Free Fatty Acid Composition of the Adipose Tissue of Intact and Castrated Lambs Slaughtered at 12 and 30 Weeks of Age

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Free fatty acids from adipose tissue of rams and wethers slaughtered at 12 and 30 weeks of age were isolated by a modified Bligh and Dyer method and analyzed by gas chromatography−mass spectrometry. A total of 15 free fatty acids were positively identified. At 12 weeks of age, rams had nonsignificantly higher levels of the flavor-significant compounds, 4-methyloctanoic and 4-methylnonanoic acids, than wethers. At 30 weeks of age, differences in the levels of these acids in rams and wethers became significant and 4-methyloctanoic and 4-methylnonanoic acids increased by 13- and 4-fold, respectively, in the noncastrated animals. Log odor unit values indicated that 4-methyloctanoic acid was present at or above its odor threshold value in rams and wethers of both slaughter ages, but that the value was much higher for 30-week-old rams. 4-Methylnonanoic acid was present below its odor threshold value in all samples.

Keywords: *Branched-chain fatty acids; fatty acids; adipose tissue; lamb; sheepmeat*

INTRODUCTION

Various classes of chemical compounds have been implicated in the characteristic aroma of cooked sheepmeat in general and of meat derived from rams in particular (Sutherland and Ames, 1995). These include branched-chain fatty acids (BCFA), especially 4-methyloctanoic, 4-ethyloctanoic, and 4-methylnonanoic acids (Wong et al., 1975; Brennand, 1989). Wong et al. (1975) identified 40 short- and medium-length chain steam volatile fatty acids from cooked ground mutton. Branched-chain and unsaturated acids with 8−10 carbon atoms were associated with the undesirable flavor. The sweaty odor of the cooked meat was considered to be mainly due to 4-methyloctanoic and 4-methylnonanoic acids. More recently, the role played by BCFA in the flavor of sheepmeat has been thoroughly investigated (Brennand, 1989; Brennand and Lindsay, 1992). In these studies, 4-methyloctanoic and 4-ethyloctanoic acids, both of which possess low odor threshold values, were established as important contributors to the characteristic flavor.

The effect of castration on flavor has been investigated in three sensory studies, (Misock et al., 1976; Crouse et al., 1981; Dransfield et al., 1990), but the impact of castration remains unclear. The age and sexual maturity of the animals at slaughter are factors that need to be considered, as well as castration.

The aim of this study was to compare the levels of fatty acids, particularly branched-chain fatty acids, from the adipose tissue of rams and wethers slaughtered at the two different ages of 12 and 30 weeks. At 12 weeks, the animals were approaching puberty and at 30 weeks, they were sexually mature (Haynes and Schanbacher, 1983).

EXPERIMENTAL PROCEDURES

Materials. Analytical grade solvents were obtained from BDH Chemicals Ltd., Poole, U.K., and authentic fatty acid methyl esters were obtained from Sigma Chemical Company, Poole, U.K. 4-Methyloctanoic and 4-methylnonanoic acids were supplied by Tastemaker, Milton Keynes, U.K.

Methods. *Preparation of Sample.* Suffolk twin male lambs (one of each pair castrated within 24 h of birth and the other left intact) were used. They were reared under exactly the same conditions prior to slaughter at 12 or 30 weeks of age. Adipose tissue was obtained from the leg area of each carcass. Thus, adipose tissue from a total of four treatments was examined: intact animals slaughtered at 12 and 30 weeks of age and castrated animals slaughtered at 12 and 30 weeks of age. All the adipose tissue (400−500 g) from each of 10 animals from each treatment group was removed and stored under reduced pressure in oxygen impermeable bags at −32 °C. Before analysis, all the adipose tissue was ground to a particle diameter of 6 mm and thoroughly mixed, and three samples were taken. Each of these samples was analyzed in triplicate, giving a total of nine measurements for each treatment. Further details are given in Sutherland and Ames (1995).

Free fatty acids were isolated from each sample of adipose tissue by a modified Bligh and Dyer method (Hornstein and Crowe, 1960). The fatty acids were derivatized to give their corresponding methyl esters, and heptadecanoic acid was used as the internal standard to obtain quantitative data. Blank extracts were prepared without adipose tissue.

