

Effect of Stress-Related Changes in Sheepmeat Ultimate pH on Cooked Odor and Flavor

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In this study sheepmeat with a moderate or high ultimate pH (mean pH 6.26 and 6.81, respectively), which was induced by preslaughter adrenaline injection, had a significantly lower overall cooking odor and flavor intensity, as assessed by a trained sensory panel, than sheepmeat of a more acceptable pH (5.66). Panelists also found that desirable odor and flavor notes decreased and undesirable ones increased as ultimate pH increased. Purge and trap gas chromatography/mass spectrometry of fat rendered during cooking identified 57 (of a total of 325) volatile compounds that significantly decreased in concentration with increasing pH. Aldehydes were the most common compounds identified. Gas chromatography/olfactometry identified 54 odor-active compounds. Ten of these were also found to be responsive to changes in meat ultimate pH. Most of these compounds were aldehydes. Therefore, these results show that sheepmeat odor and flavor are dramatically affected by elevated meat pH.

Keywords: Meat ultimate pH; flavor; odor; aldehydes; GC/MS

INTRODUCTION

The ultimate pH of meat (pH at rigor) is governed by the animal's preslaughter reserves of muscle glycogen. After death, the muscle breaks down glycogen via the anaerobic glycolytic pathway to produce lactic acid. Increased lactic acid is responsible for lowering muscle pH. If the animal's glycogen reserves were depleted preslaughter, for example by stress or exercise, insufficient lactic acid is produced to lower the pH of the muscle to its normal value, around pH 5.6 (Devine and Chrystall, 1989). Elevated pH affects several meat characteristics, including appearance and water-holding capacity. In its extreme, high ultimate pH meat is called DFD because of its dark, firm, and dry appearance.

At pH values above 5.8 the keeping quality of fresh chilled meat is adversely affected because of altered bacterial growth due to the lower content of glucose and lactic acid and the pH itself (Gill and Newton, 1981). The resulting reduction in the shelf life of high pH meat makes it unsuitable for trade in vacuum-packed fresh meat. Elevated ultimate pH can also affect eating quality, particularly tenderness (Howard and Lawrie, 1956; Hedrick *et al.*, 1961). As the ultimate pH increases from 5.5 to 6.0 the tenderness of cooked meat decreases, and above pH 6.0 the effect is reversed (Devine *et al.*, 1993). Moreover, it has also been suggested that high ultimate pH affects cooked beef flavor (Lawrie, 1985; Purchas *et al.*, 1986).

Young *et al.* (1993) compared odor and flavor differences between Coopworth and Merino lambs grazed on similar pasture, and unexpectedly found a species-related pH effect. They suggested that pH, rather than breed, might be the dominant factor affecting cooked meat odor and flavor characteristics. Panelists also registered several negative flavor and odor descriptors for the high pH meat. This result agrees with the finding of Dutson *et al.* (1981) that high-pH beef evokes negative flavor reactions by panelists. Other sensory studies show that high-pH beef is less flavorful, disliked by panelists (Dransfield, 1981; Purchas *et al.*, 1986), and

has more off-flavors than normal-pH beef (Fjelkner-Modig and Ruderus, 1983).

Studies to date have concentrated only on sensory effects of high ultimate meat pH on cooked meat flavor. Instrumental measurement of quantitative and qualitative chemical changes in cooking odor caused by high ultimate pH have been overlooked by most flavor researchers. In a brief report, Park and Murray (1975) noted large differences in the semiquantitative composition of the steam-volatile fraction from normal- and high-pH meat. No mention was made of the species tested or types of compounds involved.

Surveys have revealed that the ultimate pH of beef and sheep slaughtered in New Zealand can be variable, with some pH values being quite high. This variability and the importance of assured production of meat of good eating quality warrant detailed investigation into the effect that pH has on cooked meat odor and flavor.

In this investigation, preslaughter injections of adrenaline (epinephrine) were administered to sheep to produce carcasses of low, medium and high meat ultimate pH. The effect of this chemically induced preslaughter stress on the cooking odor and flavor of sheepmeat was assessed by a trained sensory panel, and cooking odor was evaluated by gas chromatography/mass spectrometry (GC/MS) and gas chromatography/olfactometry (GC/O). Data sets were statistically compared to help identify volatile compounds that contributed to the changes in cooked meat odor and flavor as influenced by meat ultimate pH.

MATERIALS AND METHODS

Animals. Fifty Coopworth female lambs from one flock, raised on a predominantly ryegrass and clover pasture, weighing on average 35 kg (range 29–44 kg), were held in a separate paddock near an abattoir with free access to pasture and water for 3 days before treatment. On each of 5 consecutive days, 10 randomly selected animals were removed from pasture and placed in an indoor holding pen. These animals then received two subcutaneous doses (at 17 and 3 h before slaughter) totaling between 0 and 0.3 mg of adrenaline kg⁻¹ of live weight.

Ethical approval for this study was given by the Ruakura Agricultural Centre Ethical Committee.

Animals were conventionally slaughtered by throat cut after a head-only stun. Carcasses, none of which were electrically stimulated, were held above 6 °C for 6 h followed by 22 h at 3 °C. Carcasses were then removed from cold storage, and the m. semimembranosus from the left leg of each animal was excised and stored in a permeable plastic bag (Cryovac, W. R. Grace Ltd.) with an oxygen transmission rate (OTR) of 3500 mL⁻¹ m⁻² 24 h⁻¹ at 1 atm, 23 °C, and 75% relative humidity. A portion of subcutaneous fat taken from above the m. longissimus dorsi, to the depth of the muscle, along the length of the back of the same carcass was included with each leg muscle. Samples were stored at -35 °C for 8 weeks until evaluation by a trained sensory panel.

Sample Preparation. The ultimate pH was measured on each carcass 28 h postslaughter by homogenizing a 1-g sample of m. longissimus dorsi in 10 mL of 4 mM sodium iodoacetate at pH 7 (Devine and Chrystall, 1989). From these results, 30 of the original 50 semimembranosus muscles were selected to give three distinct groups of 10 samples from carcasses with nonoverlapping pH values. The mean pH was 5.6 for the low-pH group, 6.0 for the medium-pH group, and 6.6 for the high-pH group. Immediately before sensory analysis, a sample of each minced m. semimembranosus was tested in triplicate for pH as described above.

