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Prepared by: P. Watkins and R. Warner

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Compounds associated with consumer acceptability of the flavour of lamb meat.

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Executive summary

Tenderness, sheep meat flavour, overall liking and cooking odour are regarded as important contributors to sheep meat eating quality. For cooking odour, two aromas have often been associated with cooked sheepmeat. The first aroma, known as 'mutton' flavour, is generally related to an animal's age while the second, known as 'pastoral' flavour, is related to an animal's diet.

Mutton flavour, regarded as the characteristic flavour associated with the cooked meat of older animals, becomes more pronounced as the meat is being cooked and has been cited as one of the historical reasons that sheepmeat consumption has been low in some markets. Branched chain fatty acids (BCFAs) are the chemical compounds that are accepted as the main contributors for the aroma and research continues to explore the role of these compounds and their contribution to 'mutton' odour.

'Pastoral' flavour is found with cooking the meat of pasture fed ruminants, and pasture fed animals predominantly represent the majority of the Australian domestic sheep flock, with cereal supplementation usually used in times of drought. This would imply that the pastoral note is already present in Australian sheepmeat with local consumers accustomed to the flavour of the cooked product. 3-Methylindole, also involved with 'boar' taint in pigs, and, to a lesser extent, *p*-cresol are the compounds implicated as contributors to 'pastoral' flavour.

The Co-operative Centre for Sheep Industry Innovation (Sheep CRC) has been conducting research aimed at understanding the links between a range of selected phenotypes and animal genetics and part of this work has included evaluating cooked meat product using consumer sensory panels with Meat Standards Australia protocols. To date, no study has been made which determines what, if any, relationship exists between consumer sensory attributes and the compounds responsible for 'pastoral' and 'mutton' flavours in sheepmeat. In this work, 180 sheep fat samples, representing 3 sire types and 2 sites, were selected from a cohort of 770 made available from the Sheep CRC. Three BCFAs as well as *p*-cresol and 3-methylindole were measured in each sample and statistical analysis used to identify the relationship between chemical content and three consumer sensory attributes. Two BCFAs, 4-methyloctanoic and 4-ethyloctanoic acids, were found to impact on the consumer 'Like Smell' attribute. A higher consumer acceptance of the grilled meat was found for fat samples that contained lower concentrations of these compounds. No other significant relationship was found between the other attributes and the chemical content. It is recommended that the contribution of these compounds to the variability in acceptance of sheepmeat in Australia undergo further investigation.

A review of 'Sheepmeat flavour and the effect of diet on eating quality' has been conducted and is included. The review describes flavour perception, influence of various forages and supplements on cooked sheepmeat flavour and acceptability, and the volatiles generated during cooking which are known to be associated with flavour. Condensed tannins are present in some forages and the influence of these

on pastoral flavour is discussed. The analytical techniques for detection of the volatiles and other compounds contributing to taste and aroma are presented, including gas chromatography olfactometry. The possible influence of texture and fat content of sheepmeat on the temporal flavour release in the mouth is reviewed. Finally, consumer perspectives of sheepmeat and processing techniques to ameliorate sheepmeat flavour are discussed.

It is recommended that further data should be collected. In particular, the linking of trained taste panel data with consumer data, and with chemical analysis of a greater range of compounds than those reported here is recommended. This would allow the compounds contributing to the unfavourable, and favourable, variations in consumer acceptability in domestic and export markets to be quantified. This would also allow the development of possible strategies for ameliorating any unfavourable flavours in sheepmeat. This would potentially enable the tailoring of sheepmeat and sheepmeat products to specific markets.

Contents

1 Background

In addition to tenderness, sheep meat flavour, overall liking and cooking odour are regarded as important components of sheep meat eating quality (Pleasants et al. 2005; Pethick et al. 2006). For sheep, two aromas have been associated with the cooked meat of the animal. The first aroma, known as 'mutton' flavour, is related to an animal's age while the second, known as 'pastoral' flavour, is related to an animal's diet. Mutton flavour, regarded as the characteristic flavour associated with the cooked meat of older animals, becomes more pronounced as the meat is being cooked (Young and Braggins 1998). The presence of this particular note has been cited as one of the reasons historically that sheepmeat consumption has been low in some markets (Sink and Caporaso 1977). The Chinese also have a special word, '*soo*' (meaning sweaty or sour), that describes what is regarded by these consumers as a disagreeable aroma, and is often associated with cooking sheepmeat (Wong et al. 1975). A range of fatty acids in cooked mutton fat have been reported to be responsible for this odour (Wong et al. 1975). Principally, branched chain fatty acids (BCFAs) were the compounds believed to be responsible for this odour and subsequent research has continued to elucidate the role of these compounds and their contribution to 'mutton' odour.

The 'pastoral' flavour, which has been described as 'grassy' (Young and Braggins 1998), results from cooking the meat of pasture fed ruminants (Berry et al. 1980). There is little (if any) evidence to suggest that this note can be found with grain fed animals (Berry et al. 1980). Traditionally, Australian sheep have been pasture fed with little supplementary feeding, except in times of drought when cereal and legume grains are used to supplement depleted pastures (Ashes and Rich 1987). The use of pasture for feeding Australian sheep implies that the pastoral note may be present in the cooked meat of these animals. If so then there is the risk that meat from these animals may not be palatable to consumers more accustomed to meat from grain fed sheep. This more likely to be the case with consumers in export markets as recent work has shown that Australian consumers were unable to distinguish between grilled meat taken from lambs finished on pasture or concentrated pelleted feeding systems (Pethick et al., 2005).

In a study by Watkins et al (2010a), concentrations of three BCFAs (4-methyloctanoic (MOA), 4-ethyloctanoic (EOA) and 4-methylnonanoic (MNA) acids) were measured in a survey of fat samples taken from 533 sheep carcases at abattoirs in New South Wales, Victoria and Western Australia. Watkins et al. (2010a) showed that the type of pasture the animals were grazing prior to slaughter had a significant influence on the levels of these compounds. Sheep grazing lucerne, native pasture and saltbush had up to twice the levels of MOA and EOA in their muscle, compared to sheep grazing pasture, pasture with supplement or consuming grain. To date, the relationship between these compounds and consumer scores for the acceptability of flavour has not been established and so there is scope to investigate what relationships, if any, do exist.

2 Project objectives

Objectives: To identify compounds associated with the consumer acceptability of the flavour of lamb meat from finishing systems in southern Australia.

Outcomes/Deliverables: Identification and documentation of the most important flavour compounds associated with variation in consumer panel responses to the flavour of lamb in Australia.

3 Personnel involved in the project, including review

- Peter Watkins, Robyn Warner, Tanoj Singh, Damian Franks and Conor Delahunty, CSIRO Food and Nutritional Sciences, Werribee and North Ryde
- Gavin Rose, David Allen and Joanne Bui from Department of Primary Industries, Victoria
- Gavin Kearney, statistical consultant, Hamilton, Victoria
- Owen Young, Auckland University, New Zealand

4 Analysis of MSA consumer data in relation to chemical compounds

4.1 Materials and methods

4.1.1 Fat samples

Meat and fat samples from Murdoch University and the University of New England (UNE) were delivered to CSIRO Division of Food and Nutritional Science (CFNS) at Werribee, Vic. These samples had been removed from carcasses from the Information Nucleus flock of the Cooperative Research Centre for Sheep Industry Innovation (Sheep CRC), in 2010. The meat had been assessed by MSA consumer panels for the Sheep CRC and the consumer sensory data was made available to us for this study. The original intent of this study was to conduct an initial analysis of a small sub-set of samples, measuring the mass fractions of MOA, EOA, MNA, *p*-cresol and 3-methylindole in both meat and fat samples. This would allow determination of which tissue was suitable for analysis. Previous studies had used the fat samples but we wanted to determine whether measurements on meat or fat were better related to the consumer panel scores associated with the respective sample. Unfortunately, most of the meat samples intended for Murdoch University and UNE has been lost by the respective abattoirs, and thus were not available for this study. Consequently, the analysis was performed on the fat samples. In addition, the levels of these analytes are usually too low in meat (in comparison to fat) to be detected by the analytical methods. Thus, the analysis was performed on the fat in lieu of the corresponding meat sample, and we made the assumption that the levels in the fat samples will be proportionally related to those in the meat.

Fat samples were then selected across the range of the mean consumer flavor scores for the loin portion, according to sire type (Terminal, Maternal or Merino) and

abattoir site (Tamworth and Katanning). The fat portions were delivered to DPI-Victoria (Werribee) where it was rendered molten using a microwave oven. A portion of the molten fat was used for BCFA analysis at DPI-Victoria, and the remainder was stored at -20 °C. For the analysis of *p*-cresol and 3-methylindole, the samples were collected from DPI-Victoria (Werribee) and transferred to CFNS (Werribee) for storage at -20 °C until required for analysis.

4.1.2 Measurement of branched chain fatty acids

The samples were used as received and wholly melted by heating in a microwave oven to ensure homogeneity. A sample of the liquid fat (1 g) was injected into a Unitrex sweep co-distillation unit (SGE, Ringwood) and heated at 200 °C for 1 hr under a flow (200 mL/min) of nitrogen. Each batch of ten samples included one spiked recovery fat sample containing the internal standard, undecanoic acid (1.00 µg/mL). The released compounds were purged through the Unitrex unit and collected onto a trap. The trap, consisting of Tenax®, a glass wool plug and sodium sulphate, was eluted with 5 mL diethyl ether:hexane (20:80). The organic phase was concentrated to 1 mL and, after the addition of the internal standard (1.00 µg/mL), the sample was treated with (*N*,*O*)-bisilyltrifluoroacetamide at 60 °C for 30 min and the BCFAs were derivatised as the trimethylsilyl (TMS) ester of the acids.

The fatty acid-TMS esters were separated by injection (1 µL) onto a DB5-MS fused silica capillary column (J&W, 30m x 0.25 mm i.d. x 2.5 µm film thickness) in a Varian 3400 gas chromatograph (GC) and detected by a Saturn 2000 ion trap mass spectrometer operating in full scan mode. The septumless programmable injector (SPI) was programmed starting at 45 ^{o}C and increased to 325 ^{o}C at a rate of 180 ^{o}C/min. The GC oven was held at 75 °C for 2 min then increased to 300 °C at a rate of 10 °C/min and held at this temperature for 8 min. Helium was used as the carrier gas at a constant pressure of 105 kPa. The mass spectrometer (MS) transfer line was 280 °C. Mass spectra were acquired using an ion source temperature of 220 °C and an electron multiplier voltage of 2400 V. The mass spectrometer was calibrated using FC43 (Varian, Inc., Springvale).

Quantitation of the BCFAs was performed using the Varian Saturn Workstation 2000 software. For calibration, the standards were in the range of 0.02 to 1.00 µg/mL (or mg/kg effective concentration in sheep fat) and the standard solutions were derivatised using (N,O)-bisilyltrifluoroacetamide at 60 °C for 30 min. The following ions were used for quantitation; MOA-TMS ester, *m*/*z* = 215.0, EOA-TMS ester, *m*/*z* = 229.0, MNA-TMS ester, *m*/*z* = 229.0 and the internal standard, C_{11} FA-TMS ester, *m*/*z* = 243.0, respectively. The concentrations were determined using external quantitation and the standard solutions were in the range of 0.02 to 1.00 mg/kg. Calculation of the concentration for a given BCFA was made using:

$$[\text{BCFA}]\ (\text{mg kg}^{-1}) = k.\ A_{\text{BCFAsample}} / A_{\text{ISsample}}$$

where *k* is the slope of a linear calibration curve with intercept set to zero, $A_{\text{BCFAsample}}$ is the peak area of the BCFA in the sample and A_{ISsample} is the peak area of the internal standard in the sample. The calibration curve was formed by plotting the ratio of BCFA standard peak area to peak area of the internal standard (A_{BCFA}

$_{standard}/A_{IS\ standard}$) against BCFA standard concentration where $A_{BCFA\ standard}$ and $A_{IS\ standard}$ are the peak areas of the BCFA standard and internal standard, respectively.

