyl-3(2*H*)-thiophenone	108	106	136	128	NS	ms[4]	1006
dihydro-2-methyl-3(2*H*)-thiophenone	38	31	37	44	NS	MS + LRI	1009
ethylthiapyran[g]	3[a]	6[ab]	14[c]	9[b]	<0.001	MS + LRI	1020
2-formylthiophene	33	40	46	38	NS	MS + LRI	1021
a methylformylthiophene (not 3-methyl-2-formyl or 5-methyl-2-formyl) 125, **126**(90), 97(42), 45(17), 53(11), 127(11), 69(8)	40	34	42	42	NS	se	1102
2-acetylthiophene	14	13	15	16	NS	MS + LRI	1107
2,3-dihydro-6-methylthieno-2,3c-furan[g]	109	77	87	106	NS	MS + LRI[4]	1203
an ethylformylthiophene **140**, 139(91), 111(39), 125(18), 59(16), 97(13), 141(10), 77(9), 51(7), 45(6)	14	11	12	14	NS	se	1212
2-furfuryl methyl disulfide	27	23	28	30	NS	MS + LRI	1226
dimethyl tetrasulfide	36	79	47	126	NS	ms	1236
methyldihydrothienothiophene[g]	22	13	17	20	NS	ms[4]	1385
2-furfuryl methyl trisulfide	14	11	14	14	NS	ms[5]	1472
sulfur- and nitrogen-containing							
thiazole	81	61	77	61	NS	MS + LRI	760
4-methylthiazole	44	39	42	48	NS	MS + LRI	836
4,5-dimethylthiazole	11	10	11	12	NS	MS + LRI	945
2-acetylthiazole	30	25	27	29	NS	MS + LRI	1035
2-isobutyl-4,5-dimethyl-3-thiazoline[h]	19	16	26	13	NS	MS + LRI	1241
2-isobutyl-4,5-dimethyl-3-thiazoline[h]	27	20	29	17	NS	MS + LRI	1247
2-hexyl-4,5-dimethyl-3-thiazoline[h]	7	12	13	13	NS	MS + LRI	1495
2-hexyl-4,5-dimethyl-3-thiazoline[h]	7	11	12	11	NS	MS + LRI	1504
2-heptyl-4,5-dimethyl-3-thiazoline[h]	12	15	16	15	NS	MS + LRI	1600
2-heptyl-4,5-dimethyl-3-thiazoline[h]	9	12	12	12	NS	MS + LRI	1610
2-octyl-4-methyl-3-thiazoline	10	15	10	13	NS	MS + LRI	1683
2-octyl-4,5-dimethyl-3-thiazoline[h]	27	30	34	35	NS	MS + LRI	1706
2-octyl-4,5-dimethyl-3-thiazoline[h]	23	31	29	30	NS	MS + LRI	1716
2-octyl-4-ethyl-5-methyl-3-thiazoline[h]	10	22	11	17	NS	MS + LRI	1771
2-octyl-4-ethyl-5-methyl-3-thiazoline[h]	8	17	9	13	NS	MS + LRI	1783
2-octyl-5-ethyl-4-methyl-3-thiazoline[h]	11	18	12	14	NS	MS + LRI	1790
2-octyl-5-ethyl-4-methyl-3-thiazoline[h]	9	13	10	11	NS	MS + LRI	1800

[a] Means in the same row with different superscripts are significantly different ($P < 0.05$); means are from five replicate samples. [d] Probability that there is a difference between means; NS, no significant difference between means ($P > 0.05$). [e] 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 (Kondjoyan and Berdagué, 1996); ms, mass spectrum agrees with spectrum in NIST/EPA/NIH Mass Spectral Database or with other literature spectrum: [1] Baltes and Bochmann (1987), [2] Smagula et al. (1979), [3] Stoll et al. (1967), [4] Farmer et al. (1989), [5] Madruga (1994); se, tentative identification from structure elucidation of mass spectrum. [f] Linear retention index on a BPX5 column. [g] Reported for the first time in beef. [h] Pairs of isomers.

unsaturated hydrocarbons were only tentatively identified from their mass spectra. However, they appeared in the expected LRI region for such compounds, and they could be predicted as autoxidation products of *n*−3 PUFAs. They were readily found in the beef from the fish oil treatment, which had the highest levels of C20:5 *n*−3 and C22:6 *n*−3 PUFAs, but were present at lower levels in the beef from the linseed-containing diets and were only just detected in the controls.

Other volatiles, with more than one C−C double bond, which could arise from PUFA autoxidation, are alkylfurans. In general, these compounds were present in highest concentrations in the high-PUFA meat from the fish-oil-fed cattle. It is interesting to note that the effect of diet was much greater for 2-(2-pentenyl)furan, which can be derived from an *n*−3 PUFA, than for 2-pentylfuran, which contains a long saturated alkyl chain and is derived from an *n*−6 PUFA, such as linoleic acid. The odor threshold values for furans and unsaturated hydrocarbons are relatively high, and therefore these compounds are unlikely to make a significant contribution to the flavor characteristics of the cooked meat.

Aldehydes are likely to react with other compounds, produced during cooking, to give other volatile and nonvolatile products (Mottram, 1994). Alkylthiophenes and ethylthiapyran showed a trend similar to the aldehydes, with increased quantities in the beef with higher PUFA levels. These compounds, like the corresponding alkylfurans, may be derived from dienals, but in this case involving the reaction with hydrogen sulfide from the degradation of the amino acid cysteine. Another example of the interaction of lipid-derived aldehydes with products from the Maillard reaction was the presence of a series of alkyl-3-thiazolines, which had an alkyl chain with between 4 and 9 carbon atoms in the 2-position. These compounds, together with smaller quantities of alkylthiazoles, have already been reported as volatiles of other samples of beef from the same feeding trials (Elmore et al., 1997).

The data in this paper provide further evidence for the interaction of lipid-derived compounds, especially aldehydes, with the Maillard reaction in real food systems, confirming earlier studies on model systems. Such interactions not only produce compounds which may contribute to the flavor of meat, but they also provide a mechanism by which the levels of lipid degradation products and Maillard reaction products may be controlled by the cooking process (Mottram, 1994).

In separate studies on the beef from these feeding trials, samples of grilled steaks were subjected to sensory evaluation by a panel of trained assessors (Enser et al., 1997). Although the differences in eating quality attributes across the diets was small, an examination of the aroma characteristics showed significantly higher scores for rancid, fishy notes in the meat from fish-oil-fed cattle. This latter observation could be explained by increased lipid oxidation during the cooking of these samples. Preliminary examination of the volatiles of samples of the grilled meat show much higher concentrations of aldehydes and other lipid oxidation products (Enser et al., 1997), confirming the data on pressure-cooked meat presented in this paper.

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