Gas Chromatography−Mass Spectrometry (GC-MS). A Hewlett Packard HP 5890 Series II Gas Chromatograph (Hewlett-Packard, Bracknell, U.K.) was used to separate fatty acids. The GC columns used were wall coated open tubular (WCOT), bonded phase, fused silica capillary columns coated with CP WAX (25 m × 0.32 mm i.d. × 1.0 μm film, Chrompak UK Ltd., London, U.K.). Split-splitless injection was used and the injection volume was 1 μL. The carrier gas was helium at 1.5 mL/min. The initial column temperature was held at 40 °C for 1 min followed by an increase of 4 °C/min to 250 °C. The temperature was held at 250 °C for a further 20 min, by which time all components had eluted. For each treatment, triplicate samples were analyzed. Separated volatile components of the samples were identified with a HP 5988 mass spectrometer equipped with a HP 59970 GC-MS workstation (revision 3.2) system (Hewlett-Packard). The operating parameters of the mass spectrometer in electron-impact (EI) mode were as follows: ionization voltage, 70 eV; ionization current, 100 μA; source temperature, 250 °C; accelerating voltage, 4 kV; resolution, 1000; and scan speed, 1 s/decade (repetitive throughout the run). Identifications were made with the mass spectrometer data system library and by comparison of the experimental spectra with other collections

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Table 1. Fatty Acids Identified in Intact and Castrated Lambs Slaughtered at 12 and 30 Weeks of Age

fatty acid	ref LRI[a,b]	exptl LRI[a]	mg/kg adipose tissue [c,d] castrate 12-week-old	castrate 30-week-old	intact 12-week-old	intact 30-week-old
2 (or 3-) methylbutanoic acid	991	995	nd	8.82×10^{-2} (0.78)	2.10×10^{-1} (0.36)	4.41×10^{-1} (1.00)
2-methylpentanoic acid	1088	1091	nd	7.78×10^{-1} (1.13)	nd	nd
pentanoic acid	1069	1066	9.05×10^{-2} (0.95)	2.20 (0.95)	9.05×10^{-1} (1.31)	2.59 (1.48)
hexanoic acid	1172	1168	3.00 (1.87)	1.73×10 (1.62)	3.46 (1.85)	3.81×10 (1.36)
heptanoic acid	1273	1267	4.58×10 (1.58)	7.08×10 (1.80)	4.06×10 (2.66)	4.44×10^2 (1.11)
octanoic acid	1375	1384	7.66×10 (1.45)	8.5×10^2 (2.35)	5.95×10 (2.37)	8.71×10^2 (2.00)
4-methyloctanoic acid	1389	1392	2.85 (0.70)	3.89 (1.00)	3.79 (1.40)	5.03×10 (1.01)
nonanoic acid	1476	1469	1.50×10^2 (0.07)	2.54×10^2 (0.09)	1.76×10^2 (0.64)	2.40×10^2 (2.47)
4-methylnonanoic acid	1488	1499	8.72×10^{-2} (0.97)	3.49×10^{-1} (1.2)	3.48×10^{-1} (0.65)	1.40 (0.90)
decanoic acid	1578	1579	trace (0.02)	9.67×10^{-3} (0.08)	2.41×10^{-2} (0.21)	3.23×10^{-1} (1.31)
undecanoic acid	1669	1662	trace (0.02)	3.00×10^{-3} (0.03)	1.50×10^{-1} (1.02)	1.50×10^{-1} (1.73)
dodecanoic acid	1785	1794	trace (0.03)	6.30×10^{-3} (0.01)	7.00×10^{-4} (0.03)	3.50×10^{-3} (0.05)
tetradecanoic acid	1991	1989	8.67×10^{-3} (1.52)	trace (0.02)	6.18×10^{-3} (0.06)	6.19×10^{-2} (0.30)
pentadecanoic acid	2085	2083	2.87 (0.06)	3.11 (0.31)	1.18×10^{-4} (0.15)	3.74×10 (0.33)
hexadecanoic acid	2177	2179	1.12×10 (0.89)	4.44 (0.92)	1.67 (0.50)	3.52×10 (0.91)
total			2.93×10^2	1.21×10^3	2.87×10^2	1.72×10^3

[a] LRI values for the methyl esters are quoted. [b] Laboratory database for reference compounds or the LRI determined with the authenic compound. [c] The fatty acid value (mg/kg adipose tissue) was calculated from the amount of fatty acid methyl ester: mass of fatty acid (mg/kg adipose tissue) = rel. mol. mass of the fatty acid/rel. mol. mass of the FAME × amount of FAME present (mg/kg adipose tissue). [d] The average values of three replicate samples; the standard deviations are given in parentheses; trace = fatty acid present at a level below 1.00×10^{-5} mg/kg adipose tissue; nd = fatty acid could not be detected in this sample (detection limit 6×10^{-6} mg/kg adipose tissue).

of reference spectra. Quantitative data were obtained from the mass spectral integration report.

