Novel Thiazoles and 3-Thiazolines in Cooked Beef Aroma

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Over 50 alkylthiazoles and alkyl-3-thiazolines are reported for the first time in the headspace volatiles from pressure-cooked beef steaks. The substituents in the 4- and 5-positions were methyl or ethyl, while the 2-position contained isopropyl, isobutyl, *n*-butyl, *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, or *n*-nonyl groups. The meat was obtained from cattle that had been fed diets containing linseed or fish oil supplements. Volatiles from all samples contained the thiazoles and 3-thiazolines. However, the concentrations of 2-*n*-alkyl-3-thiazolines were much higher in the steaks from the cattle fed with fish oil supplements than in the control samples. These 3-thiazolines may form from the interaction of intermediates of the Maillard reaction with aldehydes derived from lipid degradation. The cooked meat from the animals that had been fed fish oil had considerably higher concentrations of saturated and unsaturated aldehydes than meat from the control.

Keywords: *Aroma; meat; thiazoles; 3-thiazolines; aldehydes; lipid; animal feed*

INTRODUCTION

The characteristic flavor of cooked meat derives from thermally induced reactions occurring during heating, principally the Maillard reaction and the degradation of lipid. Both types of reactions involve complex reaction pathways leading to a wide range of products, which account for the large number of volatile compounds found in cooked meat. Heterocyclic compounds, especially those containing sulfur, are important flavor compounds produced in the Maillard reaction, providing savory, meaty, roast, and boiled flavors (Mottram, 1991).

The contribution of lipids to meat flavor and aroma has been the subject of considerable research since the early work of the 1950s and 1960s, which attempted to evaluate the relative contributions of fat and lean tissues to meat flavor (Hornstein and Crowe, 1960; Wasserman and Gray, 1965). That work suggested that, on heating, the fatty tissues provided species characteristics, while the lean tissues contained the precursors for the meaty flavor that is characteristic of all cooked meats. Although this view may be regarded as an oversimplification, there is no doubt that the species differences in flavor are largely explained by differences in lipid-derived volatile components. More recent work has, however, shown that interaction between precursors derived from lipid and lean portions of meat plays an important role in meat flavor (Mottram, 1994; Mottram and Edwards, 1983).

The lipids of meat comprise both the neutral triacylglycerols and the structural phospholipids. Both are capable of undergoing oxidative degradation, leading to both desirable and undesirable aroma volatiles. In model systems, interactions of lipids, and their degradation products, with intermediates from the Maillard reaction have been shown to provide routes to flavor; interactions involving the phospholipids are considered to be particularly important (Mottram, 1996). However, relatively few compounds from such interactions have been isolated from foods (Whitfield, 1992; Farmer and Mottram, 1994).

The work reported in this paper arises from studies on the quality of beef from cattle fed diets that attempted to modify the polyunsaturated fatty acid (PUFA) composition. Current human dietary guidelines for dietary fats recommend an increase in the polyunsaturated/saturated fatty acid ratio (P:S ratio), while decreasing the ω6:ω3 ratio. The fatty acids deposited in beef tissues are relatively saturated, giving a low P:S ratio in the meat, but the ratio of ω6:ω3 fatty acids is beneficially low. Strategies to improve the nutritional quality of beef need to increase the P:S ratio, while keeping the ω6:ω3 ratio low. This may be achieved by altering the diet of cattle to increase the level of components containing sources of long-chain PUFAs or by modifying the extent of their hydrogenation in the rumen. For example, linseed contains high concentrations of α-linolenic acid (C18:3ω3), and fish oil contains eicosapentenoic acid (C20:5ω3) and docosahexenoic acid (C22:6ω3). However, an increase in PUFA concentrations in meat may compromise its oxidative stability, resulting in flavor changes in the processed meat.

This paper reports on some novel compounds isolated from the volatiles of pressure-cooked beef reared on such modified diets.

MATERIALS AND METHODS

Sample Preparation. Steaks were obtained from four groups of Charolais steers fed, for 120 days, on grass silage and concentrates (barley and sugar beet) containing different fat sources: palm oil (control), bruised linseed, fish oil, and bruised linseed plus fish oil in equal amounts (Scollan et al., 1997). The steaks were all taken from similar sections of M. longissimus lumborum, posterior to the ninth rib. The mean carcass weight across all teatments was 334 kg (±12 kg). Animals received approximately 45% of their oil intake from the fat supplements, and they were grown to similar weights. All diets had a similar vitamin E content (345 IU/kg).