4.1.3 Measurement of *p*-cresol and 3-methylindole

Headspace solid phase microextraction (SPME)/GC-MS was performed using an Agilent GC-MS system (Palo Alto, CA, US), comprising of a Model 6890 gas chromatograph, HP-VOC column (Agilent, 60m X 0.32 mm i.d. X 1.8 µm film thickness) and Model 5973 Mass Selective Detector with a CombiPAL SPME autosampler (CTC, Switzerland).

Chemicals - *p*-Cresol (4-methylphenol) and 3-methylindole were purchased from Sigma-Aldrich without purification. Divinylbenzene/Carboxen®/polydimethylsiloxane (DVB/Car/PDMS) SPME fibres were obtained from Sigma-Aldrich. The SPME fibres were pre-conditioned at 280 °C for 90 min.

Headspace solid-phase microextraction (SPME) - After heating at 60 °C for 30 min, aliquots (1g) of rendered sheep fat was transferred to a 20 mL glass headspace vial and sealed with polytetrafluoroethylene (PTFE, Teflon®)/silicone septa and steel caps. For analysis, the vials and their contents were heated at 100 °C for 2 min prior to the insertion of the DVB/Car/PDMS fibre into the headspace above the sample where it was held for 30 min. Subsequently, the autosampler withdrew the fibre and inserted it into the GC injector where the adsorbed compounds were desorbed for transfer to the analytical column. The fibre was held in the injector for 7 min, which was in the splitless mode for the first 2 min and then split (20:1) for the remainder of the analysis.

Measurement by gas chromatography-mass spectrometry (GC-MS) - The GC oven temperature was initially held at 100 °C and then increased to a final temperature of 280 °C at a rate of 6 deg/min. Helium was used as the carrier gas with a constant flowrate of 1.2 mL/min. The mass selective detector was operated in electron ionisation mode (70 eV) and the data was collected with single ion monitoring with the electron multiplier voltage held at 400 V above the autotune value. The detector response of each analyte was quantified by measuring the abundance of a characteristic target ion using the Agilent Chemstation software. A qualifying ion was also used to confirm the analyte's identification. The respective target and quantifying ions were for *p*-cresol, m/z = 107 and 108, and 3-methylindole, m/z = 130 and 131.

For calibration purposes, working standards were prepared by spiking dehydrogenated coconut oil with *p*-cresol and 3-methylindole. The standard concentration range for *p*-cresol was 0 to *ca* 300 µg/kg while, for 3-methylindole, it was 0 to *ca* 250 µg/kg, spanning the expected range for both compounds in sheep fat. Quantification was performed using the external standard technique.

4.1.4 Statistical analysis of consumer sensory attributes

Initial models tested the significance of each chemical compound in relation to the three consumer attributes 'Overall like', 'Flavour' and 'Like Smell', using models with

fixed and random terms similar to those used by the Sheep CRC. The restricted maximum likelihood method (REML) was used for all data analyses with abattoir (Katanning, Tamworth), slaughter date, sex (wether, female), age of dam (2, 3, 4, 5, 6 - 7 years), dam breed (Merino or crossbreed), birth-rear type (11, 21, 22, 31, 32, 33, with the first number being the number of lambs born and the second number being the number of lambs reared), sire type (Merino, maternal or terminal) and sire, and interactions thereof, where appropriate, as fixed effects. Sire was included as a fixed effect rather than a random effect due to the low number of samples per sire. Dam was not included in the models as a random effect since 95% of the dams only had a single record. The consumer sampling session was included as a random term, to take into account any variation which occurred from session to session. The models used for these analyses also allowed for separate residual variance for each site by slaughter date. For all analyses, terms were included in the final model only if they were statistically significant ($P < 0.05$), except in the case of interactions where the main affects must also be included, even if not significant. The following covariates were tested in the models; EOA, MNA, MOA, *p*-cresol and 3-methylindole. The most parsimonious model for each variate was chosen using Wald tests using approximate *F* statistics (Kenward and Roger 1997). All statistical analyses were performed using GENSTAT software (12th Edition, VSN International Ltd, Hemel Hempstead, UK).

4.1.5 Statistical classification of predicting 'Flavour' sensory attribute using fatty acid profiles

The total ion chromatograms (TICs), representing the total abundance against time, were exported from the Star Workstation software as files in the comma separated value (CSV) format. A selection of 40 files was made from the complete dataset, and consisted of the chromatograms associated with ten samples that had the lowest and highest consumer "flavour" scores, obtained at the Katanning and Tamworth abattoirs. The twenty chromatograms of the lower flavour score were assigned as the "Low" category while the remainder were assigned to the "High" category. The efficacy of two statistical classification algorithms, support vector machines (SVMs) and randomForests (RF), were evaluated using 10-fold cross validation. Using this approach, for a single iteration one third of the dataset was randomly assigned as a test set and the remainder was used as a training set. Based on the training set, a model was developed using the classification algorithms and then applied to the test set to test the predictive capability of the model. A class confusion matrix, comprising of the correctly classified and misclassified samples, was produced and used to generate a class confusion ratio matrix. The ratio matrix was formed by expressing each column entry as a proportion of the column total. A tally was made for each column entry for 10 iterations and the average value was determined.

Two different pre-treatments were applied to the data; (a) no transformation (i.e. original data) and (b) range transformation. For range transformation, each TIC was scaled in the range of 0 to 1, using $x_j^* = \frac{x_j - x_{j,\min}}{x_{j,\max} - x_{j,\min}}$ where x_j^* is the scaled for each row *j*, x_j is the original measured TIC response, and $x_{j,\min}$ and $x_{j,\max}$ are the minimum and maximum values of the TIC, respectively. After data-pretreatment, the dataset was analysed using R (R Development Team, 2011) using SVMs and RF. The

implementations of these algorithms can be found in the "e1071" and "randomForests" packages available at http://cran.r-project.org. Each algorithm was used "as is" with the default settings provided in each software package. For the comparison, the same training and test set was used for each algorithm.

4.2 Results and discussion

4.2.1 Sample description

In total, a subset of 180 fat samples was selected from the cohort supplied by the CRC of Sheep Industry Innovation (Sheep CRC). The samples were selected across the range of the mean 'Flavour' consumer sensory scores to be representative of the dataset. Figure 1 shows a histogram for the distribution of 'Flavour' and 'Like Smell' values for samples of 'Terminal' sire type. Approximate Gaussian curves are seen for each attribute, indicating no bias was present in the dataset. Of the final cohort, 124 samples belonged to the 'Terminal' sire type category while 22 and 34 samples belonged to the 'Maternal' and 'Merino' sire categories, respectively.

The BCFA content was measured for each fat sample, and MOA was the most abundant BCFA in the samples (Table 1). For example, for the samples taken at Katanning, the mean MOA concentration for sheep of Terminal sire type was 0.230 mg/kg while, for EOA and MNA, the mean concentrations were 0.051 and 0.050 mg kg, respectively. These results are comparable to the range of BCFAs that have been reported in a survey of the Australian meat sheep flock (Watkins 2010a). As in this study, MOA was the most abundant of the BCFAs while MNA was the less abundant and EOA was intermediate between the two compounds.

The concentration of 3-methylindole and *p*-cresol was also measured. These results in Table 1 are comparable with those that have been reported for these compounds previously. *p*-Cresol lies within the range of 5 to 246 ng/g reported for sheep fat (Ha & Lindsay, 1991) while 3-methylindole was in the range of 31 to 154 ng/g found by Schreurs *et al* (2007).

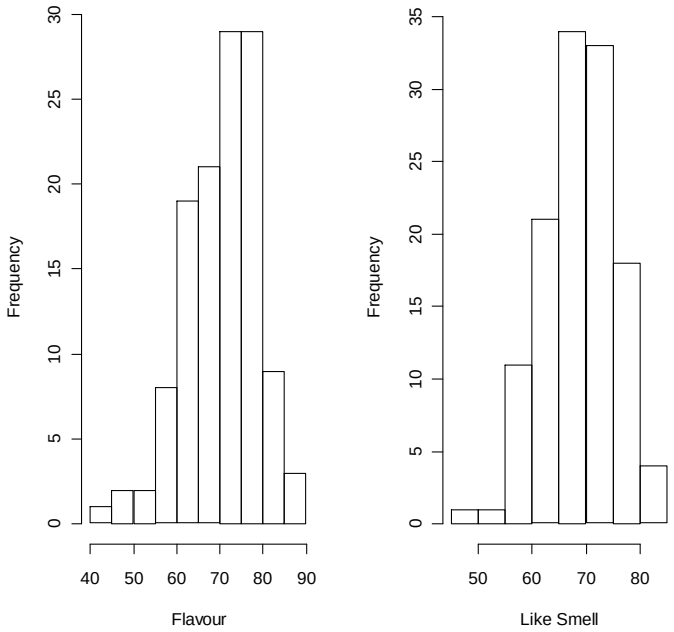

Figure 1. Histograms of the mean 'Flavour' and 'Like Smell' consumer sensory scores for the 'Terminal' sire type.

4.2.2 Statistical modelling of 'Like Smell' consumer sensory score

Initial models tested the significance of each chemical compound in describing the variation in the three consumer attributes 'Overall like', 'Flavour' and 'Like Smell', using models similar to those used in the Sheep CRC. MOA and EOA were the only statistically significant covariates which contributed to the 'Like Smell' and 'Overall Like' sensory attributes (see Appendix 1). As none of the chemical compounds were significant in the model for 'Flavour' (see Appendix 1 for detail of analyses conducted), this attribute will not be discussed any further. 'Like Smell' was highly correlated with 'Overall Like' and so the latter score was not given further consideration. Other parameters were also tested for their significance but were not significant ($P > 0.05$) except for site.DATE and the kill date. In the final model for 'Like Smell', EOA and MOA were included as covariates and Site.DATE, site, and 'Sire type' were included as fixed effects.