Linear Retention Indices (LRI). Identifications were confirmed for components relative to a solution of standard alkanes run under the same GC-MS conditions. LRI values were compared with those of authentic compounds.

Statistical Analysis. Statistical evaluation of the data was performed by analysis of variance (ANOVA) using Microsoft Excel version 4.0 software.

RESULTS AND DISCUSSION

The fatty acids identified by combined GC-MS, together with the amount of each component present, are summarized in Table 1. Reference LRI values confirmed the identities of the compounds in Table 1. A total of 15 free fatty acids, including 4 BCFA, were positively identified. All the fatty acids, apart from 2-methylpentanoic acid, have previously been reported as components of sheepmeat (Wong et al., 1975; Lorenz et al., 1983; Suzuki et al., 1985; Brennand, 1989). Chromatograms of blank isolates had six minor peaks due to volatile impurities that are not included in Table 1.

Differences were observed among the total amounts of fatty acids in the adipose tissue of the four samples (see Table 1). Levels of total fatty acids identified in rams and wethers slaughtered at 12 weeks of age were almost the same, whereas at a slaughter age of 30 weeks, the level of total fatty acids in rams was ~1.5-fold higher than in wethers. In contrast, amounts of total fatty acids increased by 4 and 6 times, respectively, for wethers and rams on increasing the slaughter age to 30 weeks.

Differences in levels of some individual fatty acids were observed with castration. When the samples from intact and castrated animals slaughtered at 12 weeks of age were compared, levels of 4-methyloctanoic and 4-methylnonanoic acids increased by 1.3- and 4-fold, respectively, in the rams, but these differences were not significant. When the age at slaughter was increased to 30 weeks, levels of these two acids increased significantly, by 13- and 4-fold, respectively, in the rams (see Tables 1 and 2). The *F* value for 4-methyloctanoic acid is particularly high at 6.46×10^7 for 30-week-old rams and wethers. Significantly higher levels of several straight-chain acids were also present in the 30-week-old intact animals.

Slaughter age also influenced levels of individual fatty acids. 4-Methylnonanoic acid increased 4-fold in both wethers and rams on increasing the slaughter age from 12 to 30 weeks (see Table 1). In contrast, levels of 4-methyloctanoic acid increased 13-fold in the older rams but only 1.3-fold in the older wethers (see Table 1). Differences in these acids with slaughter age were only significant for the intact animals. Levels of 4-methyl branched-chain acids were lowest in the samples from castrated animals slaughtered at 12 weeks and highest in samples from intact animals slaughtered at 30 weeks, with the values for the samples from intact animals slaughtered at 12 weeks and castrated animals slaughtered at 30 weeks being broadly similar. Significantly higher levels of several straight-chain acids were observed in both intact and castrated animals on increasing the slaughter age from 12 to 30 weeks.

Far more important than the levels of the free fatty acids in the adipose tissue of the different treatments are the effects of the free fatty acids on flavor. The odor threshold of a fatty acid in water depends on the pH of the medium, because only the protonated form is volatile. The adipose tissue used in this study had a pH of 6.5. Amoore et al. (1968), Baldwin et al. (1973), and Brennand et al. (1989) determined odor threshold values of several fatty acids in water. Brennand et al. (1989) and Baldwin et al. (1973) used pH values of 2.0 and 6.0, respectively. Amoore et al. (1968) did not specify the pH but, presumably, it was between 4.0 and 5.0. Many fatty acids have a pK_a in the region of 4.8 (Weast and Astle, 1980); that is, at this pH, 50% of the acid exists in the undissociated form. By applying the Henderson−Hasselbalch equation, it is possible to calculate the percentage of fatty acid that would exist in the undissociated form at other pH values. Thus, at pH values of 2, 5.2, and 6.0, 99.8, 29, and 0.007%, respectively, of the acid is present in the volatile,