One steak from each animal was sliced into approximately 50 g pieces, which included fat. Each piece was placed in a

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S0021-8561(97)00066-6 CCC: $14.00

separate 100 mL bottle fitted with an airtight, PTFE-lined screw top. The samples were cooked at 140 °C in an autoclave for 30 min, after which time they were allowed to cool. The samples were combined and minced twice, and 40 g samples were taken for volatile analysis. Analyses were performed on steaks from three animals from each treatment group.

Volatile Extraction. Aroma isolates were collected on Tenax TA, using a method based on that of Madruga and Mottram (1995). The sample (40 g) was held at 60 °C for 1 h while nitrogen, at 40 mL/min, swept the volatiles onto a glass-lined, stainless steel trap (105 mm × 3 mm i.d.) containing 85 mg of Tenax TA (Scientific Glass Engineering Ltd., Ringwood, Australia). 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.

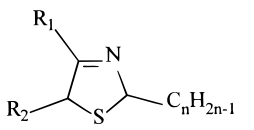
Gas Chromatography/Mass Spectrometry. All analyses were performed on a Hewlett-Packard 5972 mass spectrometer, fitted with a HP5890 Series II gas chromatograph and a G1034C Chemstation. A CHIS injection port (Scientific Glass Engineering Ltd.) was used to thermally desorb the volatiles from the Tenax 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 10 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. Analyses of the volatiles were also carried out on a BP20 column (50 m × 0.32 mm i.d., 0.5 μm film thickness; Scientific Glass Engineering Ltd.), providing additional LRI data. Gas chromatographic conditions were the same as for the BPX5 column, except that the maximum oven temperature was 250 °C.

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 400 at 1.9 scans/s. The identities of alkylthiazoles and alkyl-3-thiazolines were confirmed by comparison of mass spectra and LRI with those of similar compounds prepared in our laboratory (Elmore and Mottram, 1997). Other compounds 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.

Gas Chromatography/Olfactometry. The effluent from the BPX5 column was split (50:50) between the flame ionization detector of a Hewlett-Packard 5890 gas chromatograph and an odor port. Sample preparation and gas chromatographic conditions were the same as for the equivalent GC/MS experiment, except that the sample size was 100 g and extraction was carried out for 4 h.

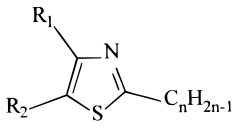
RESULTS AND DISCUSSION

Nearly 350 compounds were identified across the four different samples, of which nearly 150 had not been identified in cooked beef before. Of particular interest was a group of over 50 compounds, which were identified as 3-thiazolines (**1**) and thiazoles (**2**). All of the compounds had an alkyl chain of up to nine carbon atoms in the 2-position of the five-membered ring and no substituent greater than ethyl in positions 4 and 5. None of these compounds had been reported in beef before, and only a few had been identified in any food (Tang et al., 1983; Ho and Carlin, 1989; Werkhoff et al., 1991). We have recently reported such compounds as the major products from the reaction of α-hydroxyketones or α-diones with aliphatic aldehydes and ammonium sulfide in aqueous solution. Mass spectra and retention indices were in agreement with these data (Elmore and Mottram, 1997).



1

$R_1 = CH_3$ or C_2H_5
R_2 = H, CH_3 or C_2H_5
n = 3 - 9



2

R_1, $R_2 = CH_3$ or C_2H_5
n = 4 - 8

The 3-thiazolines and thiazoles identified are shown in Tables 1 and 2 respectively, together with the approximate concentrations in the headspaces of the samples from the different feeding regimes. 3-Thiazolines that contained substituents in the 5-position (methyl or ethyl) were observed in pairs of geometric isomers.

The 3-thiazolines and thiazoles were present at similar concentrations in the control, and there were only slight differences in the thiazoles found in the control and the supplement-fed meat. However, there were much larger quantities of 2-*n*-alkyl-3-thiazolines in the meat from animals fed on both the diets containing fish oil than in the control samples. The differences were not so pronounced with meat from cattle fed on linseed-containing diets.