For sensory analysis, the whole semimembranosus from each carcass was tempered to -5 °C over 2 h, diced, and passed twice through a 3-mm plate mincer with enough diced backfat from the same animal to produce a mince with a 20% (w/w) fat content [calculated on the basis of an estimated lean fat content of 5% (w/w) and the added weight of backfat]. To minimize lipid oxidation, samples were prepared less than 1 h before cooking and kept at 4 °C in sealed low O₂ permeability plastic bags (Cryovac) with a stated OTR of 30 mL⁻¹ m⁻² 24 h⁻¹ at 1 atm, 23 °C, and 75% relative humidity. Samples were cooked to an internal temperature of 75 °C (measured by a temperature probe) by placing the minced meat into stainless steel beakers that were positioned in a water bath at 100 °C. Minces were stirred regularly, with individual spoons, to ensure even cooking.

After cooking, the rendered fat and broth from each sample were carefully poured into glass beakers. The separated fat was removed for instrumental analysis, and the remaining broth was quickly returned to the cooked mince. The mixture was then reheated for about 1 min before presentation to panelists.

The fat samples were held at 60 °C and centrifuged for 2 min at 2000 rpm to separate any remaining lean tissue and water from the fat. The clear supernatants were transferred to glass vials with Teflon-coated screw caps and flushed with high-purity nitrogen. After the screw caps had been tightly sealed, vials were placed in gas-impermeable foil-laminated bags (Borden NZ Ltd., Auckland, New Zealand), which were vacuum packed, placed in a second foil-laminated bag and vacuum packed again, and then stored at -35 °C until instrumental analysis.

Sensory Analysis. *Selection and Training.* Selection and training of panelists were done principally as described by Winger and Pope (1981) for the sensory evaluation of meat flavors. Selected panelists were further trained for the present study on two occasions (3 and 4 days) before the first of 5 consecutive daily evaluation sessions. Training samples for overall and foreign flavor and odor intensity were prepared from a low-pH (5.6) and a high-pH (6.8) meat sample as described below for the evaluation samples.

Training samples for sheepmeat flavor and odor intensity were derived by mixing various proportions of mince meat known to vary in sheepmeat intensity. Intensity differences were discussed and defined among the group of panelists. Foreign odor and flavor were identified by panelists as an attribute considered not normally present in sheepmeat of acceptable eating quality. Individual self-generated odor and flavor descriptors were also discussed, and a consensus of significant descriptors was made.

Sample Evaluation. In any one daily session, hot samples (about 20 g) of cooked mince, from two randomly selected samples from each of the three pH groups, were transferred

from the stainless steel beakers to small (50 mL) screw-capped glass jars placed in a holding waterbath set at 70 °C. The samples, coded with three-digit random numbers, were immediately served fully randomized to each booth, one at a time. The order of panelists for each booth was changed each session. A reference sample of low-pH semimembranosus meat (5.60), obtained from the carcass of an animal from the original flock but not part of the treatment groups, was also included at each session. Apple juice, flat Coca-Cola, and dry crackers were presented between samples to clean the palate. Evaluations were made in individual positive air-pressured sensory booths at 22 °C and under subdued red lighting to mask any possible variation in meat color.

The 12 panelists were asked to score for overall odor, sheepmeat odor, and foreign odor immediately after the screw cap was removed from the jar. They were then asked to remove samples from each jar with individual clean spoons and score for overall flavor, sheepmeat flavor, and foreign flavor. All attributes were scored on a scale of 0–100: 0 signified no odor or flavor and 100, extreme. Panelists were also asked to record self-generated descriptors of the odors and flavors.

Instrumental Analysis of Volatile Compounds. *GC/MS.* Fat samples were melted by placing vials in 60 °C water for a brief time. Two grams (± 0.005 g) of melted fat was placed into the bottom of a 50-mL (25 × 150 mm) clean glass purge vessel fitted with a ground-glass stopper joint. An internal standard consisting of 2.05 μ g of 2-octanone in 5 μ L of pentane was rapidly injected into the fat, and the tube was stoppered, mixed, and left to equilibrate at room temperature for 5 min. A glass nitrogen gas purge tube was then positioned 5 mm above the surface of the fat, and a Tenax TA collection trap (200 mg in a 150 mm × 4 mm i.d. glass tube plugged at each end with silylated glass wool) was attached to the outlet of the purge tube. The Tenax trap had been preconditioned for 10 min at 260 °C with a helium flow of 20 mL min⁻¹. The purge vessel was immersed in a glycerol bath maintained at 100 (± 0.01) °C. Instrument grade purge nitrogen was passed through molecular sieve and activated charcoal filters (Alltech) and a tube filled with Tenax TA (200 mg) before finally passing over the fat surface.

Volatile compounds generated from the heated fat were dynamically purged from the headspace above the sample by a flow (60 mL min⁻¹) of this high-purity nitrogen gas for 30 min and collected on the Tenax TA.

The glass purge and trap assembly was checked for contamination by purging without a fat sample, using the sampling conditions just described. Also, no volatile compounds, as detected by smell and GC/MS analysis of a second trap attached in series, broke through the collection trap when fat samples were analyzed.

The volatile compounds were then thermally desorbed at 250 °C for 10 min under a flow (20 mL min⁻¹) of redirected gas chromatograph helium carrier gas onto the head of a cryogenically cooled (-10 °C) DB5-MS capillary column (30 m × 0.25 mm, 1.0- μ m film thickness) housed in a Fisons 8000 GC. The chromatography conditions were as follows: injector temperature, 260 °C; split flow, 64 mL min⁻¹; column head pressure, 82.7 kPa; split ratio of 32:1 and column flow of 2.0 mL min⁻¹ (measured at -10 °C); temperature program, -10 °C for 10 min, raised to 40 °C at a rate of 50 °C min⁻¹, held for 5 min, raised to 150 °C at 3 °C min⁻¹, and then to 280 °C at 6 °C min⁻¹ with a final hold time of 5 min.