Table 2. Statistical Analysis of Free Fatty Acids in Lamb Adipose Tissue

	castrate 12 vs castrate 30[a,b]		castrate 12 vs intact 12 [a,c]		castrate 30 vs intact 30[a,d]		intact 12 vs intact 30 [a,e]	
fatty acid	*F* value	p^f	*F* value	p^f	*F* value	p^f	*F* value	p^f
2 or 3-methylbutanoic acid		nd		nd	7.72	ns	3.91×10^{-1}	ns
2-methylpentanoic acid		nd		nd		nd		nd
pentanoic acid	7.00	ns	7.6×10^{-1}	ns	1.64	ns	2.95×10^{2}	**
hexanoic acid	1.00×10^{2}	***	9.20×10^{-2}	ns	1.92×10^{4}	***	1.50×10^{4}	***
heptanoic acid	3.27×10^{2}	***	8.48	*	8.78×10^{5}	***	2.03×10^{5}	***
octanoic acid	2.35×10^{5}	***	1.14×10^{2}	***	1.08×10^{4}	***	1.44×10^{7}	***
4-methyloctanoic acid	2.18	ns	1.08	ns	6.46×10^{7}	***	4.27×10^{4}	***
nonanoic acid	2.50×10^{6}	***	4.89×10^{3}	***	1.04×10^{2}	**	3.67×10^{3}	***
4-methylnonanoic acid	8.60×10^{-2}	ns	1.50×10^{-1}	ns	3.70×10	*	5.30×10	*
decanoic acid	4.10×10^{-2}	ns	3.90×10^{-2}	ns	1.95×10^{-1}	ns	2.22×10^{-1}	ns
undecanoic acid	2.10×10^{-2}	ns	6.50×10^{-2}	ns	2.20×10^{-2}	ns	0.00	ns
dodecanoic acid	1.19×10^{-1}	ns	1.00×10^{-3}	ns	1.50×10^{-2}	ns	5.90×10^{-2}	ns
tetradecanoic acid	0.00	ns	8.04×10^{-6}	ns	1.47×10^{-1}	ns	1.62×10^{-1}	ns
pentadecanoic acid	1.73	ns	9.47×10^{2}	***	8.82×10^{6}	***	1.30×10^{5}	***
hexadecanoic acid	8.40×10	***	2.61×10^{2}	***	2.84×10^{7}	***	2.01×10^{4}	***

[a] One-way ANOVA. [b] Castrated 12-week-old compared with castrated 30-week-old. [c] Castrated 12-week-old compared with intact 12-week-old. [d] Castrated 30-week-old compared with intact 30-week-old. [e] Intact 12-week-old compared with intact 30-week-old. [f] ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; nd = fatty acid could not be detected in one of the samples.

Table 3. Theoretical Odor Thresholds at pH 6.0 and Log Odor Unit Values for Fatty Acids Identified in Adipose Tissue of Intact and Castrated Lambs

		theoretical log odor unit value[b]			
		castrate		intact	
fatty acid	theoretical odor threshold at pH 6.0[a] (mg/kg water)	12-week-old	30-week-old	12-week-old	30-week-old
2-methylbutanoic acid	456	—[c]	−3.71	−3.34	−3.01
3-methylbutanoic acid	9.98	—[c]	−2.05	−1.68	−1.35
2-methylpentanoic acid	1682	—[c]	−3.33	—[c]	—[c]
pentanoic acid	157	−3.24	−1.85	−2.24	−1.78
hexanoic acid	657*	−2.34	−1.58	−2.28	−1.24
heptanoic acid	39.9	0.06	0.25	0.01	1.05
octanoic acid	1357*	−1.25	−0.20	−1.36	−0.19
4-methyloctanoic acid	2.85	0.00	0.14	0.12	1.25
nonanoic acid	342	−0.36	−0.13	−0.29	−0.15
4-methylnonanoic acid	92.7	−3.03	−2.42	−2.43	−1.82
decanoic acid	157*	—[c]	−4.21	−3.81	−2.69
undecanoic acid	14.3	—[c]	−3.68	−1.98	−1.98
dodecanoic acid	—[d]				
tetradecanoic acid	—[d]				
pentadecanoic acid	—[d]				
hexadecanoic acid	—[d]				

[a] Values were calculated by the Henderson−Hasselbalch equation with experimental odor threshold data from Brennand et al. (1989), except for values marked by * in which data from Amoore et al. (1968) were used. [b] Values calculated according to Guadagni et al. (1972). [c] Compound not detected or detected at trace levels in this sample. [d] Odor threshold data not available.