It has been demonstrated that 3-thiazolines and thiazoles can be formed from a hydroxyketone or a dione, hydrogen sulfide, ammonia, and an aldehyde (Takken et al., 1976; Vernin and Párkaacunyi, 1982; Elmore and Mottram, 1997). Hydroxyketones tended to form mainly 3-thiazolines, particularly 4-substituted, whereas diones gave similar amounts of thiazoles and thiazolines, with no preferential substitution. The only hydroxyketones detected in the headspace volatiles were 1-hydroxy-2-propanone, 1-hydroxy-2-butanone, 3-hydroxy-2-butanone, 2-hydroxy-3-pentanone, and 3-hydroxy-2-pentanone, which would give 4-methyl-, 4-ethyl-, 4,5-dimethyl-, 4-ethyl-5-methyl-, and 5-ethyl-4-methyl-3-thiazolines, respectively. These are products of the Maillard reaction between amino acids and reducing sugars. The related Strecker degradation of cysteine provides a source of both ammonia and hydrogen sulfide. It seems likely that these thiazoles are formed from the oxidation of 3-thiazolines, because the thiazoles detected are the homologues of the thiazolines present in the largest amounts. A possible formation pathway for both thiazoles and thiazolines is shown in Figure 1.

The 2-position on the 3-thiazoline (or thiazole) ring was occupied by an alkyl group derived from an aldehyde. For the 3-thiazolines and thiazoles with *n*-alkyl chains containing four to nine carbon atoms reported in this paper, the aldehydes appear to be derived from lipid. The meat from animals fed on the fish oil supplemented diets contained much higher amounts of C_5−C_{10} alkanals than the control beef, with the highest concentrations in the fish oil fed samples (Table 3). For example, levels of heptanal were over 10 times greater and levels of nonanal approximately 4 times greater in the meat from the fish oil fed animals than in the control steaks. These samples also showed the highest concentrations of 3-thiazolines. The linseed-containing diet resulted in cooked meat with increased concentrations of aldehydes compared with the control, but levels were considerably lower than those of the meat from animals

Table 1. 3-Thiazolines Identified in the Volatiles of Cooked Beef from Animals Grown on Diets Modified with Different Lipid Supplements