The capillary column was connected to a Fisons MD 800 mass spectrometer with a transfer line temperature of 280 °C and source temperature of 200 °C. Mass spectra were generated at 70 eV and a detector setting of 350 V. Data were recorded from 40 to 350 mass range by MASSLAB integration software (Fisons) in the total ion monitoring mode, and spectra were compared with an NIST mass spectral data base supplied with MASSLAB. In nearly all cases samples were analyzed in duplicate.

GC/O. Three grams of fat rendered from each of six animals per pH group was pooled and stored at -35 °C until analysis. Not all 10 animals from each pH group were sampled because four samples from one pH group were

compromised due to accidental procedural loss at this stage of the experiment. To ensure balanced sampling between each pH group, six randomly selected samples from each of the remaining two pH groups were selected for pooling. Tenax TA traps containing volatile compounds from duplicate 2 ± 0.005 g aliquots of these pooled samples, prepared without internal standard, were thermally desorbed onto a 30 m \times 0.53 mm, 1.0- μ m film DB5 capillary column. The chromatographic conditions were similar to those for the GC/MS analysis, except that the initial temperature was -40 °C to aid retention of low boiling point compounds on the larger diameter (0.53 mm as opposed to the GC/MS 0.25 mm) column, and the initial ramp to 40 °C was at 70 °C min^{-1} . The column head pressure was 34.5 kPa and the split flow was 28 mL min^{-1} to give a split ratio of 2.4:1 and column flow of 11.8 mL min^{-1} measured at -40 °C. The effluent from the end of the capillary column was split 1:1 between a flame ionization detector (FID) and the olfactometer (SGE, Australia).

The odors emitting from the column were sniffed by the author (who has extensive experience in this technique) after being combined with a flow of humidified air (near 100% relative humidity at 22 °C). Retention times of the odors were recorded by push button that sent an electronic signal to a Maxima integration software package. The button remained depressed for as long as the author could detect the specific odor. Odor port evaluation was carried out for the first 60 min of the run. The author also recorded a descriptor for the odor and scored its intensity on a 9-point hedonic scale: 1 was minimal odor and 9 was extreme.

Data Analysis. For GC/MS data, all peaks were integrated and peak areas were normalized to the internal standard, 2-octanone, to remove analytical variation of the purge and trap and GC/MS steps, and expressed as total ion peak areas. All samples were analyzed at least once and in most cases in duplicate. Eight of the 30 samples were analyzed only once either due to insufficient sample or because of MS malfunction.

Compounds were identified by comparison of peak mass spectra with those in the NIST MS data base (supplied with MASSLAB), Kovats retention indices (Kovats and Keulemans, 1964), and, when available, authentic standards. Peak identification of GC/O and GC/FID was done by overlaying FID and olfactometer signal traces and using Kovats indices. Comparison between GC/MS data and GC/FID/O data was done using Kovats retention indices. Olfactometer signals were interpreted as the area under the step/impulse response "curve" using Maxima integration software. Corresponding "area counts" represented a combination of nonlinear odor intensity and odor duration and are hereinafter referred to as "odor responses". These values give only an approximate relative intensity of each odor.

The residual maximum likelihood (REML) routine in the GENSTAT (U.K.) statistical software was used to interpret sensory panel data and \log_{10} -transformed GC/MS chromatography peak area counts normalized to the internal standard. \log_{10} transformation of GC/MS data was preferred, as preliminary data analysis indicated a skewed data set. The REML routine adjusts for possible imbalance of data, caused by missing data, across multiple levels of variation (Patterson and Thompson, 1971). In this study REML accounted for variation among carcasses, replicate thermal desorptions, pH and sensory panelists and session. Mean \log_{10} area counts for each of the pH groups were then back-transformed to geometric means and converted to concentrations (nanograms per gram of fat) by using the known concentration of the added 2-octanone internal standard (see Table 4).

An unsupervised pattern recognition multivariate analysis (principal component analysis, Unistat, Ltd., U.K.) was also done to seek to explain the maximum proportion of variance of the collective relationship of all volatile compounds as affected by meat ultimate pH (data from Tables 4 and 5). For an in-depth explanation of principal component analysis, the reader should refer to Zervos and Albert (1992) or Manly (1994).

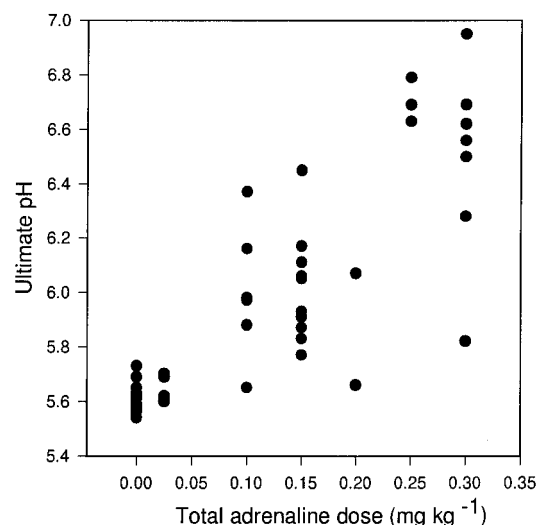


Figure 1. Effect of total adrenaline dose on meat ultimate pH. Each point represents one animal.

Table 1. pH Values for Longissimus Dorsi (LD) and Semimembranosus (SM) Muscles for Selected Carcass Groups ($N = 10$ for Each Group)

	low pH		medium pH		high pH	
	LD	SM	LD	SM	LD	SM
mean	5.56	5.66	6.07	6.26	6.69	6.81
max	5.68	5.71	6.40	6.36	7.07	6.98
min	5.44	5.60	5.75	6.13	6.30	6.45
SD	0.08	0.03	0.21	0.07	0.22	0.15

Table 2. Summary of Semimembranosus Weight and Rendered Fat Weight of Selected Subgroups ($n = 10$ for Each Group)

	low pH		medium pH		high pH	
	muscle (g)	rendered fat (g)	muscle (g)	rendered fat (g)	muscle (g)	rendered fat (g)
mean	359	12.4	347	12.4	327	11.5
max	467	20.4	443	23.9	383	19.3
min	292	5.6	302	7.0	283	6.6
SD	48	5.5	43	5.5	26	4.2

RESULTS AND DISCUSSION

Ultimate pH and Sampling. The wide range of ultimate pH values generated by adrenaline injection (Figure 1) is consistent with results of others (Hedrick *et al.*, 1961; Watanabe *et al.*, 1996) who have attempted to generate a range of pH values.