Table 1. Number (*n*), mean, standard error (s.e..), minimum (Min.) and maximum (Max.) values for the mass fractions of 4-methyloctanoic acid (MOA), 4-ethyloctanoic acid (EOA), 4-methylnonanoic acid (MNA), *p*-cresol (PC) and 3-methylindole (MI) in fat taken from sheep of three different sire types (Terminal, Maternal, Merino) at two different sites (Katanning and Tamworth)

			Terminal			Maternal			Merino		
Site	Compound	*n*	Mean ± s.e.[A]	Min.	Max.	Mean ± s.e.	Min.	Max.	Mean ± s.e.	Min.	Max.
Katanning	MOA	180	0.230 ± 0.02	0.07	0.537	0.215 ± 0.032	0.073	0.64	0.093 ± 0.009	0.047	0.149
	EOA	180	0.051±0.006	0.007	0.229	0.039 ± 0.007	0.007	0.118	0.0185 ± 0.004	0.009	0.054
	MNA	180	0.050 ± 0.002	0.018	0.094	0.043 ± 0.003	0.024	0.071	0.030 ± 0.003	0.01	0.042
	PC	179	121.0 ± 14.5	13	482	88.7 ± 16.7	13	243	34.5 ± 6.4	12	69
	MI	179	26.1 ± 4.2	0	103	30.3 ± 10.3	0	193	18.7 ± 4.2	0	50
Tamworth	MOA	180	0.53 ± 0.04	0.137	1.697	0.344 ± 0.061	0.079	0.78	0.172 ± 0.040	0.073	0.284
	EOA	180	0.170 ± 0.020	0.013	0.828	0.078 ± 0.018	0.011	0.249	0.0338 ± 0.0179	0.007	0.07
	MNA	180	0.097 ± 0.006	0.041	0.32	0.104 ± 0.009	0.05	0.186	0.038 ± 0.06	0.018	0.072
	PC	179	171.5 ± 37.2	16	1899	113.1 ± 18.2	22	280	118.2 ± 21.0	21	340
	MI	179	34.4 ± 7.7	0	439	17.93 ± 4.43	0	56	30.4 ± 7.4	0	79

[A]For MOA, EOA, and MNA mass fraction = mg/kg while, for PC and MI, mass fraction = µg/kg

Both MOA and EOA made significant impacts on the 'Like Smell' consumer sensory score (Table 2). With the increase in concentration for each BCFA, a corresponding reduction occurs in the consumer's acceptance of the 'Like Smell' for the grilled meat product. The impact of MOA and EOA on 'Like Smell' is clearly seen in Figure 2, which shows a plot of the predicted 'Like Smell' score against the concentration range for MOA and EOA. In some ways, this result is not unexpected since BCFAs, in general, are regarded as the main contributors to the 'mutton' aroma found in the cooked meat of older sheep, and the presence of these compounds can reduce consumer acceptance of this type of product. It is also interesting to note that, with a range of animal age from 214 to 365 days, that these animals can be regarded as 'lambs' and even for this category, MOA is an important contributor to the smell of the cooked meat perceived by the consumers. This would suggest that ameliorating the impact of this compound in the cooked meat could result in higher acceptance of the final product. Having stated that though, it is also important to recognise that other factors, chemical and/or otherwise not examined in this study, would also affect the 'Like Smell' sensory score and, in these cases, other strategies would need to be considered to counter their impact.

Table 2 Coefficients (s.e. in parenthesis) and level of significance (*P*-value) of the coefficient for covariates in models relating 'LikeSmell' to site, kill date (Site.DATE) and siretype with 4-methyloctanoic acid (MOA), 4-ethyloctanoic acid (EOA) and combined terms (MOA+EOA) with adjustment for sensory session fitted as random effect.

		Alternative models for 'Like Smell'		
Term	Numbers	1	2	3
Site	*P*-value	0.798	0.879	0.866
Site.DATE	*P*-value	0.062	0.122	0.082
Siretype	*P*-value	0.591	0.635	0.412
EOA	*P*-value		0.040	0.086
	Coefficient		-45.34 (20.30)	-35.70 (20.64)
MOA	*P*-value	0.013		0.031
	Coefficient	-7.49 (2.98)		-6.66 (3.06)

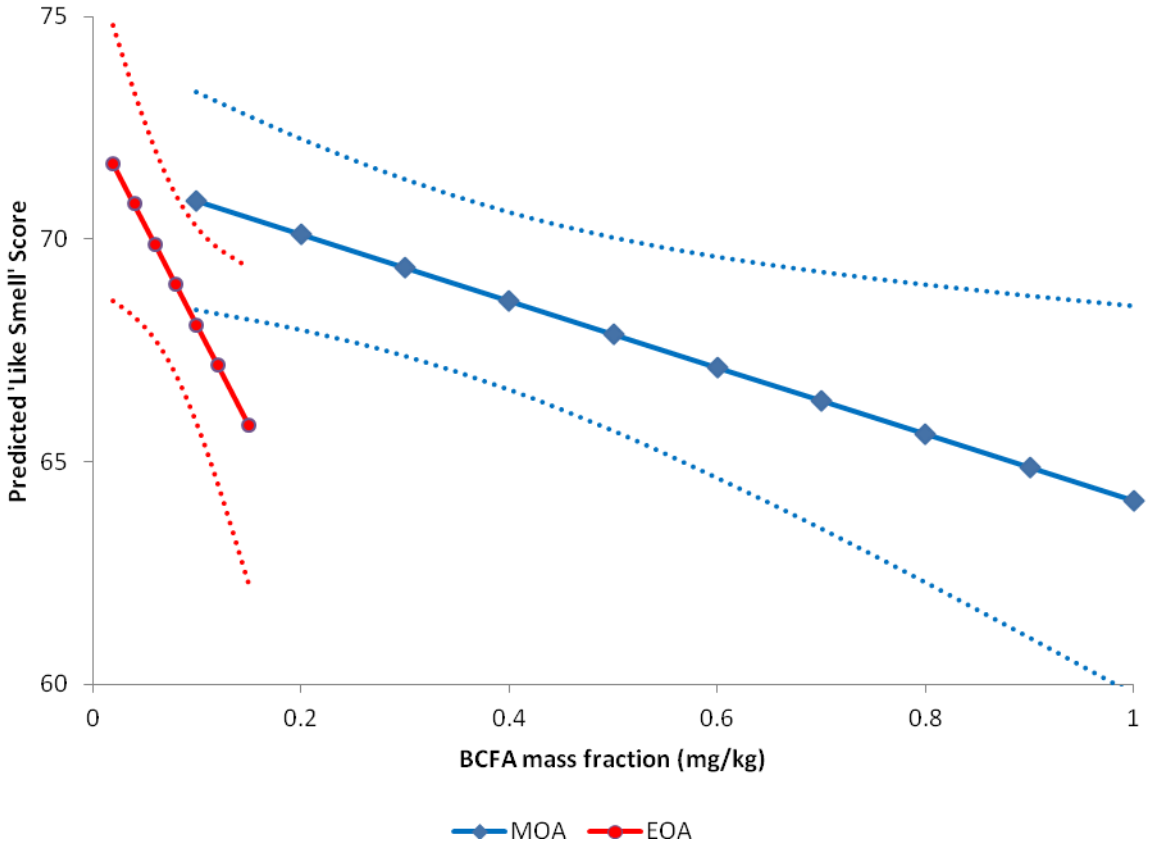

Figure 2 Plot of predicted 'Like Smell' consumer sensory score against 4-methyloctanoic (MOA) and 4-ethyloctanoic (EOA) mass fraction (mg/kg)
The dashed lines indicate ± 2 times the standard error.

4.2.3 Statistical classification algorithms for predicting 'Flavour' consumer sensory attribute

Two classification algorithms (support vector machines and random Forests) were evaluated as possible tools for testing the quality of cooked meat, based on the mean 'Flavour' consumer score. In a separate study (Watkins et al 2010b), the use of total ion chromatogram (TICs) as a 'fingerprint' for sheep category with statistical classification algorithms has been shown to be useful for the prediction of sheep category. In a similar way, we wished to test whether TICs could be used as a 'fingerprint' for predicting the 'Flavour' sensory score.

The two classification algorithms Support Vector machines and Random Forest, were not suitable for predicting the category of 'high' and 'low' of the mean flavour consumer score as the accuracy for correct prediction of class was around ~ 0.60. For use as a predictive tool, it would be preferable that the accuracy of the algorithm was higher (> 0.90). Additionally, a wide variation in the range of predictive accuracy was also evident; for example, for predicting the 'High' category using support vector machines, the accuracy ranged from 0.250 to 1.000. While this latter result does indicate that correct classification is achievable, it is not consistent enough for this

approach to be routinely used. Increasing the number of samples might improve the accuracy but this would need to be tested and shown to be true. Additionally, the use of range scaling of the data set made no demonstrable difference to improving the predictive accuracy for either algorithm.

Table 3 Performance of two classification algorithms for predicting 'low' and 'high' mean flavour consumer scores.

Algorithm[A]	Treatment[B]	Predicted	Actual High[C]	Actual Low	Range[D]
SVM	None	High	0.607	0.393	0.250 - 1.000
		Low	0.378	0.622	0.300 - 0.800
RF	Scaled	High	0.640	0.360	0.333 - 1.000
		Low	0.505	0.495	0.200 - 0.750

[A]SVM - support vector machine RF - randomForest [B]None - raw data Scaled – data scaled between 0 and 1 [C]mean of 10 iterations [D]Range of minimum to maximum values for prediction of same class (eg 'High' to 'High')

4.3 Summary

The aim of this study was to determine what impact, if any, that BCFAs (responsible for 'mutton' aroma) as well as *p*-cresol and 3-methylindole (responsible for 'pastoral' flavour) measured in fat samples has on a set of consumer sensory attributes of the associated grilled meat samples obtained from the Sheep CRC. Of these compounds, MOA and EOA were the most significant contributors to impact on the perceived smell of the cooked meat. A higher consumer acceptance of the grilled meat was found for fat samples that contained lower concentrations of these compounds.

4.4 References

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5 Draft Literature review

Sheepmeat Flavour and the Effect of Diet on Eating Quality – A Review

P.Watkins[1], D. Frank[2], T. Singh[2], R. Warner[1] and O.Young[3]

[1]CSIRO, Food and Nutritional Sciences, 671 Sneydes Rd, Werribee. Vic., 3030, Australia
[2] CSIRO, Food and Nutritional Sciences, North Ryde, NSW, Australia
[3]Auckland University, New Zealand

5.1 Introduction

Flavour is an important component of the eating quality of meat, and refers to the components of food responsible for chemosensory stimulation – volatile aroma and non-volatile taste compounds. Flavour molecules must by definition interact with sensory receptors to be perceived; flavour information is normally integrated together with texture, visual and other sensory cues by the brain to create a unique sensory signature. Not only are the type, quantity and balance of flavour molecules critical to meat flavour, but also the way the structure and composition of the meat affect the way they are released during cooking and eating. Also, flavour perception is determined not just by the chemical composition of flavour active compounds, but also by the release of these compounds and their availability to receptors. The composition of the meat, particularly, the fat content, and the structure (e.g. toughness) can determine release of flavour compounds. In this respect the preparation and cooking may also have a large effect on the overall flavour and eating quality.

In its fresh uncooked state, meat has very little flavour and it is only as a result of cooking that meat develops a flavour, often that can be regarded as species specific flavour for an animal. During cooking, a complex set of thermally induced reactions occurs between the non-volatile components of lean and fat tissues that results in the generation of a large number of products (Mottram, 1998). The major precursors of meat flavour are either lipids or water-soluble components which are subject to two sets of reactions during the cooking process; Maillard reactions between amino acids and reducing sugars, and thermal degradation of the lipid content. The lipid-derived volatile compounds are the ones primarily responsible for explaining the differences between the volatile profiles of meat species, and thus are the compounds that contribute to the species specific flavour for each animal.

Historically, focus of attention for sheepmeat flavour has been on aroma, specifically for ‘mutton’ and ‘pastoral’ flavours but others (eg from other feedtypes – rape/vetch) as well eg ‘potato’ from microbial contamination. This review will focus on the effect of diet on sheepmeat (and fat) flavour.

5.2 Effect of diet on the aroma/taste of cooked sheepmeat assessed by sensory panels

The use of a pasture-based finishing diet for sheep, compared to one which is grain-based, can significantly impact on the sensory properties of the associated cooked meat (Table **1**). Pasture introduces a different flavour to the final product which is

perceptible by trained sensory panels(Crouse *et al*, 1981) (Tudor *et al*, 1982)(Bailey M. E. *et al*, 1994)(Rousset-Akrim *et al*, 1997)(Priolo *et al*, 2002a)(Young *et al*, 2003)(Borton *et al*, 2005)(Pethick *et al*, 2005)(Font i Furnols *et al*, 2009)(Resconi *et al*, 2009), and has been also regarded as a species specific flavour as well (e.g. "sheepmeat" (Rousset-Akrim *et al*, 1997)(Young *et al*, 2006), "lamb"(Priolo *et al*, 2002b)). For some consumers, the presence of a pasture-based flavour has been regarded as a taint by some consumers (in the US) but this observation is more likely to related to a bias within the taste panel since these consumers would be more accustomed to grain-fed meat than product derived from pasture-fed animals (Borton *et al*, 2005).