protonated form in aqueous solution. By assuming that the pH of the water used in the study of Amoore et al. (1968) was 4.8, and using the data of Brennand et al. (1989) and Baldwin et al. (1973), it was possible to calculate the odor threshold values of all the fatty acids identified at pH 6.0 (representative of adipose tissue). The theoretical odor threshold value of each acid at pH 6.0 and its theoretical log odor unit value in each sample of adipose tissue is shown in Table 3. Lipid comprises 71.8% of adipose tissue (Paul and Southgate, 1978), and the acids would be expected to partition between the aqueous and lipid phases, with a greater proportion of each acid being present in the lipid phase with increasing acid hydrophobicity. Therefore, although the log odor unit values in Table 3 provide an indication of the relative contribution of each fatty acid to the aroma of each sample of adipose tissue, they should be interpreted with caution.

From these data, it seems that only heptanoic and 4-methyloctanoic acids are present in sufficiently high levels to contribute to the aromas of the adipose tissue (i.e., they possess positive log odor unit values). However, it would seem reasonable for the fatty acids in a sample to act synergistically and thus to contribute to the total aroma. Overall, log odor unit values are lowest for the tissue samples from castrated animals slaughtered at 12 weeks and highest for the tissue samples from intact animals slaughtered at 30 weeks. Any synergistic effects would thus be greatest for this latter sample.

4-Methyloctanoic acid is of particular interest. It had a very high log odor unit value in all four samples, implying that it made an important contribution to the aroma. The related compound, 4-methylnonanoic acid, was also present in each sample, but below its odor threshold value. Both these branched chain acids have been associated with the sweaty odor of cooked mutton, which is described as “soo” by the Chinese (Wong et al., 1975). Brennand et al. (1989) described the odor of 4-methyloctanoic acid as waxy and goaty at 1 mg/kg and as goaty-muttony at 25 mg/kg. At a level of 1 mg/kg, 4-methylnonanoic acid was described as waxy-sweet, soapy, fatty and acid-like, but the odor was modified to muttony, wet wood, and fatty at 25 mg/kg. More recently, Brennand (1989) and Brennand and Lindsay (1992) studied the role of fatty acids in sheepmeat

aroma and concluded that 4-methyloctanoic acid, which has a "mutton-like" odor, gives the backbone to the typical flavor of sheepmeat, with 4-methylnonanoic acid playing a lesser role, because of its higher odor threshold value (see Table 3). 4-Ethyloctanoic acid is another branched-chain acid associated with "sweaty" and the species-specific notes of mutton (Brennand, 1989). It was not isolated in detectable quantities in the current study. It is more usually associated with older animals and has been isolated in much higher levels from mature sheep (rams and ewes) than lambs by previous workers (Ha and Lindsay, 1990).

The significantly higher levels of some straight-chain fatty acids present in the adipose tissue of 30-week-old rams compared with those in rams slaughtered at 12 weeks and wethers slaughtered at 30 weeks may also contribute to the typical sheepmeat flavor. For example, octanoic acid has been described as goaty at 50 mg/kg (Brennand et al., 1989) and, when comparing intact animals slaughtered at 12 and 30 weeks of age, the F value was very high at 1.44×10^7 (see Table 2).

The results of this study demonstrate that although castration has no significant effect on the levels of BCFA when the animals are slaughtered at 12 weeks of age (before sexual maturity), when slaughtering is performed at 30 weeks (when animals are sexually mature), the levels of 4-methyloctanoic and 4-methylnonanoic acids and many of the straight-chain acids are significantly increased in noncastrated animals. These significant differences may be expected to increase the undesirable sweaty, goaty-muttony odor of the tissue from the older animals.

Fatty acids are formed in the rumen of sheep by the action of microorganisms (Henderson, 1990), and the specific metabolic processes that occur result in the formation of methyl-substituted BCFA (Mottram, 1991). The results of this study show that castration affects fatty acid levels. In particular, levels of 4-methyloctanoic acid are significantly higher in sexually mature rams compared with wethers of the same age or immature rams. Rams possess higher levels of testosterone than wethers (Henderson, 1990), and it seems that this hormone may influence their formation. 4-Methyloctanoic and 4-methylnonanoic acids, as well as some of the straight-chain acids, possess sweaty odors, so it is possible that they act as semiochemicals (sexual attractants), that could account for their presence at higher levels in mature rams.

ABBREVIATIONS USED

ANOVA, analysis of variance; BCFA, branched-chain fatty acids; GC-MS, gas chromatography−mass spectrometry; LRI, linear retention indices.

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