For the three groups of 10 carcasses with nonoverlapping ultimate pH values, selected using the m. longissimus dorsi pH values, subsequent analysis of semimembranosus muscles gave mean pH values of 5.66 for the low group, 6.26 for the medium group, and 6.81 for the high group (Table 1). The mean pH values for the semimembranosus muscles were about 0.2 unit higher than for the corresponding longissimus dorsi, a difference that represents normal intermuscle pH variation (Talmant *et al.*, 1986). Raw m. semimembranosus weights for each pH group covered a wide range, and although mean weights decreased with increasing pH, the differences were not significant ($P > 0.2$). The amount of rendered fat recovered after cooking also covered a wide weight range since each sample was prepared to a constant fat concentration. No difference ($P > 0.9$) was observed in rendered fat recovered among the pH groups (Table 2), nor was there any correlation between pH and semimembranosus weights in the original 50 animals treated with adrenaline.

Table 3. Mean Sensory Panel Intensity Scores for Cooked Semimembranosus Mince from Animals in Three pH Groups^a

	low (pH 5.66)	medium (pH 6.26)	high (pH 6.81)	low vs medium	low vs high	medium vs high
overall odor	51.1	44.8	39.8	*	***	*
overall flavor	50.3	41.7	27.0	*	***	**
sheepmeat odor	28.5	26.9	25.9	NS	NS	NS
sheepmeat flavor	38.1	34.8	21.7	NS	**	**
foreign odor	21.9	19.6	16.6	NS	*	NS
foreign flavor	13.4	14.8	16.0	NS	NS	NS

^a Samples were scored on a scale of 0–100: 0 signified none and 100, extreme. NS, not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

On average, 12.1 g of rendered fat was recovered from the gently strained mince. This represented about 20% (w/w) of the fat content of the raw mince, after allowing for the moisture content (25%) of the added backfat. Samples of mince presented to sensory panelists would therefore contain close to 16% (w/w) fat—sufficient to maintain adequate mouth feel and carry a significant fraction of the fat-derived flavor and odor compounds.

Sensory Analysis. Table 3 shows the intensity score means and levels of statistical significance among the pH groups. The panelists found that overall odor and flavor decreased significantly as pH increased. The change was significant among the three pH groups ($P < 0.05$) but was most obvious between the Low and High pH groups ($P < 0.001$). Panelists could not detect a change in sheepmeat odor between any of the groups, but did detect a decrease in sheepmeat flavor between the low and high and the medium and high groups ($P < 0.01$). A smaller decrease ($P < 0.05$) in foreign odor was observed between the low- and high-pH groups only. There was no significant difference in foreign flavor among any of the groups.

The observation by the sensory panel that overall flavor decreased with increasing pH agrees with similar work on beef (Dransfield, 1981; Fjelkner-Modig and Ruderus, 1983) and pork (Buscailhon *et al.*, 1994). The most frequent descriptors from panelists' comments on odors and flavors are shown graphically, by frequency of occurrence, in "radar" plots of odors and flavors (Figure 2). These plots show a general movement of emphasis from desirable descriptors to undesirable descriptors as pH increased. For example, the frequency of bland/flat/low and stale/musty flavor descriptors, considered undesirable attributes of cooked meat, increased as pH increased. Others (Dutson *et al.*, 1981; Fjelkner-Modig and Ruderus, 1983) have also found an increase in negative or off-flavors in high-pH beef. At the same time, strong, beefy, meaty, and sweet flavor descriptors, considered desirable attributes, decreased.

As pH increased, foreign odor scores decreased ($P < 0.05$), suggesting that whatever compounds were responsible, they were not the same as those responsible for undesirable odor notes such as metallic and stale/musty. Although panelists comments have not been subjected to rigorous statistical analysis, these descriptor plots show a general movement of emphasis from desirable descriptors to undesirable descriptors as pH increased. These results should not go unreported as they do represent the perception of a number of panelists. In retrospect, it would have been useful for the panelists to have also scored for changes in all descriptors. However, giving panelists too many attributes to consider can confuse and jeopardize the integrity of the sensory session. Young *et al.* (1993) compared odor and flavor differences between Coopworth and Merino lambs grazed on similar pasture and noted that panelists' descriptors for Coopworth samples (mean pH 5.77) were

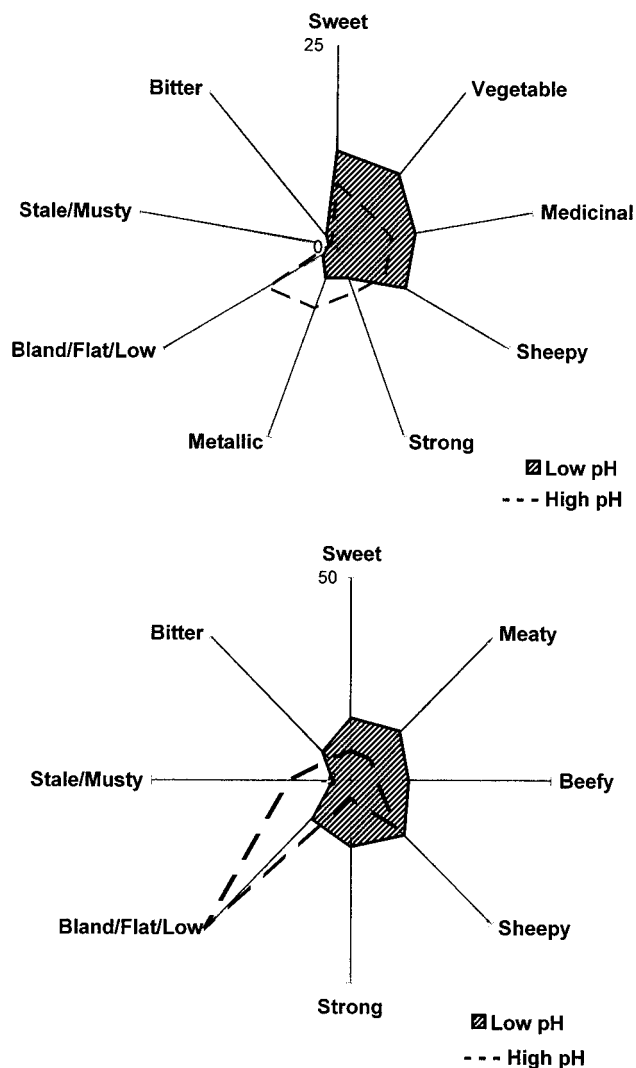


Figure 2. Frequency of odor descriptors (top) and frequency of flavor descriptors (bottom) for low (shaded area) and high (dashed line) ultimate pH sheepmeat.