Within pasture types, differences in sheepmeat flavour can also occur because of the feed material. For example, in comparative trials of different pasture species, unacceptable flavours have been found by trained panels for white clover (Shorland *et al*, 1970), lucerne and phalaris (Nicol & Jagusch, 1971)(Park *et al*, 1972a)(Young *et al*, 1994), rape (*Brassica*)(Park *et al*, 1972b)(Wheeler *et al*, 1974)(Hopkins *et al*, 1995a), saltmarsh and related pastures (Whittington *et al*, 2006)(Lind *et al*, 2009) (see Table 2). In contrast, other comparative studies have reported that no differences exist between other forage species; e.g. tropical legumes *vs* grass (Park & Minson, 1972), chicory *vs* lucerne (Hopkins *et al*, 1995b) and clover (Houdjik, 2010), saltbush (Pearce *et al*, 2003), lotus, ryegrass and white clover (Farouk *et al*, 2007).

In some instances, the impact of the pasture species on the sheepmeat flavour has been quite significant. One such example is forage rape (*Brassica*) where the flavour has been reported as "strong and attractive" by a trained sensory panel (Wheeler *et al*, 1974) as well as "unacceptable" by an untrained consumer panel (Hopkins *et al*, 1995a). In these cases, it seems likely that the volatile compounds responsible for the differentiation in the flavour of the cooked meat would be present in appreciable concentrations and thus could be measured by technique such as gas chromatography-mass spectrometry. However, this was not reported as having been done. However, in the case of forage rape, the literature suggests a plausible mechanism which could explain the presence of this "unacceptable" flavour in the cooked meat. Australian cultivars of rapeseed (*Brassica*) are known to contain glucosinolates in concentrations greater than 30 □mol g^{-1} (dry weight) (Mailer & Wratten, 1985). Once consumed, glucosinolates become available to the animal and thus are potential contributors to meat flavour. Additionally, these compounds can be metabolised by the animal and form products such as isothiocyanates, nitriles and thiocyanates (Tripathi & Mishra, 2007). Isothiocyanates are volatile compounds and are known to be extremely pungent (see, for example, in Wasabi (Depree *et al*, 1998)) and so could significantly contribute to the flavour of the final cooked product. There is some evidence that glucosinolates are metabolised by sheep to produce isothiocyanates which supports this contention. High levels of serum isothiocyanate have been found in blood of sheep which had been fed high glucosinolate mustard (*Brassica juncea*) meal (Tripathi *et al*, 2001). These authors attribute these levels directly to the consumption of glucosinolate which was metabolised by the myrosinase enzyme in the animal and released into the blood stream. Such compounds would then be available for deposition in either muscle or fat. Thus, it is

possible that metabolites from the hydrolysis of glucosinolates in sheep are responsible for the “unacceptable” flavour in animals which consume forage rape prior to slaughter. Of course, this is speculative and would need further substantiation. In addition, there will be other compounds present in the feed material that could contribute to the volatile profile as well. Rapisarda and co-workers (2011) have measured the aroma profiles of different concentrate systems suitable as feed sources for lambs. In this work, differences did exist between the volatile profiles of the feed systems and this does have potential to impact on the final meat product, depending on the level of transfer of these compounds from the rumen for deposition into the muscle and fat of the animal.

5.3 Effect of diet on volatile compounds present in cooked sheepmeat

Table **2** shows a summary of the significant differences found in the volatile composition of cooked sheepmeat, reported in comparative studies of pasture-based and grain-based feeding systems. For pasture systems, volatile compounds such as terpenes and diterpenoids have been found in the cooked meat, and are derived from the feeding system. 2,3-Octanedione has been a common compound found in the cooked meat from pasture fed animals and has been noted by Young *et al* (1997) to be an excellent indicator of pasture diet. Prolio *et al* (2004) have also substantiated this observation while recent work have suggested that 2,3-octanedione would be a suitable biomarker for authenticating a pasture diet (Sivadier *et al*, 2010).

Higher levels of □-lactones have been associated with the use of grain feeding regimes for sheep. Free fatty acids available in the grain are likely to the precursors for these compounds (Stark *et al*, 1978) as these workers have suggested a mechanism for the biosynthesis of □-dodecalactone from oleic acid. □-Lactones have also been reported as high in the meat from pasture-finished animals (Bailey M. E. *et al*, 1994) and in bovine milk as well (Urbach & Stark, 1978).

3-Methylindole and *p*-cresol have been implicated as the main contributors to the ‘pastoral’ flavour evident in the cooked meat of pasture fed animals (Young *et al*, 2003). Pasture has been noted as having a high protein to readily fermentable carbohydrate ratio, and the protein is more readily digestible in the rumen compared to that available in grain and concentrate diets (Schreurs *et al*, 2008). Additionally, substantial degradation of feed protein to amino acids occurs in the rumen which allows a higher availability of peptides and amino acids which cannot be fully incorporated into microbial protein as insufficient energy is released from carbohydrate metabolism (Ulyatt et al 1975).

3-Methylindole is formed in the rumen from the anoxic metabolism of L-tryptophan (Deslandes *et al*, 2001)(Mohammed *et al*, 2003). Rumen bacteria and protozoa transform tryptophan to 3-methylindole in a 3-step process (Mohammed *et al*, 2003)(Yokoyama & Carlson, 1981). Initially, tryptophan is deaminated to form indolepyruvic acid which undergoes two successive decarboxylation steps via an intermediate, indoleacetic acid, to form 3-methylindole (Deslandes *et al*,

2001)(Mohammed *et al*, 2003)(Tavendale *et al*, 2005). Usually, 3-methylindole would be metabolised by the liver after release into the blood supply from the rumen. When in excess though, some can escape liver metabolism and be released into the blood supply for deposition into fat tissue (Schreurs *et al*, 2008). *p*-Cresol is also produced by rumen bacteria using another amino acid, tyrosine (Ha & Lindsay, 1991)(Martin, 1982)(Yokoyama & Carlson, 1981). Tyrosine undergoes successive transamination and decarboxylation steps to produce the intermediate, *p*-hydroxyphenyl acetic acid, which then undergoes further decarboxylation to form *p*-cresol (Ha & Lindsay, 1991), which would then be deposited into the fat tissue.

Diet is also implicated with the formation of BCFAs, regarded as the main contributors to 'mutton' aroma in cooked sheepmeat. Higher concentrations of these compounds have been observed in animals receiving a grain based finishing diet prior to slaughter (Wong *et al*, 1975)(Johnson *et al*, 1977)(Duncan & Garton, 1978)(Young *et al*, 2003). This has been attributed to availability of carbohydrate within the diet since higher amounts are associated with grain and concentrates compared to those diets which are based on pasture (Young & Braggins, 1998). Thus, it would be logical to conclude that grain-dominated diets will result in increased sheepmeat flavour in the cooked meat but Young & Braggins (1998) have noted cereal grains differ in their propensity to generate BCFAs so some care is required in extrapolating this observation. Recent work (Watkins *et al*, 2010) supports this view where higher levels of BCFAs have been found in animals fed on finishing diets composed of native pasture, saltbush or mixed lucerne compared to that derived from grain.

It can be seen that the effect of diet on the volatile compounds in cooked sheepmeat is not simple and that further work is required to elucidate what is, a complex relationship between diet and volatile composition. In fact, this has been noted by other authors where multilinear regression has been used to relate volatile chemical composition to 'grassy' and 'lamb' flavour intensities in cooked sheepmeat (Bailey *et al*, 1994).

5.4 Effect of condensed tannins on pastoral flavour

Lush pasture, containing readily degradable protein, can be a rich source of tryptophan and lead to the formation of indole and 3-methylindole, compounds responsible for 'pastoral' flavour in sheepmeat. Recent work has described the effect of condensed tannins (CTs), a class of naturally occurring polyphenols which can be present in certain forage legumes, on the formation of indole and 3-methylindole (Tavendale *et al*, 2005). Specifically, CTs (from *Dorycinum rectum*) were demonstrated to inhibit the conversion of protein to 3-methylindole and indole by rumen microbes, and particularly inhibited the transformation of indoleacetic acid to 3-methylindole by rumen bacteria. Other workers investigated sulla (*Hedysarum coronarium* L.), another source of CTs, but reported no effect of CT on the fat concentrations of these compounds which was attributed to the low CT content in sulla (Priolo *et al*, 2005). Further confirmation of the impact of CTs on the formation of indole and 3-methylindole was made using *Lotus corniculatus* for grazing lambs (Schreurs *et al*, 2007a). These workers found lower concentrations of these

compounds in rumen fluid and blood plasma taken from slaughtered animals that had grazed on *L. corniculatus* compared to those grazed on ryegrass/white clover. Similar results were found for the concentration of 3-methylindole fat. Of interest, a trained sensory panel evaluated the odour emanating from molten (intra-muscular) fat, taken from the animals fed on the two different feeding regimes, and no discernible difference was found between the odours of the two different fats. These workers concluded that the reduction in indole and 3-methylindole concentrations was not sufficient to impact on the odour arising from the heated fat. In fact, no significant difference between the mean indole concentration of the tail-stub fat was found for the two grazing treatments and only a marginal effect was found for 3-methylindole (P<0.06, Schreurs *et al*, 2007). Thus, it is feasible that this observation could be related to the fact that similar concentrations of these compounds are present in the intra-muscular fat and are not differentiable by sensory analysis. This assumes of course that the concentrations of these compounds in the intra-muscular fat are the same as those in tail-stub fat. Grape seed extract (as a source of CT) has also been used as a supplement to diets of white clover and perennial ryegrass but this only resulted in small reductions in indole and 3-methylindole concentrations in rumen fluid and blood plasma as well as odour scores in associated fat samples (Schreurs *et al*, 2007d). Adding CT as an oral supplement (prepared as extracts from *Lotus pedunculutus* and grape seeds) reduced the formation of indole and 3-methylindole in the rumen (Schreurs *et al*, 2007b). Additionally, the CT content of forage also impacts on the formation of these compounds as plants with higher CT concentrations tend to be more effective in reducing the production of these compounds (Schreurs *et al*, 2007c). The impact of quebracho (*Schinopsis loretzii*) tannins on lambs fed on production systems derived from forage as well as concentrates has been assessed and the use of the tannins reduced the production of 3-methylindole in the animals from both systems (Priolo *et al*, 2008). Notably though these authors found that tannins were more effective in reducing 3-methylindole production in animals fed concentrate diets than forage based diets. Presumably, the tannins forms complexes with protein which then makes it unavailable for subsequent transformation to 3-methylindole resulting in lower concentrations of this compound in the animal. The impact of polyphenols as supplements to diets (with added oil) on the volatile profile of heated muscle has also been examined (Vasta *et al*, 2010). The addition of grape seed extract to diets with, and without, oil supplementation caused no differences to be observed in the concentrations of lipid oxidation products (heptanal, 2-nonenal, 4-heptenal and 3-hydroxy-2-butanone), implying that the extract prevented the formation of these compounds. A further reduction in 4-heptenal, an oxidation product of linolenic acid, was found with *Cistus* supplementation. Higher levels of CTs and phenolic compounds were found in the diet derived from *Cistus* supplementation and this could be related in reducing the formation of 4-heptenal from linolenic acid.