		LRI		amount of 3-thiazoline in extract (ng)[b]			
3-thiazoline derivative[a]	MW	BP20	BPX5	control	linseed	fish oil	linseed + fish oil
2-isopropyl-4,5-dimethyl	157	1487	1149	tr[c]	1.2 (0.7)	4.4 (0.7)	3.5 (0.4)
2-isopropyl-4,5-dimethyl	157	1518	1169	tr	1.4 (0.6)	3.1 (0.7)	3.0 (0.4)
2-isobutyl-4-methyl	157	1630	1224	2.2 (1.5)	7.9 (6.4)	6.5 (5.1)	11.0 (2.9)
2-butyl-4-methyl	157	1698	1266	tr	tr	2.6 (0.8)	4.7 (2.0)
2-isobutyl-4,5-dimethyl[e]	171	1603	1249	3.0 (1.3)	6.5 (5.0)	9.0 (2.4)	11.5 (3.3)
2-isobutyl-4,5-dimethyl[e]	171	1612	1256	4.3 (1.6)	10.1 (7.8)	13.5 (4.0)	17.3 (3.3)
2-butyl-4,5-dimethyl	171	1669	1294	tr	1.2 (0.6)	3.7 (1.0)	3.6 (1.0)
2-butyl-4,5-dimethyl	171	1683	1303	1.6 (0.9)	3.2 (2.4)	6.1 (1.3)	7.1 (1.8)
2-pentyl-4-methyl	171	1804	1372	tr	1.8 (0.8)	2.5 (2.3)	4.8 (1.1)
2-isobutyl-4-ethyl-5-methyl	185	1632	1316	2.0 (1.3)	2.9 (1.9)	2.6 (0.7)	3.2 (0.5)
2-isobutyl-4-ethyl-5-methyl	185	1645	1326	2.3 (1.3)	3.5 (2.2)	3.0 (0.8)	3.4 (0.5)
2-isobutyl-5-ethyl-4-methyl	185	1671	1331	1.4 (1.0)	2.4 (1.7)	2.0 (0.4)	2.5 (0.2)
2-isobutyl-5-ethyl-4-methyl	185	1687	1342	1.2 (0.8)	2.7 (1.8)	2.1 (0.6)	2.7 (0.0)
2-pentyl-4,5-dimethyl	185	1773	1397	1.8 (0.6)	2.0 (0.6)	7.0 (2.6)	6.7 (1.8)
2-pentyl-4,5-dimethyl	185	1787	1407	1.5 (0.5)	1.9 (0.4)	6.1 (2.1)	5.9 (1.9)
2-pentyl-4-ethyl	185	1847	1453	tr	tr	2.0 (0.5)	2.1 (0.4)
2-hexyl-4-methyl	185	1915	1478	tr	3.0 (1.5)	4.6 (4.1)	12.4 (4.5)
2-pentyl-4-ethyl-5-methyl	199	1797	1465	tr	1.2 (0.5)	2.5 (1.1)	2.0 (0.5)
2-pentyl-4-ethyl-5-methyl	199	1816	1477	tr	tr	2.5 (1.1)	2.6 (0.5)
2-pentyl-5-ethyl-4-methyl	199	1844	1481	1.0 (0.4)	1.3 (0.7)	2.2 (0.9)	1.8 (0.4)
2-pentyl-5-ethyl-4-methyl	199	1853	1493	tr	tr	1.2 (0.6)	1.1 (0.2)
2-hexyl-4,5-dimethyl	199	1882	1503	tr	3.9 (0.9)	14.6 (6.5)	19.4 (5.7)
2-hexyl-4,5-dimethyl	199	1896	1512	tr	2.9 (0.9)	12.9 (5.6)	9.9 (7.6)
2-hexyl-4-ethyl	199	1955	1558	tr	1.3 (0.8)	3.7 (1.2)	5.3 (1.3)
2-heptyl-4-methyl	199	2026	1584	tr	2.0 (1.0)	1.9 (1.9)	9.0 (4.5)
2-hexyl-4-ethyl-5-methyl	213	1905	1569	1.1 (0.7)	2.4 (1.0)	5.1 (2.8)	6.2 (0.4)
2-hexyl-4-ethyl-5-methyl	213	1923	1581	tr	2.0 (0.8)	3.9 (2.1)	4.9 (0.0)
2-hexyl-5-ethyl-4-methyl	213	1947	1587	tr	1.7 (0.3)	3.6 (1.5)	4.5 (0.6)
2-hexyl-5-ethyl-4-methyl	213	1947	1598	tr	tr	2.1 (1.1)	2.7 (0.1)
2-heptyl-4,5-dimethyl	213	1996	1608	tr	2.1 (0.4)	9.8 (5.2)	13.8 (9.7)
2-heptyl-4,5-dimethyl	213	1996	1617	tr	1.7 (0.3)	8.7 (5.0)	11.8 (9.0)
2-heptyl-4-ethyl	213	2064	1663	tr	tr	2.1 (0.5)	3.2 (1.4)
2-octyl-4-methyl	213	2138	1691	tr	3.8 (1.8)	4.4 (2.4)	20.2 (18.3)
2-heptyl-4-ethyl-5-methyl	227	2012	1673	tr	1.4 (0.2)	3.6 (2.0)	4.3 (1.9)
2-heptyl-4-ethyl-5-methyl	227	2030	1685	tr	tr	2.7 (1.8)	3.4 (1.4)
2-heptyl-5-ethyl-4-methyl	227	2068	1691	tr	tr	2.2 (1.2)	3.2 (1.8)
2-heptyl-5-ethyl-4-methyl	227	2068	1702	tr	tr	1.3 (0.7)	1.7 (1.0)
2-octyl-4,5-dimethyl	227	2108	1713	tr	5.5 (1.9)	25.2 (13.9)	37.9 (36.8)
2-octyl-4,5-dimethyl	227	2108	1723	tr	4.4 (1.5)	20.3 (12.3)	31.8 (30.9)
2-octyl-4-ethyl	227	2175	1770	tr	1.7 (0.9)	4.2 (0.7)	7.7 (5.4)
2-octyl-4-ethyl-5-methyl	241	2121	1778	1.1 (0.4)	3.2 (0.9)	9.1 (5.5)	11.5 (8.3)
2-octyl-4-ethyl-5-methyl	241	2137	1790	tr	2.2 (1.3)	6.9 (4.1)	9.1 (6.5)
2-octyl-5-ethyl-4-methyl	241	2179	1797	tr	1.3 (0.6)	4.7 (3.1)	6.6 (6.1)
2-octyl-5-ethyl-4-methyl	241	2179	1809	tr	1.2 (0.4)	3.4 (2.2)	4.7 (4.4)
2-nonyl-4,5-dimethyl	241	2199	1818	–[d]	–	1.9 (0.4)	2.7 (1.6)
2-nonyl-4,5-dimethyl	241	2199	1829	–	–	1.5 (0.3)	2.3 (1.6)
2-nonyl-4-ethyl-5-methyl	255	>2200	1883	–	–	tr	1.0 (0.4)
2-nonyl-4-ethyl-5-methyl	255	>2200	1895	–	–	tr	tr