also dominated by beefy, sweet, and big-flavored, whereas bland, fishy/stale/rancid, grassy, and bloody descriptors dominated in Merino samples (mean pH 6.16).

GC/MS Analysis. In this dynamic headspace analysis, thermal desorption of the Tenax TA traps eluted more than 300 compounds that were recorded as total ion chromatogram peaks by GC/MS for each sample of rendered fat. Generally, total volatile compound concentration decreased with increasing pH. Summation of total mean area counts for each sample showed a relative reduction from 100 for the low-pH group to 80 and 75 for the medium- and high-pH groups, respectively.

Twenty-eight compounds decreased ($P < 0.05$, most cases $P < 0.001$) among all sample groups as pH

Table 4. Mean Approximate Concentrations (Nanograms per Gram of Rendered Fat) of Compounds That Changed Significantly from Low (L) to Medium (M), Low to High (H), and Medium to High pH Groups^a

peak	Kovats index	compound	ng g ⁻¹			significance		
			L	M	H	L vs M	L vs H	M vs H
Aldehydes								
28	715	pentanal	293	160	121	***	***	*
155	1103	nonanal	277	112	91	***	***	*
102	963	benzaldehyde	120	48	28	***	***	***
117	1004	octanal	92	39	30	***	***	***
193	1265	(E)-2-decenal	48	15	9	***	***	**
140	1058	(E)-2-octenal	41	15	9	***	***	***
219	1379	2-undecenal	28	7	4	***	***	***
120	1012	(E,E)-2,4-heptadienal	23	14	9	***	***	***
208	1330	2,4 decadienal	21	5	2.5	***	***	***
69	852	(E)-2-hexenal	13	6.3	4.3	***	***	*
203	1302	(E,E)-2,4 decadienal	11	2.9	1.7	***	***	***
16	672	(E)-2-butenal	7	3.3	2	***	***	*
Alcohols								
109	980	1-octen-3-ol	170	56	25	***	***	***
110	968	heptanol	97	153	50	NS	NS	**
22	702	1-penten-3-ol	95	36	16	***	***	***
144	1068	1-octanol	44	22	16	***	***	**
142	1065	2-octenol	37	12	5	***	***	***
46	774	2-penten-1-ol	19	6	2.9	***	***	***
158	1112	2-phenylethanol	8	4.4	2.9	***	***	*
131	1033	2-phenylmethanol	6	3.4	2.3	***	***	*
47	778	3-penten-2-ol	2.9	3.7	1.9	NS	NS	***
Acids								
14	631	acetic acid	278	224	187	NS	*	NS
48	782	butanoic acid	23	29	18	NS	NS	*
141	1063	heptanoic acid	17	7	2.5	***	***	***
Alkanes								
237	1473	2,6,10,14-tetramethylheptadecane	127	96	135	*	NS	(**)
246	1512	pentadecane	39	29	35	*	NS	NS
259	1606	hexadecane	19	14	18	*	NS	(*)
204	1306	tridecane	13	6.3	4.3	***	***	*
229	1414	a methylalkane	12	7.6	8.9	**	NS	NS
239	1484	a methylalkane	5	3.3	4.5	*	NS	NS
Alkenes								
265	1645	unknown (57, 97, 55, 111)	3	2.4	3.7	*	NS	(**)
271	1687	unknown alkene	3	1.8	2.3	***	NS	NS
285	1783	2,6,10,14-tetramethyl-2-hexadecene	224	161	255	*	NS	(**)
Ketones								
143	1067	1-phenylethanone	103	85	70	NS	**	NS
201	1297	2-undecanone	20	14	15	*	NS	NS
Miscellaneous								
258	1599	a phthalate	10	7.6	9	*	NS	NS
45	772	unknown (42, 55, 41, 70)	142	63	40	***	***	***
55	800	unknown (59, 80)	94	50	25	***	***	***
73	867	unknown (56, 55, 43, 69)	60	27	16	***	***	***
43	762	unknown (55, 83, 84, 41)	16	8.9	5.3	***	***	***
183	1217	unknown (43, 88, 99, 144)	11	7.8	5.8	**	***	*
54	797	unknown (83, 55, 98)	8	5.4	6.8	*	NS	NS
93	923	unknown (57, 41, 59, 81)	8	4.7	3.5	NS	*	NS
146	1077	unknown (68, 81, 119, 134)	7	3.8	2.9	***	***	*
251	1536	unknown (124, 137, 55, 180)	6	4	2.6	*	***	*
175	1189	unknown (43, 58, 70, 83, 97)	3.2	2.6	2	NS	*	NS
27	713	unknown (86, 57)	2.7	2.5	1.8	NS	*	NS
275	1704	unknown (57, 71, 43, 97)	3	2	3	**	NS	NS

^a MS identifications were by comparison with the NIST MS database and Kovats retention indices. Numbers in parentheses refer to the principal ions observed, in decreasing order of intensity, for unidentified compounds. Asterisks represent significant decreases (increases) in concentration among pH groups. NS, not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$.

increased (Table 4). Of this group of compounds, 12 were identified as aldehydes, 7 were alcohols, 1 was an alkane, 1 was a fatty acid, and 7 remain unidentified.