5.5 Effect of dietary supplementation

Lipid supplementation of pre-slaughter diets has been employed to incorporate higher levels of fatty acids of nutritional value into the lipids of ruminants. Protection of the supplement from ruminal hydrogenation was afforded by encapsulating oil

droplets in a formaldehyde-treated protein coating (Ford & Park, 1980). The meat from lambs fed on a lipid-protected sunflower supplement displayed levels of up to 30% higher of the total fatty acid content (Park *et al*, 1974). A 'sweet-oily' aroma was reported in the cooked meat of the product and the source of the 'sweet' aroma was identified as □-dodeceno-6-lactone while *trans*, *trans*-2, 4-decadienal, an oxidation product of linoleic acid (C18:2 *n*-2), was implicated as the contributor to the 'oily' (Park *et al*, 1974). Similar observations were reported for dairy cattle where the lactone was present in butterfat taken from animals that were fed the same supplement (Stark & Urbach, 1974). Further work showed that a trained sensory panel found that an unacceptable flavour was found in meat taken from animals fed on the supplement over a period of 6 weeks, and this flavour note increased in intensity with the length of the experiment (Park & Ford, 1975). A corresponding increase in the amount of □-dodecen-6-lactone was also reported for the meat samples, which Park and Ford (1975) report as the main contributor to the unacceptable flavour. The use of this supplement has also been reported to ameliorate 'mutton' flavour. Meat taken from pasture-fed animals, which received one to two weeks of treatment of the sunflower supplement, was reported to have a small but significant decrease in mutton aroma and flavour intensity compared to lot-fed animals where no such reduction was evident (Park *et al*, 1978). Following from the earlier studies, these authors were interested in the impact of the diet on □-dodeceno-6-lactone and decadienal, and not on BCFAs which are the main contributors to 'mutton' flavour. If we assume though that the lipid supplement directly impacts on the BFCA concentration then this could prove useful as a means for reducing the 'mutton' flavour. Of course, this is speculative and would require further substantiation. Additionally, the animals in this study were also pasture fed so this suggests the sensory panel could have assessed 'pastoral' flavour instead, given the reduction of the aroma with the introduction of the lipid supplement. In the event that an assessment is made on the effect of lipid supplementation to reduce BCFA levels, then this aspect would also need to taken into consideration.

More recently, attention has been given to other polyunsaturated fatty acids (PUFAs), namely, α-linolenic (C18:3 *n*-6), eicosapentaenoic (EPA, C20:5 *n*-3) and docasahexaenoic (DHA, C22:6 *n*-6) acids and their impact on the volatile aroma compound profile found for cooked and grilled lamb meat (Elmore *et al*, 2000)(Elmore *et al*, 2005). For cooked meat, higher levels of lipid oxidation products were found in product derived from animals fed on a supplement based on fish oil, a source of EPA and DHA (Elmore *et al*, 2000). Notably, unsaturated aldehydes, unsaturated hydrocarbons and alkylfurans were up to fourfold higher compared to the control, and resulted from the auto-oxidation of the PUFAs during cooking. While no sensory evaluation of the cooked meat was performed in this study, presumably the use of the fish oil as a supplement to the diet would also impact on the sensory properties of the cooked meat. Later, these workers substantiated this when comparing volatile profiles for grilled lamb using a protected lipid supplement, similar to the one used by Park and co-worker as mentioned above, and marine algae and fish oil which are good sources of EPA and DHA (Elmore *et al*, 2005). Higher levels of oxidation products from *n*-3 fatty acids were found for the meat from the lambs fed fish oil/algae diets, while compounds derived from *n*-6 fatty acids were highest in the meat from the lambs fed the protected lipid supplement. Interestingly, these authors

state the results of sensory profiling of the grilled lamb samples and noted that less than desirable scores were associated with the meat derived from the diets based on the fish oil/algae diets. Fishy odours were reported for the samples derived from fish oil containing diets while abnormal and rancid flavours were found for the animals fed the algal diets. These authors note that, while increasing PUFA content in muscle may be nutritionally desirable, poor sensory quality could result if the PUFA levels were excessive. Recent work with kid goats confirms this observation as high levels of DHA were added as a supplement to a pre-slaughter diet in order to manipulate fatty acid profiles of goat muscle which resulted in a meat product with unusual odours, unpleasant flavours and low overall sensory appreciation scores (Moreno-Indias *et al, in press*).

5.6 An integrated view of the effect of feed on lamb flavour

The mechanisms by which feed may affect final lamb flavour are complex and multiple. In the simplest form, feed may affect the final flavour of lamb by a direct transfer of specific plant derived compounds into the meat, which may impart specific flavour notes. For example phytol, phytene, terpenes and sesquiterpenes may accumulate within the muscle tissue (Priolo 2004, Chevance & Farmer 1999). Once within the sheep meat these compounds may directly affect the final flavour if present at sufficient concentration, or they may undergo degradation during thermal processing to form new flavour active compounds.

The composition and final fatty acid profiles of meat are affected by feed (Vasta & Priolo 2004). During cooking, extensive oxidation reactions result in potent lipid derived odour active volatiles. These oxidation pathways are affected by the initial types of fatty acids present (pattern of unsaturation), meat pH, antioxidant status (presence of α-tocopherol and carnosine etc) and also the presence of haeme and non-haeme iron (Min *et al.* 2008). Different fatty acids produce different odour-active volatiles during cooking induced thermal oxidation. Significant diet induced changes in initial fatty acid profiles will influence the volatiles produced. A diet higher in a particular metal ion may affect the rate of lipid oxidation, resulting in elevated aroma volatiles. A higher concentration of generic volatiles may result in noticeable sensory differences in the final cooked meat, although no unique volatile compounds may be present.

A specific feed type may affect the final fat content and distribution of intramuscular saturated and unsaturated lipid (Vasta & Priolo 2006). Apart from effects on texture, other factors being equal, an increased meat fat content will act as a reservoir for lipophilic volatile compounds directly affecting the rate and extent of release during oral processing. Although not demonstrated extensively in meat systems, the effects of fat on release in dairy products and emulsions are well known. The presence of fat attenuates the release of volatiles and increases the relative amount released post-swallow compared to preswallow (Frank *et al.* 2011, others).

Lamb meat flavour

In order to understand flavour differences which may be impacted by feed and pasture it is necessary to have a good working knowledge of the essential components required for “characteristic” or baseline lamb flavour (see Figure 1). Integrated flavour perception is brought about by the interaction of non-volatile and volatile (meat) components with human chemosensory receptors; taste and olfactory receptor cells as well as other sense networks. Textural components, such as tenderness, juiciness, chew resistance, muscle structure and breakdown may also directly affect or attenuate overall perceived flavour. The overall content and intramuscular distribution of fat within the muscle structure may also play an essential role in the way flavour compounds are released and hence, perceived. The separation and quantitative measurement of non-volatile meat flavour components (free amino acids, flavour nucleotides, peptides, fat globules, free fatty acids, sodium ions etc.) is a considerable analytical challenge.

Measurement of volatile flavour compounds is less analytically demanding; for this reason quantitative measurement of non-volatile flavour compounds are most often overlooked and the focus is placed on volatile flavour compounds. The relative contribution of both non-volatile and volatile molecules to the final sensory attributes has been widely debated in the literature. It is clear that both non-volatile and volatile compounds present at the right concentrations and relative ratios is essential to create desirable flavour attributes. Measurement by gas chromatography olfactometry is the usual approach used to characterise complex aroma and enables categorisation of volatiles.

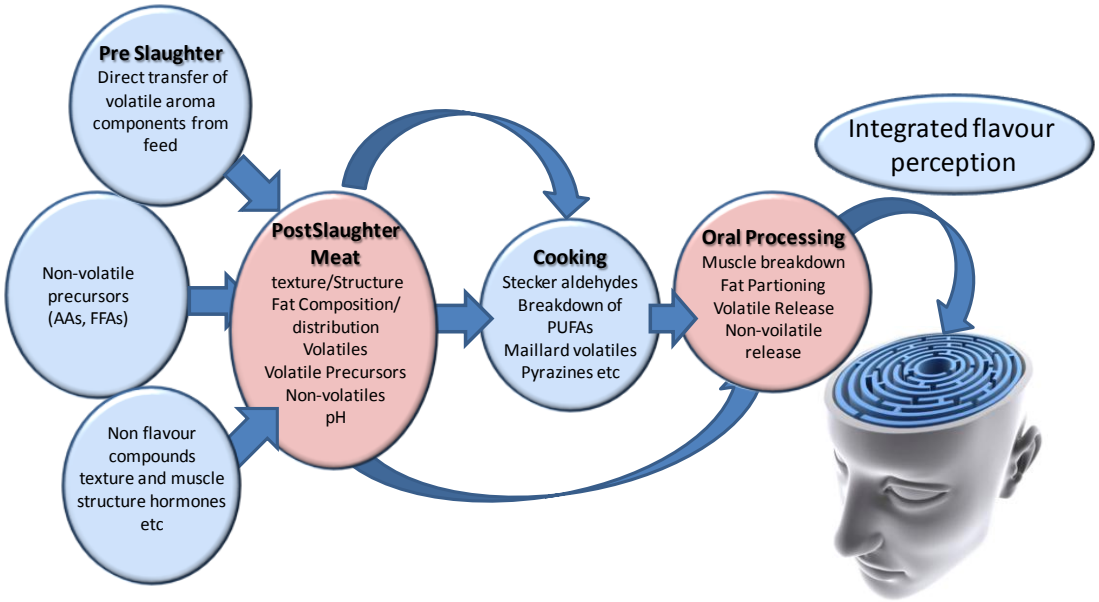

Figure 1: Diagrammatic summary of important variables where the interactions of feed and processing may directly or indirectly affect the final flavour attributes of lamb (meat) and perception.

5.7 Meta analysis of GC-O data

Despite many published works on the volatile constituents of lamb and other meats, there are surprisingly few studies which include comprehensive aroma analysis by gas chromatography olfactometry (GC-O). GC-O allows the relative odour activity of different volatiles to be ascertained; in most cases a relatively smaller subset of all volatiles measured are found to have significant odour impact. In any aroma characterisation, the validity of the findings depend strongly on the aroma extraction method employed. In the recent literature, X-GC-O studies were found for lamb aroma specifically (Bueno et al. 2011, Resconi et al. 2010,). Surprisingly few credible GC-O studies of the aroma of other cooked meat have been published. The several found were in ham (Song & Cadwallader 2007), beef and pork stock (Christlbauer & Schieberle 2009) and in dry cured sausages (Söllner & Schieberle 2009, Marco et al. 2007) The rationale behind GC-O is to be able to assign a relative odour-intensity or impact value to individual volatiles. GC-O allows a degree of data reduction, whereby volatiles with odour activity can be identified. In practise the number of odour active volatile compounds is always considerably less than volatiles identified by GC-MS. For example, very extensive lists of sulphur volatiles identified in meat extracts have been published (Rochat *et al.* 2007), yet few GC-O data convincingly show a role for most of them in the actual meat aroma extracts, with the exceptions of methanethiol, 2-methylthiophene, 2-methy-3-furanethiol, dimethyltrisulphide, Methional, methionol and 2-acetylthiazoline (Rochat *et al.* 2007 and others). In order to understand baseline lamb, or in fact any cooked meat aroma, an analysis of available meat GC-O literature was conducted. Data from at least eight published studies were compiled and the data was scaled to percent of total rated stimulus. The average stimulus across all studies was compiled to create a “meta-aromagram” of meat/lamb (Figure 2). More GC-O studies, directed at lamb

meat specifically, are required to better understand what a quality lamb flavour volatile profile looks like.