[a] All 3-thiazolines substituted in the 5-position were present as pairs of *cis/trans* isomers. [b] Mean values of three replicates with standard deviations shown in parentheses. [c] tr, <1 ng in headspace. [d] Not detected. [e] Reported in yeast extracts by Werkhoff et al. (1991).

Table 2. Thiazoles Identified in the Volatiles of Cooked Beef from Animals Grown on Diets Modified with Different Lipids Supplements

		LRI		amount of thiazole in extract (ng)[a]			
thiazole derivative	MW	BP20	BPX5	control	linseed	fish oil	linseed + fish oil
2-isobutyl-4,5-dimethyl[d,e]	169		1224	tr[b]	tr	tr	tr
2-pentyl-4,5-dimethyl[e,f]	183		1384	tr	–[c]	tr	tr
2-pentyl-4-ethyl-5-methyl	197		1433	tr	–	tr	–
2-hexyl-4,5-dimethyl[e,f]	197		1486	1.1 (0.5)	tr	1.4 (0.7)	tr
2-hexyl-4-ethyl-5-methyl	211		1534	1.1 (0.1)	tr	tr	tr
2-heptyl-4,5-dimethyl[e,f]	211		1588	1.1 (0.4)	tr	1.2 (0.4)	tr
2-heptyl-4-ethyl-5-methyl[e,f]	225		1635	1.1 (0.2)	tr	tr	tr
2-octyl-4,5-dimethyl[e,f]	225		1692	1.6 (0.5)	1.6 (0.2)	2.5 (0.7)	1.5 (0.6)
2-octyl-4-ethyl-5-methyl	239		1737	1.9 (0.3)	2.3 (0.7)	1.8 (1.1)	tr
2-octyl-5-ethyl-4-methyl[e]	239		1762	tr	tr	tr	tr

[a] Mean values of three replicates with standard deviations shown in parentheses. [b] tr, <1 ng in headspace. [c] Not detected. [d] Reported in yeast extracts by Werkhoff et al. (1991). [e] Reported in French-fried potatoes by Ho and Carlin (1989). [f] Reported in fried chicken by Tang et al. (1983).

fed fish oil. The concentrations of 3-thiazolines appeared to correlate with the levels of aldehydes found in the samples from the different treatments.

The mechanisms resulting in increased levels of C_5–C_{10} alkanals in the cooked meat from animals fed fish oil are at present unclear, since the characteristic fish

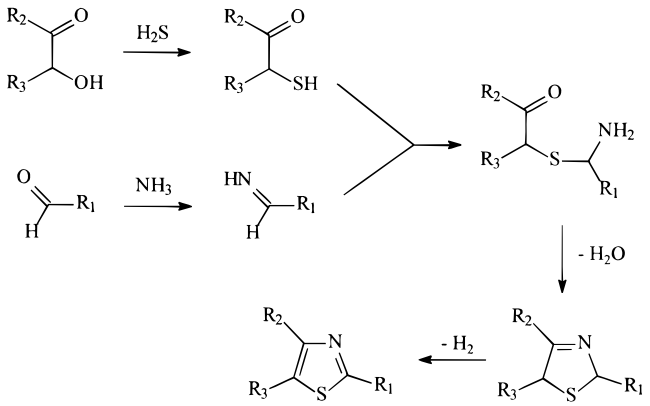


Figure 1. Route to the formation of alkyl-3-thiazolines and alkylthiazoles.