In addition, nine compounds (one ketone, five alkanes, and three unknowns) decreased in concentration ($P < 0.05$) from the low- to medium-pH group, three compounds (two alcohols and one fatty acid) decreased ($P < 0.05$) from the medium- to high-pH group, and five compounds (one ketone, one acid, and three unknowns) decreased ($P < 0.05$) from the low- to high-pH group.

Most interestingly, only four compounds (two alkanes

and two alkenes) showed an increase ($P < 0.02$) in concentration with increasing pH among any of the treatment groups. Hydrocarbons have high odor thresholds and are not considered significant contributors to meat odor (Shahidi *et al.*, 1986). These compounds therefore do not explain the panelist's observations that the frequency of occurrence of undesirable odor descriptors increased as pH increased. It may be that the purge and trap GC/MS technique as used here lacks the sensitivity to detect odors that possibly are in the parts per billion range but are still above the odor threshold.

Table 5. Compounds Specifically Targeted* with Mean Approximate Concentrations (Nanograms per Gram of Rendered Fat) of Those Compounds That Changed Significantly from Low (L) to Medium (M), Low to High (H), and Medium to High pH Groups^a

Kovats index	compound	ng g ⁻¹			significance		
		L	M	H	L vs M	L vs H	M vs H
1050	2-methylphenol	1.1	0.73	0.5	***	***	*
1070	4-methylphenol	2.8	1.5	1.3	***	***	NS
1223	unknown (135, 150, 121) phenol ?	0.34	0.08	0.04	***	***	***
1019	2-acetylthiazole	0.85	0.42	0.44	*	*	NS
774	2-methylthiophene	0.33	0.24	0.32	NS	NS	NS
991	2-pentylfuran	5.1	1.3	0.61	***	***	***
1089	2-hexylfuran	0.9	0.31	0.26	***	***	NS
751	dimethyl disulfide	0.82	2.4	0.84	NS	NS	*
985	dimethyl trisulfide	0.8	2.4	0.71	(*)	NS	***

^a MS identifications were by comparison with the NIST MS database and Kovats retention indices. Numbers in parentheses for unknown compounds refer to the principal ions observed, in decreasing order of intensity. Asterisks represent significant decreases (increases) in concentration between pH groups. NS, not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$. Compounds specifically targeted but not found in any of the samples: (phenols) thio-, 2-ethyl-, 3,4-dimethyl-, 2,4-dimethyl-, 2-isopropyl-, 2,4,6-trimethyl-, 4-isopropyl-, 2-methyl-5-(1-methylethyl)-, 5-methyl-2-(1-methyl ethyl)-; (pyrazines) methyl-, 2,5-dimethyl-, 2,6-dimethyl-, 2,3-dimethyl-, 2-ethyl-(5)methyl-, 2-ethyl-(6)methyl-, 2-ethyl-3,6-dimethyl-, 2,3-diethyl-5-methyl-, ethyl-, ethenyl-, 2-ethyl-3,5-dimethyl-; (thiazoles) 4,5-dimethyl-, 2,4-dimethyl-; (thiophenes) 2,4-dimethyl-, 3-ethyl-, 5-methyl-2-thiophene carboxyaldehyde, 3-methyl-2-thiophenecarboxyaldehyde, 3-methyl-, 2,3-diformyl-; (furanones) 2(5H), dihydro-2-methyl-3(2H)-, dihydro-5-methyl-3(2H)-, dihydro-3-methyl-2(3H)-; (furans) 2-methyl-, 2-ethyl-, 3-pentyl-, 3-phenyl-, 3-heptyl-, carboxyaldehyde (furfural), 2,5-dihydro-2,5-dimethyl-, 2,5-dihydro-3-methyl-, 2,3-dihydro-5-methyl-, 2,3-dihydro-4-methyl-, 2,3-dihydro-3-methyl-, methylthio-, 2-methylfuranthiol, 2-furanylmethanethiol sulfide, dimethyl-

Alternatively, favorable odor notes present in low-pH meat may mask less desirable notes and only when these masking odors are reduced—due to an increase in meat pH—do the undesirable odors dominate (St. Angelo *et al.*, 1987).

Of the identified volatile compounds that decreased significantly as pH increased (Table 4), the aldehydes and alcohols were the main contributors (42%), as expressed by compound class, to the significant changes in concentration among all three pH groups ($P < 0.001$ in at least two and in most cases in all three pH group comparisons). These compounds are generated from the oxidation of lipid-derived fatty acids at cooking temperatures. Many have distinct odors, such as the French fry aroma associated with 2,4-decadienals and the "green" or "grassy" aroma attributed to hexanal. They also play a major role in the Maillard reaction (Reineccius, 1994). The aldehydes 2,4-decadienal, (*E,E*)-2,4-decadienal, nonanal, and 2-undecenal, which were more prominent in low-pH samples, were identified by Gasser and Grosch (1990), using an aroma dilution technique, as being highly significant contributors to the odor of meat broths.

Changes in the concentration of volatile compounds detected by GC/MS reflect the sensory panelists' perceptions (Table 3) and are consistent with biochemical changes expected to occur in postrigor muscle. Proteolysis and lipolysis operate more favorably at lower pH (Buscaillon *et al.*, 1994). These hydrolytic reactions may produce the vital precursors required for generation of odor and flavor compounds produced during cooking. Alternatively, the greater water-holding capacity of high-pH meat may influence the release of volatile compounds and affect flavor perception (Lawrie, 1985).

Madruga and Mottram (1995) observed increases in a number of volatile heterocyclic compounds in cooked meat (also thought to contribute to meaty flavor), when they titrated meat from pH 5.6 to 4.0 before cooking. They also found that the total number of volatile compounds increased as the pH decreased.

In this study, 44 heterocyclic compounds [including those observed by Madruga and Mottram (1995)] were specifically targeted using Kovats indices and quantified using the mass spectral FIND routine within the MASSLAB (Table 5). Only 2-pentylfuran, 2-hexylfuran,

2-methylthiophene, and 2-acetylthiazole were observed in cooked sheepmeat volatiles. All of these, except 2-methylthiophene, showed a significant decrease with increasing pH.