On the basis of the meat/lamb aroma meta-analysis, the top fifteen impact compounds in baseline aroma were identified as likely to be, in decreasing rank; methional (*potato, savoury*), (*E,E*)-2,4-decadienal (fatty,), 2-acetyl-1-pyrroline (*popcorn, roasted*), 1-octen-3-one (*mushroom*), hexanal (*green, fatty*), furaneol (*caramel, cooked sugar*), (E)-2-nonenal (fatty), isovaleric acid (*cheesy, vomit*), (*Z*)-2-heptenal (*fatty, oxidized*)), butanoic acid (*cheesy, rancid*), diethyl methyl pyrazine (*nutty, roasted*), (E,Z)-2,4-decadienal (*fatty, cooked*), *p*-cresol (*barnyard, urine*) and 2,4,5-trimethyltriazole. These odour compounds are well known generic products of either unsaturated fat oxidation or the Strecker degradation of amino acids. None of these compounds are likely to be directly transferred from the feed, but rather are formed from non volatile and semi-volatile precursors which are transformed by thermal processing. Diet may however directly affect the free amino acid and fatty acid profiles of the meat potentiating formation of these volatiles. Many of the lower impact volatiles in the meta-aromagram are generated via oxidation of unsaturated fat or amino acid degradation.

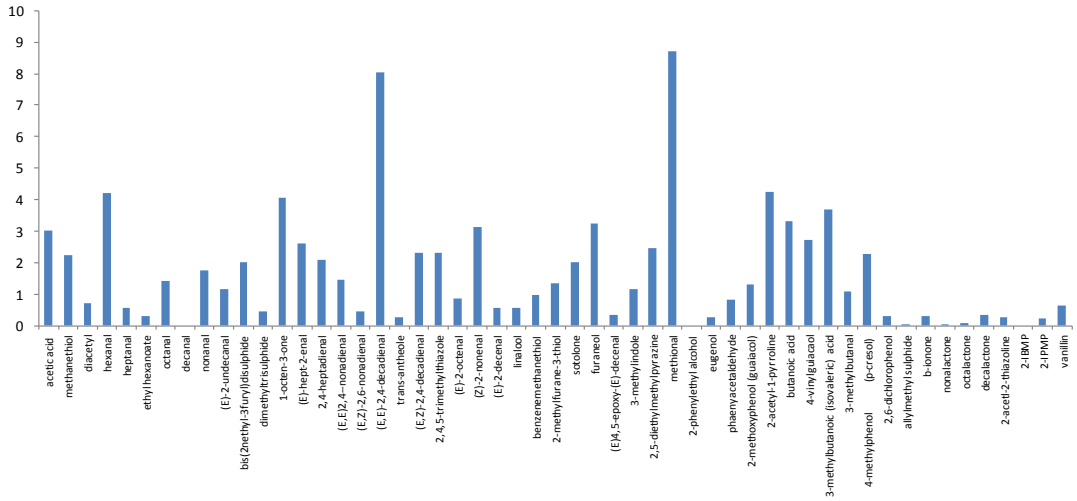

Figure 2. Composite aromagram based on a meta-analysis of available credible GC-O data from lamb and other meat aroma studies. Data from individual studies were scaled to a percent of total aroma stimulus before averaging.

5.8 Flavour release

Flavour and perception have important temporal components which are influenced by the composition and structure of food and oral processing. This applies to both non-volatile e.g. salt (de Loubins et al. 2011, Ventanas et al. 2010, Saint-Eve et al. 2010), amino acids (Toelstede & Hofmann 2008) and volatile compounds (e.g. aroma). The timing, rate and amount released from the food matrix are an intrinsic part of the sensory properties of a food. The presence of fat for example has a singularly profound effect on the release of volatiles in vivo (Frank et al. 2011, 2010). Other food components such as protein, peptides (Gianelli *et al.* 2003),

carbohydrates and water have also been shown to affect release. Meat is largely composed of protein, water and fat. The unique structure of muscle meat adds another layer of complexity, with muscle fibres, fat globules and intra- and intercellular water creating a unique flavour delivery matrix. During oral processing (mastication), the breakdown of muscle fibre and subsequent release of flavour form part of the unique sensory characteristics of meat. The ability of skeletal dipeptides, carnosine and anserine and sarcoplasmic protein (myoglobin) to interact with volatiles and affect their release has been demonstrated in a few studies (Gianelli et al. 2003). The role of fat on volatile release in meat products has also been reported (Chevance & Farmer 1999, Ventanas et al. 2010, Herrera-Jiménez et al. 2007, Carraipso 2007). Volatile release was measured by GC and olfactometry in frankfurters with different fat contents (5, 12 and 30% fat) (Chevance & Farmer 1999). In low-fat frankfurters, the volatile release of a number terpenes, sesquiterpenes and phenols was significantly increased leading to greater perceived smoky and spicy odours. A higher intensity of mushroom aroma was perceived in low-fat bologna sausages and the perceived juiciness was significantly higher and had longer duration in high fat variants (Ventanas et al. 2010). Few attempts have been made to measure dynamic in vivo release during consumption of meat products (Carrapiso 2007). In this study, although the release of volatiles was reduced by fat, the time to maximum concentration in the mouth (T_{max}) was not affected. In vivo studies using meat systems are required to understand implications for flavour development and perception. Dietary changes that affect the type, amount and distribution of fat within animal muscle – intramuscular fat (IMF) must be considered as having the potential to significantly affect the final flavour and sensory quality of meat (Ventanas *et al.* 2008, Bindon 2004). In a study comparing pork loins with high and low IMF, volatile compounds derived from lipid oxidation, such as 1-hexanol, octanal, (*E*,*E*)-2,4-heptadienal and (*E*)-2-decenal, as well as amino acid derived products such as dimethylsulfide, 3-methylbutanal or phenylacetaldehyde, were significantly higher in the headspace in high IMF samples (Ventanas *et al.* 2008). Further research on the effects of IMF on volatile generation and its effect on in vivo temporal release and perception are required.

5.9 Indirect flavour effects

Any changes in diet that affect the final protein or antioxidant status of muscle can theoretically affect the final flavour characteristics. Carnosine is the most abundant dipetide in skeletal muscle and has antioxidant activity (Namgung, et al. 2010). The histidine rich compound has been to shown to decrease lipid oxidation and minimise formation of odour active aldehydes and other volatiles. Diets high in histidine may affect the final carnosine content of meat and its overall antioxidant potential and hence the amount of lipid derived volatiles formed during cooking.

5.10 Other factors

It is important to note that, while diet does impact on ‘aroma’ and ‘taste’, these are not the only factors which will contribute to the overall consumer’s flavour perception

of the meat product. For example, different rearing systems have been reported to impact on the overall sensory quality of grilled lamb meat (Napolitano *et al*, 2002) and the volatile profile of broiled lamb meat (Osorio *et al*, 2008). Napolitano and co-workers found that a semi-trained sensory panel was able to discriminate between meat samples taken from lambs reared on a milk substitute, and animals raised on maternal milk. Milk replacer was used in the study by Osorio *et al*. and a large range of lipid oxidation products were evident in the volatile profile from the cooked meat of animals fed on milk replacer compared to those animals raised on maternal milk.

Diet can affect the fatty acid content of the meat (Cooper *et al*, 2004) as described above. Concentrate based feeds generally modify the fatty acid profile to closely resemble that recommended for human diet. The time of grazing is also known to impact on the profile of volatile compounds in lamb fat (Vasta *et al*). These workers were able to discriminate the profiles of animals grazed on pasture in the morning and those who were grazed in the afternoon using two compounds, 6-methyl-2-heptanone and 4-hydroxy-2,5-dimethyl-3-furanon. The latter compound is more commonly known as furaneol and is an aroma compound detected in wine (Ferreira *et al*, 2003) and fruit (Chen & Sidisky, 2011).

5.11 Consumer perspectives of sheepmeat and sheepmeat products

The sheep flocks of the world total about 1 billion animals (FAOSTAT, 2011), for wool and milk production, and after slaughter for meat, skins and wool. The largest flock is in China, about 130 million, followed by Australia (70), India (65), Iran and Sudan (50 each), Nigeria, New Zealand and the U.K. (30 each). Plots of sheep number by tonnes of sheep (and goat) meat available for consumption shows a rough linear relationship, meaning that much of each country's production is consumed locally. China is a clear example of dominant local consumption. Australia and New Zealand, by contrast, are major sheepmeat exporters, but even there consumption is high, particularly in New Zealand. In all these major production and consuming countries it may be safely assumed that a significant fraction of the population accept the characteristic flavour of sheepmeat as normal, whether cooked and eaten as primal cuts or as an ingredient in processed foods. These populations can be described as habituated. The source of sheep meat for processed foods is usually from older ovines, typically mutton, which is a cheaper source than lamb. In these processed foods the meat is usually comminuted, which eliminates any problem of toughness due to muscle origin and animal age. However, mutton is more strongly flavoured and in at least New Zealand – where although consumers are well habituated to the flavour – inclusion of mutton is viewed negatively. Thus, mainstream sausages prepared with mutton are often labelled 'beef flavoured sausages'; the converse, 'mutton flavoured sausages' is never seen.

The mental constructs of this phenomenon in New Zealand were explored by Lim (2001), who conducted a survey at a major agricultural show with 400 respondents. The female to male ratio was 1.6:1, with a wide spread of ages. Most respondents were of European descent. With no meat consumed or on view, they were asked to score seven meats for how good was their taste, their quality, and their healthiness

(Figure 3). Only the first of these attributes has an objective base, but all respondents scored the three attributes without comment. The left-to-right sequence of species on the ballot had lamb, hogget and mutton well spaced, but is presented in Figure 3 for easy comparison of lamb, hogget and mutton results.

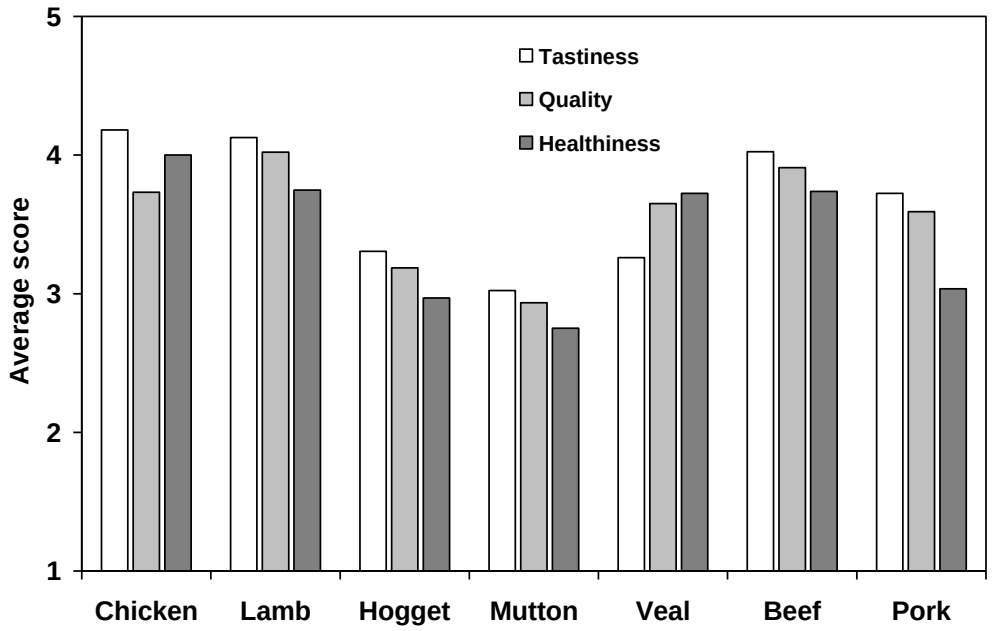

Figure 3. Consumer perceptions of meat attributes where 1 was low and 5 high. Data are means for 400 consumers. Standard deviation bars have been deleted for clarity, but very many of the differences were highly significant.