Table 3. Aldehydes Isolated from the Volatiles of Cooked Beef from Animals Grown on Diets Modified with Different Lipids Supplements

		amount of aldehyde in extract[a] (ng)			
aldehyde	LRI BPX5	control	linseed	fish oil	linseed + fish oil
3-methylbutanal	626	833 (9)	987 (29)	3861 (18)	4106 (21)
2-methylbutanal	645	366 (9)	871 (49)	1812 (16)	1742 (32)
pentanal	711	144 (18)	221 (46)	1011 (8)	640 (30)
hexanal	812	212 (4)	527 (9)	1486 (8)	816 (15)
heptanal	913	109 (7)	625 (9)	1843 (12)	1344 (12)
2-ethylhexanal	963	tr[b]	tr	19 (29)	10 (12)
octanal	1015	84 (8)	286 (8)	681 (12)	478 (7)
nonanal	1117	128 (8)	307 (2)	428 (12)	380 (18)
decanal	1218	tr	tr	tr	12 (2)
undecanal	1320	–[c]	tr	tr	tr
(*E*)-2-pentenal	767	–	tr	23 (31)	tr
3-hexenal	818	–	–	tr	–
(*E*)-2-hexenal	867	–	tr	33 (21)	20 (18)
4-heptenal	911	tr	tr	tr	tr
(*E*)-2-heptenal	972	–	tr	62 (23)	45 (8)
(*E*)-2-octenal	1073	–	24 (27)	95 (9)	46 (28)
5-nonenal	1107	–	–	tr	tr
(*Z*)-2-nonenal	1161	–	tr	tr	tr
(*E*)-2-nonenal	1175	tr	22 (27)	109 (11)	73 (18)
(*Z*)-2-decenal	1263	–	–	tr	tr
(*E*)-2-decenal	1278	tr	15 (33)	122 (7)	88 (20)
(*Z*)-2-undecenal	1365	–	–	tr	tr
(*E*)-2-undecenal	1381	–	tr	67 (15)	46 (5)
2,4-heptadienal	1030	–	tr	43 (6)	tr
2,4-octadienal	1130	–	–	tr	tr
2,6-nonadienal	1165	–	–	tr	tr
2,4-nonadienal	1236	–	–	tr	tr
2,4-decadienal	1313	–	tr	tr	11 (5)
2,4-undecadienal	1444	–	–	tr	tr

[a] Mean values of three replicates with standard deviations shown in parentheses. [b] tr, <10 ng in headspace. [c] Not detected.

oil fatty acids are eicosa-5,8,11,12,17-pentaenoic acid and docosa-4,7,10,13,16,19-hexaenoic acid, which will not degrade to these aldehydes. However, free radical propagation during lipid oxidation induced by these highly unstable fatty acids would involve other fatty acids, including oleic acid, which is the major fatty acid in meat.

During the evaluation of the aroma of the volatiles from the meat samples using GC/O, odors were not detected in the regions where the long-chain 3-thiazolines eluted, indicating that these compounds did not possess very low odor threshold values. Certain thiazolines with methyl or acetyl substituents possess thresholds in the low micrograms per kilogram range, but it appears that the larger molecules with the long alkyl substituents do not have such low thresholds. However, the presence of these compounds in meat confirms that interactions between lipid degradation products and the Maillard reaction occur during the cooking of meat. Such reactions may be important in modifying the nature and concentration of the products of the Maillard reaction, thus modifying the aroma from the cooked meat. Increased concentrations of aldehydes, resulting from feeding animals on diets containing fish oil, increased the concentrations of these Maillard interaction products.

ACKNOWLEDGMENT

We are most grateful to N. D. Scollan (Institute of Grassland and Environmental Research, Aberystwyth), who conducted the animal feeding trials.

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Received for review January 21, 1997. Revised manuscript received May 19, 1997. Accepted May 28, 1997.[⊗] We gratefully acknowledge funding for this project from the Ministry of Agriculture, Fisheries and Food, and the assistance of the International Fish Oil and Meal Manufacturers Association, Roche Products Ltd. and Trident Feeds.

JF970066M

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.