The low incidence of volatile heterocyclics may be due to the mild cooking conditions employed here. Ground meat was cooked for only 10 ± 1 min to a final temperature of 75 °C in a beaker immersed in a 100 °C waterbath. No significant browning, known to aid the production of Maillard reaction products, occurred. MacLeod and Ames (1986), Spanier *et al.*, (1990) and Drumm and Spanier (1991) found an increase in the production of heterocyclic and sulfur compounds as cooking temperatures and times increased.

Volatile compounds thought to contribute to the species-related odor and flavor of cooked sheepmeat include branched-chain fatty acids (BCFAs) (Wong *et al.*, 1975a) and a number of alkylphenols (Ha and Lindsay, 1991). Two branched-chain fatty acids, 4-methyloctanoic acid and 4-methylnonanoic acid, responsible for sheepmeat odor (Wong *et al.*, 1975b; Ha and Lindsay, 1990; Young and Berdague, 1996) could not be detected in any of the samples under these conditions. The absence of branched-chain fatty acids is probably due a combination of the mild cooking temperature, the short purge and trap time, the lower volatility of these acids, and the fact that they are present in low concentrations (Ha and Lindsay, 1990), particularly in leg muscles (Brennand and Lindsay, 1992a).

In this experiment, mean sensory scores for sheepmeat odor decreased with increasing pH, but the decrease was not statistically significant. In contrast, the decrease in sheepmeat flavor was significant ($P < 0.01$) between low- and high-pH and between medium- and high-pH groups. These results can be explained by the mild cooking conditions employed. When comparing volatiles from boiled, fried, or roasted mutton collected during cooking, Brennand and Lindsay (1992b) found that a lesser concentration of volatile free fatty acids (VFFA) was collected from the headspace of boiled and fried mutton than from the headspace of roasted mutton. One explanation given was that the former two methods had shorter cooking times and the surface roasting temperature was much higher. These workers

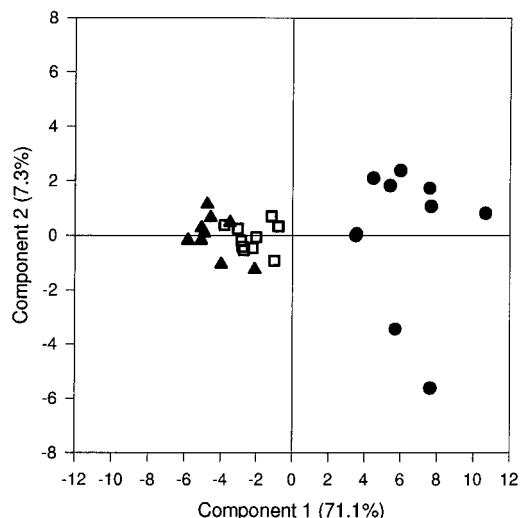


Figure 3. Principal component analysis used to discriminate animals on the basis of volatile compounds. Only compounds that decreased in concentration among all three pH groups were used in the data set: low pH group (●); medium pH group (□); high pH group (▲). Values in parentheses represent the percent of variance explained by each principal component.

also observed higher concentrations of VFFA in rendered fat from fried samples than in rendered fat from boiled mutton, for which a large portion of the VFFA could dissolve in the aqueous phase. Thus, under the mild cooking conditions used in this experiment, it is probable that the BCFAs were not completely liberated into the fat fraction but mostly were dissolved and retained in the aqueous meat broth. This would explain the absence of BCFAs in the trapped volatiles and account for the changes in sheepmeat flavor as observed by panelists. Lower pH conditions would favor liberation of the BCFAs from their parent triacylglycerols. The BCFAs would enter the aqueous phase and be detected as flavor.

Only three of the phenols listed in Table 5 [2-methylphenol, 3(or 4)-methylphenol (*m*- or *p*-cresol), and an unknown phenol (MW = 150)], were detected, and all showed a decrease in concentration with increasing pH. *p*-Cresol (2-methylphenol) has been implicated as a contributor to muttoney/sheepyard aroma of cooked sheep meat at low (ppb) concentrations (Ha and Lindsay, 1991).

Principal Component Analysis. PCA was performed on a restricted data set derived from GC/MS data of all compounds that showed a significant decrease in concentration with increasing meat ultimate pH. A plot of the principal components (Figure 3) shows that 78% of the variability is accounted for in the first two components. Component 1 explains 71% of the variability and shows that the low-pH samples are well discriminated from the medium- and high-pH groups. This shows that increases in pH, from that of the low-pH group (5.6–5.7) to the medium (6.1–6.4) and high (6.5–7.0) groups, had a marked effect on the production of cooked meat volatile compounds, even under mild cooking conditions. Component 2 reveals a degree of variability within the low-pH group that is less evident in the other two groups. Principal component analysis of the same data transformed to \log_{10} (plot not shown) reduced the spread of data points in the low-pH group but had the opposite effect on the high-pH group. It also explained more of the variability in the first two components (75.7 and 4.8% for components 1 and 2, respectively). This suggests that another factor, inde-

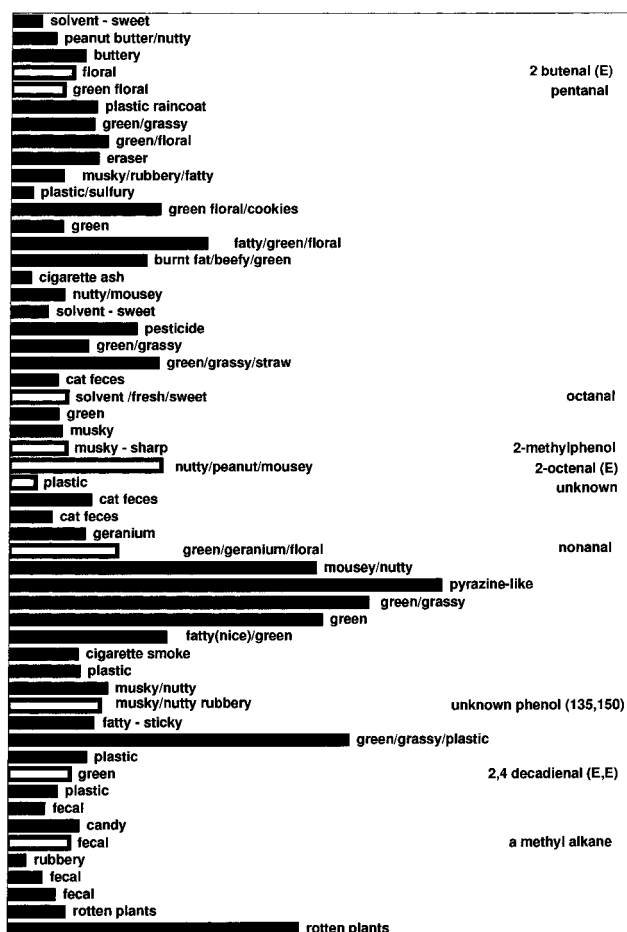


Figure 4. GC/odor responses and their descriptors of odor-active compounds identified in a composite rendered fat sample from the low pH meat group. Compounds are listed in order of increasing retention time. Those compounds labeled on the right of the figure were also found to change significantly with meat pH. For full identifications and retention indices see Table 6.