Means for lamb and beef were similar, but the perceptions of hogget and mutton attributes were strikingly lower. The perception for mutton taste may be based on reality, and for mutton quality may have its origins in tenderness, but with hogget the name is probably the driver of perception. This is because the objective eating quality attributes of sheepmeat are closely similar for lamb (roughly to age one year) and hogget (one to two years) (Young et al., 2005). Considering that at one point in a sheep's life a hogget is only day older than a lamb, the name hogget has an unfortunate marketing consequence. With the exception of a veal/beef distinction, no other meat type in Figure 3, nor venison, is fraught by names based on age.

Looking beyond perceptions within a population, there is no doubt that populations around the world vary in their liking of sheepmeat. Sheepmeat consumption in Australasia is hundreds of time greater than in Japan (FAOSTAT, 2011), and this is reflected in habituation. Prescott et al. (2001) spiked grain-finished beef with zero, low and high concentrations of mixed branched chain fatty acids, and of skatole to simulate nine flavour combinations of sheepmeat raised on pasture. These combinations were assessed as 'minced meat' by female Japanese and New

Zealand consumers. For Japanese, there was strict linear decrease in liking as fatty acid concentration increased. New Zealand consumers by contrast, liked the combination low fatty acid concentration best, confirming the effect of habituation. The results for skatole were more complicated, but the highest concentration was clearly most disliked by both populations.

Had males been included in these trials the results may well have been different. Young et al. (2009) assessed liking of nitrite/salt-cured sheepmeat sausages where sugars were added to reduce perceptions of sheepmeat flavour through generation of Maillard reaction products that could mask the flavour. Xylose addition was the most useful, but of more interest was a gender effect on identification. The source meat was not identified and respondents had to identify the species from a proffered list of five. Misidentification was greatest with xylose, but much more so for males than females. Based on some disparate research on perceptions of volatile fatty acids, it was proposed that misidentification may be associated with the likely greater sensitivity of females to these fatty acids, which are components of sweat.

The concept that females are more sensitive to volatile fatty acids, which include the branched chain fatty acids of interest, has support in an unrelated study of goat yoghurt (Young et al., 2011). Goat fats also contain the branched chain fatty acids that occur in sheep fats, which along with other (free) fatty acids can be rendered mostly non-volatile by forming inclusion complexes with cyclodextrins added to liquids like milk and yoghurt. β-Cyclodextrin was much less effective with females than males in masking goaty flavour in yoghurt.

Given that unhabituated populations are less accepting of sheepmeat, the challenge remains to reduce perceptions of branched chain fatty acids for those populations. Masking flavours with herbs and spices is the obvious path to take and each culinary tradition has its 'flavour principles' (Rozin & Rozin, 1981) that could be used. The importance of this was confirmed by Prescott et al. (2004) who compared the reactions to lamb – labelled as such – of ethnic Chinese females in Singapore with those of New Zealand females of European descent. The lamb was flavoured to characterise Chinese cuisine. In spite of the fact that the Singaporeans ate much less lamb, their liking of lamb flavoured with Chinese spices far exceeded the liking of that product by New Zealanders.

Lu (2010) extended the work of Prescott et al. (2001) by spiking beef with high concentrations of branched chain fatty acids and skatole (together called sheep flavour). This meat was used in a glucose-fermented sausage, with which she compared the effects of sheep flavour, nitrite curing, and spicing (rosemary plus garlic extracts) in the eight possible combinations, all under lighting to hide colour. As isolated treatments, neither curing nor spicing affected the marked difference in liking between sausage treatments with and without added sheep flavour (respondents did not like the sheep flavour). However, combined curing and flavouring almost entirely overrode the negative effect of added sheep flavour. Thus, ovine and pastoral flavours should be more acceptable to unhabituated consumers where fermented sausage flavoured with 'flavour principles' is simultaneously cured.

Given that intense sheepmeat flavours can be masked by a number of treatments, there is no fundamental impediment to creating a range of sheepmeat products designed for the unhabituated. For the habituated – those who accept or even seek the sheepy flavour – only one impediment remains in producing desirable products from lower cost sheepmeat. It is the names hogget and mutton.

Different forages/grains available

- – to be inserted by Robyn Warner
- including characteristics, ME, CP, other compounds (glucosinylates, etc.).
- Brief overview of metabolism in animal and necessity for growth and fat deposition

Include anti-oxidants here

Influence of diet on fat deposition and growth

Effects of diet on 'taste' of sheepmeat

- - to be inserted by Tanoj Singh
- Points as for aroma

5.12 Conclusions and Recommendations

To be inserted

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Table 1: Impact of various feeding regimes on flavour of sheepmeat

Feeding system	Impact on flavour	Type[A]	Attribute[B]	Reference
White clover *vs* ryegrass	Stronger flavour/odour for white clover	T	T+O	(Shorland *et al*, 1970)
Lucerne *vs* perennial ryegrass	More intense flavour/odour for lucerne	T	T+O	(Nicol & Jagusch, 1971)
Lucerne *vs* phalaris	Lucerne > phalaris	T	T	(Park *et al*, 1972a)
Rape, vetch, oats *vs* pasture	Low acceptability for rape	T	T	(Park *et al*, 1972c)
	Some differences found for vetch & oats			
Tropical legumes *vs* grass	No significant difference	T	T	(Park & Minson, 1972)
Brassica rapus vs pasture	Brassica - strong, unattractive odour/flavour	T	T	(Wheeler *et al*, 1974)
Lucerne	Lucerne related flavour increased	T	T	(Park *et al*, 1975)
Alfalfa *vs* Corn/soybean	Flavour more intense for alfalfa	T	T	(Crouse *et al*, 1981)
Parthenium weed *vs* grain	Panel could differentiate "taint" - differences small	T	T	(Tudor *et al*, 1982)
White clover, lucernce, lotus, ryegrass *vs* corn, corn + fescue	Corn finished samples < forage finished	U	T+O	(Bailey M. E. *et al*, 1994)
Cowpea varieties, alfalfa *vs* grain/oat hay	Grain < cowpea, alfalfa			
Ryegrass, tall fescue, cocksfoot, Phalaris, lucerne, chicory, prairie grass	Phalaris ("foreign flavour") > others	T	T+O	(Young *et al*, 1994)
Chicory *vs* lucerne	No difference	U	T	(Hopkins *et al*, 1995)
Rape *vs* pasture	Stronger, less acceptable flavour for rape	U	T	(Hopkins *et al*, 1995)
Perennial ryegrass + other grasses *vs* grain-based	"Sheepmeat" higher for pasture than grain	T	T+O	(Rousset-Akrim *et al*, 1997)
Saltbush *vs* barley/lupin/hay	No difference	U	T	(Pearce *et al*, 2003)
Pasture *vs* grain concentrate	"Lamb" flavour higher in concentrate	T	T+O	(Priolo *et al*, 2001)
	Grass-fed animals; higher in"liver" flavour	T	T+O	
Pasture *vs* lucerne or maize concentrate	"Sheepmeat" higher for pasture	T	T+O	(Young *et al*, 2003)

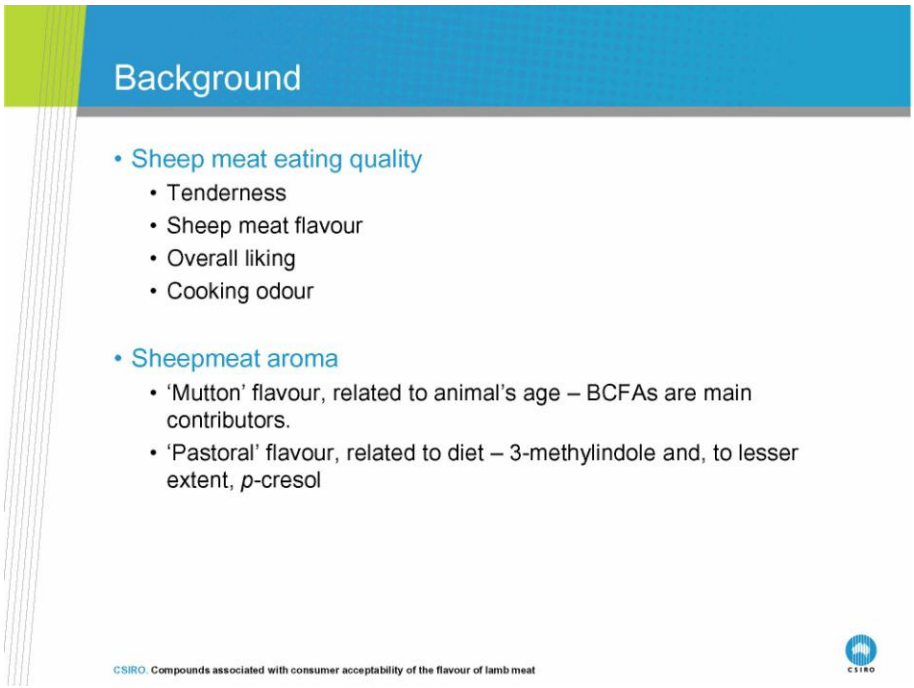
Ryegrass *vs* concentrate	"Off" odours/flavours in pasture-fed meat	T	T	(Borton *et al*, 2005)
Mixed pasture *vs* grain-based or poor quality dry feed	No difference bn pasture *vs* grain	U	T	(Pethick *et al*, 2005)
Ryegrass *vs* saltmarsh, heather, moorland	ryegrass < others	nr		(Whittington *et al*, 2006)
Lotus *vs* ryegrass *vs* white clover	No influence on meat flavour;	T	T	(Farouk *et al*, 2007)
	(*p*-cresol negatively corr'd with sheepy odour)			
Milk *vs* milk replacer (rearing system)	No discrimination	U	T	(Osorio *et al*, 2008)
Pasture *vs* concentrate *vs* pasture/concentrate	Differences based consumer (country)preference	U	T	(Font i Furnols *et al*, 2009)
Cultivated pasture *vs* mountain pasture (Norway)	Minor differences in 'metallic' and 'rancid	T	T+O	(Lind *et al*, 2009)
Pasture *vs* concentrate *vs* pasture/concentrate	Lower acceptance of pasture-fed animals	T	T	(Resconi *et al*, 2009)
Grass/clover *vs* chicory	No appreciable difference	T	T+O	(Houdjik, 2010)
Cottonseed meal *vs* corn dried distillers grains	No difference	T	T+O	(Whitney & Braden, 2010)

Note [A]Type = type of sensory panel T = trained U = Consumer (untrained) nr = not recorded [B]Flavour attribute tested T = taste O = odour/aroma

Table 2. Chemical compounds known to be associated with pasture based and grain based diets in ruminants

Pasture-based	Reference	Grain-based	Reference
Diterpenoids	(Suzuki & Bailey, 1985)(Young *et al*, 1997), (Sivadier *et al*, 2010)	γ-Lactones	(Bailey M. E. *et al*, 1994)
2,3-Octanedione	(Suzuki & Bailey, 1985)(Young *et al*, 1997), (Priolo *et al*, 2004)	Longer chain aldehydes (2-undecanal)	(Young *et al*, 1997)
3-hydroxyoctan-2-one	(Suzuki & Bailey, 1985)	short-branched, non-branched FAs	(Sebastián *et al*, 2003)
δ-Lactones	(Bailey M. E. *et al*, 1994)	4-heptanone 2-octanone	(Sebastián *et al*, 2003), (Resconi *et al*, 2010)
Long chain alkanes	(Sebastián *et al*, 2003)	3-Hydroxy-2-butanone	(Almela *et al*, 2010)
C7 aldehydes	(Sebastián *et al*, 2003)	alkenals, alkadienals, Strecker aldehydes and ketones	(Resconi *et al*, 2010)
Sesquiterpenes/terpenes	(Priolo *et al*, 2004), (Sivadier *et al*, 2010)		
Hexanoic acid	(Almela *et al*, 2010)		
BCFA	(Young *et al*, 1997)		
3-Methylindole	(Young *et al*, 1997)(Young *et al*, 2003) (Almela *et al*, 2010)		
Phenols	(Lane & Fraser, 1999), (Almela *et al*, 2010)		
Toluene	(Sivadier *et al*, 2010)		