pendent of pH, may affect volatile compound production. No improvement was found for discrimination between the medium- and high-pH groups

GC/O. Figure 4 shows the odor response for a combined composite sample of rendered fat from the low-pH group. No significant difference was observed in odor responses among the pH groups (data not shown), probably because these values were derived by a very subjective method (a single observer and low number of replicates). Relative potencies of each odorous compound would be better estimated by other, more quantitative techniques (Acree *et al.*, 1984; Ullrich and Grosch, 1987). However, qualitatively, 10 of the 54 odorous compounds identified (Table 6) were also some of the compounds found by GC/MS to decrease with increasing pH. Six of these were aldehydes. This confirms that these compounds contribute to the odor intensity changes due to pH and also confirms proposals (Gasser and Grosch, 1990; Reineccius, 1994) that they play a major role in cooked meat odor.

A number of aldehydes have been shown to be responsible for rancidity changes in cooked meat flavor (St. Angelo *et al.*, 1987). Samples in this experiment were stored in such a way that rancidity onset is not considered a significant contributor to the changes in aldehyde concentrations observed. The results suggest that, at certain concentrations, some aliphatic aldehydes play a major role in the development of a favorable

Table 6. Odor Descriptors and Tentative MS Identification of Odorous Volatile Compounds Eluting from the Gas Chromatograph/Olfactometer for a Mixed Sample of Rendered Fat (Low pH Group)^a

Kovats index	odor descriptor	tentative identification
513	sweet solvent	cyclopentane
560	peanut butter/nutty	unknown (56, 57, 41)
590	buttery	2,3-butanedione
648	floral	(E)-2-butenal
682	green floral	pentanal
735	plastic raincoat	unknown (55, 83, 84)
792	green/grassy	hexanal
796	green/floral	hexanal
810	eraser	ND
812	musky/rubbery/fatty	ND
816	plastic/sulfury	1,3-octadiene
866	green floral/cooked cookies	ND
882	green	2- or 3-heptanone
897	fatty/green/floral	4-heptenal
901	burnt fat/beefy/green, lingers	heptanal
913	cigarette ash	3-methylthiopropional
918	nutty/mousey	dihydro-2(3)-furanone
949	solvent/sweet	propylbenzene
968	pesticide	phenol
978	green grassy	2,3-octanedione
981	green/ketoney/grassy, straw	2,3-octanedione
987	cat feces	ND
1000	solventlike, fresh/sweet	octanal
1011	green	2,4-heptadienal
1033	musky	benzyl alcohol
1045	musky, sharp	2-methylphenol
1052	nutty/peanut/mousey	(E)-2-octenal
1069	plastic	ND
1080	cat feces	dimethylbenzyl alcohol
1083	cat feces	ND
1094	geranium	3-hexene-2,5-diol
1097	green/geranium, strong/floral	nonanal
1100	mousey/nutty	unknown (124)
1115	pyrazine-like, lingers	unknown
1136	green/grassy, lingers	2-nonenal (E or Z)
1153	green	ethylbenzaldehyde
1171	slightly fatty (nice) green	unknown (127, 57, 43)
1204	cigarette smoke	unknown
1211	plastic	unknown (43, 88, 99, 144)
1213	musky/nutty (pyrazine?)	unknown
1226	musky/nutty/rubbery, lingers	unknown phenol (135, 150)
1245	fatty, sticky	nonanoic acid
1248	green/grassy/plastic	(Z)-2-decenal
1312	plastic	2,6,7-trimethyldecane
1315	green	(E,E)-2,4-decadienal
1335	plastic	ND
1423	fecal	ND
1425	candies	ND
1475	fecal	a methylalkane
1488	rubbery	cyclopentadecane
1509	fecal	unknown
1516	fecal	unknown
1524	rotten plants	unknown
1587	rotten plants	unknown

^a Relative odor responses for each of the compounds are shown in Figure 4. ND, peak not detected by MS. MS identifications were by comparison with the NIST MS database and Kovats retention indices. Numbers in parentheses for unknown compounds refer to the principal ions reliably observed, in decreasing order of intensity.

cooked sheepmeat odor and flavor. Similar observations have been reported for cooked chicken (Ho and Chen, 1994).

Conclusions. This study demonstrated that volatile compounds isolated by headspace analysis from cooked meat and fat change quantitatively with pH and that these changes are consistent with changes observed by sensory panelists. Many of these compounds are pro-

duced from the oxidation of lipids during cooking and are considered to be important contributors to meat odor and flavor. The findings indicate that even moderate increases in meat ultimate pH from 5.6 to 6.3 significantly alter the quality and quantity of odors and flavors of cooked sheepmeat. This conclusion can almost certainly be extended to meat of other species.

As with taste, odors in mixtures tend to suppress each other and it is difficult to identify the qualities of individual components that make up the mixture (Laing, 1994). GC/O/MS provides us with the tools to separate these mixtures and identify compounds that are most influential in the determination of food odors and flavors. A more detailed analysis of the odors produced during cooking using these and similar techniques will go a long way toward helping us to understand the intricate nature of food odors and flavors.

Current work is concentrating on the effects of manipulating high ultimate pH meat to lower its pH level and studying changes in volatile compounds and their precursor compounds that are thought to be responsible for odor and flavor changes at high ultimate pH.

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