6 Slides for presentation



Sheep CRC

- Information Nucleus Flock
  - Sheep CRC measured various genotypes
  - Relate genetics to genotypes
  - Consumer sensory scores (MSA) of grilled meat
  - 'Flavour', 'Like Smell', 'Juicy', 'Overall Like' MSA sensory attributes
- Aim
  - Test relationship between consumer sensory scores with 'pastoral' and 'mutton' flavour compounds

Chemical analysis

		Terminal	Maternal	Merino
Site	Compound	Mean ± s.e.	Mean ± s.e.	Mean ± s.e.
Katanning	MOA[A]	0.230 ± 0.02	0.215 ± 0.032	0.093 ± 0.009
	EOA	0.051 ± 0.006	0.039 ± 0.007	0.0185 ± 0.004
	MNA	0.050 ± 0.002	0.043 ± 0.003	0.030 ± 0.003
	PC[B]	121.0 ± 14.5	88.7 ± 16.7	34.5 ± 6.4
	MI	26.1 ± 4.2	30.3 ± 10.3	18.7 ± 4.2
Tamworth	MOA	0.53 ± 0.04	0.344 ± 0.061	0.172 ± 0.040
	EOA	0.170 ± 0.020	0.078 ± 0.018	0.0338 ± 0.0179
	MNA	0.097 ± 0.006	0.104 ± 0.009	0.038 ± 0.06
	PC	171.5 ± 37.2	113.1 ± 18.2	118.2 ± 21.0
	MI	34.4 ± 7.7	17.93 ± 4.43	30.4 ± 7.4

[A]MOA = 4-methyloctanoic acid, EOA = 4-ethyloctanoic acid, MNA = 4-methylnonanoic acid (mg/kg)
[B]PC = *p*-cresol, MI = 3-methylindole (μg/kg)

REML analysis

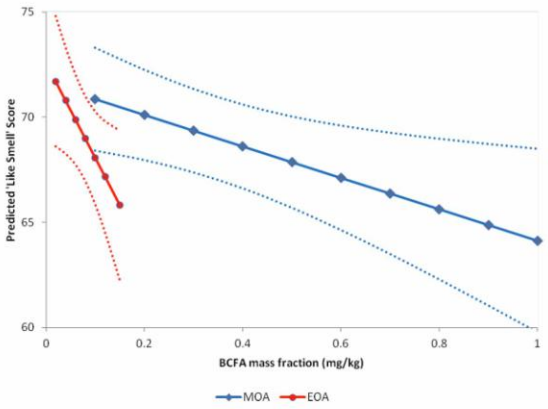
- Effect on 'Like Smell'

Term	Numbers	MOA	Model EOA	MOA + EOA
Site	*P*-value	0.798	0.879	0.866
Kill date	*P*-value	0.062	0.122	0.082
Siretype	*P*-value	0.591	0.635	0.412
EOA	*P*-value		**0.040**	0.086
	Coefficient		-45.34	-35.70
MOA	*P*-value	**0.013**		**0.031**
	Coefficient	-7.49		-6.66

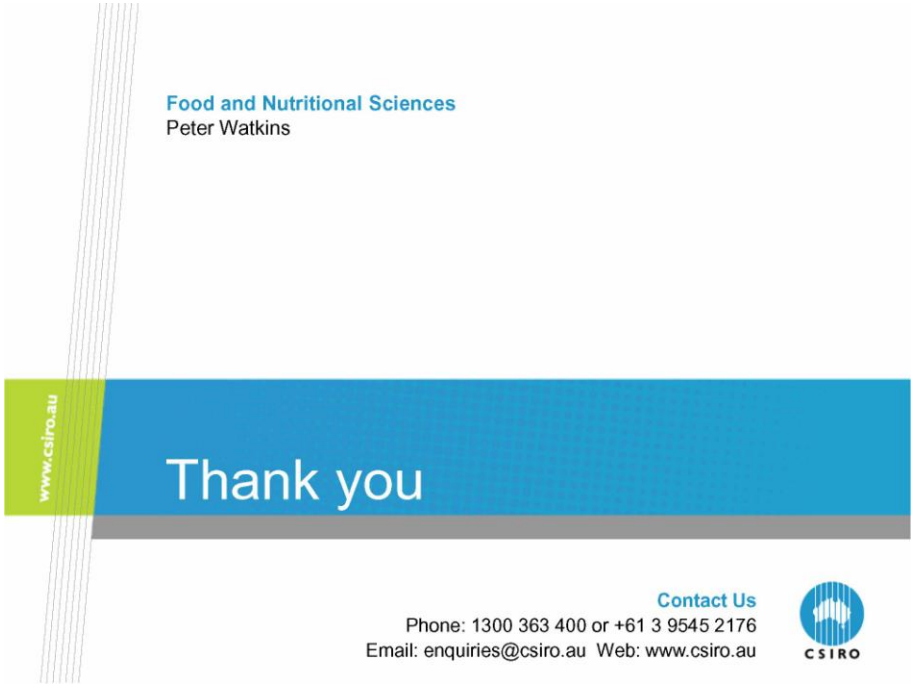
- Other models
  - 'Flavour' not significant ($P > 0.10$)
  - 'Overall Like' highly correlated with 'Like Smell', ∴ excluded

CSIRO. Compounds associated with consumer acceptability of the flavour of lamb meat

Effect of MOA and EOA on 'Like Smell'



CSIRO. Compounds associated with consumer acceptability of the flavour of lamb meat


Food and Nutritional Sciences
Peter Watkins
www.csiro.au
Thank you
Contact Us
Phone: 1300 363 400 or +61 3 9545 2176
Email: enquiries@csiro.au Web: www.csiro.au
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7 Conclusions and recommendations

The aim of the analysis of the consumer data in relation to chemical compounds was to determine what impact, if any, that BCFAs, *p*-cresol or 3-methylindole had on a set of consumer sensory attributes of the associated grilled meat samples obtained from the Sheep CRC. Of these compounds, MOA and EOA were the most significant contributors to impact on the perceived smell of the cooked meat. A higher consumer acceptance of the grilled meat was found for fat samples that contained lower concentrations of these compounds. It is recommended that the contribution of these compounds to the variability in acceptance of sheepmeat in Australia undergo further investigation.

A review of 'Sheepmeat flavour and the effect of diet on eating quality' has been conducted and is included. The review describes flavour perception, influence of various forages and supplements on cooked sheepmeat flavour and acceptability, and the volatiles generated during cooking which are known to be associated with flavour. Condensed tannins are present in some forages and the influence of these on pastoral flavour is discussed. The analytical techniques for detection of the volatiles and other compounds contributing to taste and aroma are presented, including gas chromatography olfactometry. The possible influence of texture and fat content of sheepmeat on the temporal flavour release in the mouth is reviewed. Finally, consumer perspectives of sheepmeat and processing techniques to ameliorate sheepmeat flavour are discussed.

It is recommended that further data should be collected. In particular, the linking of trained taste panel data with consumer data, and with chemical analysis of a greater range of compounds than those reported here is recommended. This would allow the compounds contributing to the unfavourable, and favourable, variations in consumer acceptability in domestic and export markets to be quantified. This would also allow the development of possible strategies for ameliorating any unfavourable flavours in sheepmeat. This would potentially enable the tailoring of sheepmeat and shepmeat products to specific markets.

Appendices

7.1 Appendix 1

Table A1.1 *P* values for terms in the models relating the **'Like Smell', 'Flavour'** and **'Overall Like'** consumer sensory scores to the mass fractions of 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA), 4-methylnonanoic (MNA) acids, 3-methylindole (MI) and *p*-cresol (PC) as covariates with Site and Siretype included as fixed effects. Significant terms ($P < 0.05$) are shown in bold. Terms close to significance ($P < 0.1$) are shown as italicised and underlined.

Attribute	Site	Siretype	MOA	EOA	MNA	PC	MI
Like Smell	0.128	0.394	**0.010**				
Flavour	0.810	0.185	0.171				
Overall Like	*0.088*	0.243	*0.070*				
Like Smell	*0.075*	0.657		**0.011**			
Flavour	0.841	0.102		*0.085*			
Overall Like	**0.036**	0.118		**0.022**			
Like Smell	0.535	0.603			0.242		
Flavour	0.429	0.122			0.553		
Overall Like	0.255	0.148			0.417		
Like Smell	0.873	0.786				0.601	
Flavour	0.235	**0.050**				*0.085*	
Overall Like	0.452	*0.059*				0.116	
Like Smell	0.816	0.744					0.994
Flavour	0.332	*0.092*					0.879
Overall Like	0.353	*0.096*					0.812

Table A1.2. *P* values for terms in the models relating **'Flavour'** consumer sensory score to the mass fractions of 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA), 4-methylnonanoic (MNA) acids, 3-methylindole (MI) and *p*-cresol (PC) as covariates with Site and Siretype included as fixed effects. Significant terms ($P < 0.05$) are shown in bold. Terms close to significance ($P < 0.1$) are shown as italicised and underlined.

Site	Siretype	MOA	EOA	MNA	MI	PC
0.712	0.119		0.158		0.630	
0.900	0.212	0.238			0.631	
0.510	0.142			0.795	0.591	
0.265	**0.044**				0.578	0.114
0.550	0.191	0.326	0.221			
0.924	0.175	0.106		0.283		
0.858	0.120	0.121				0.153
0.569	0.168		0.114	0.474		
0.799	*0.060*		0.142			0.195
0.444	*0.077*			0.464		0.163
0.535	0.117		0.124	0.294		0.179
0.956	0.113	*0.094*		0.330		0.169
0.564	0.127	0.243	0.273			0.182
0.536	0.216	0.273	0.362		0.716	
0.550	0.193		0.151	0.496	0.709	
0.829	*0.061*		0.178		0.649	0.139
0.564	0.127	0.243	0.273			0.182
0.923	0.125	0.110			0.702	*0.094*
0.481	*0.079*			0.456	0.655	0.106
0.556	0.121		0.156	0.290	0.755	0.121
0.978	0.117	*0.084*		0.315	0.657	0.105
0.574	0.135	0.210	0.347		0.754	0.118
0.576	0.129	0.606	0.374	0.991		0.184
0.607	0.214	0.499	0.488	0.762	0.703	
0.612	0.136	0.497	0.499	0.899	0.749	0.120

Table A1.3. *P* values for terms in the models relating **'Like Smell'** consumer sensory score to the mass fractions of 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA), 4-methylnonanoic (MNA) acids, 3-methylindole (MI) and *p*-cresol (PC) as covariates with Site and Siretype included as fixed effects. Each row shows an individual model that was tested. Significant terms ($P < 0.05$) are shown in bold. Terms close to significance ($P < 0.1$) are shown as italicised and underlined.

Site	Siretype	MOA	EOA	MNA	MI	PC
0.055	0.643		**0.017**		0.397	
0.113	0.366	**0.014**			0.499	
0.527	0.600			0.399	0.445	
0.837	0.822				0.400	0.319
0.015	0.387	**0.056**	**0.055**			
0.067	0.430	**0.003**		*0.059*		
0.128	0.498	**0.010**				0.346
0.022	0.474		**0.010**	0.196		
0.061	0.756		**0.014**			0.460
0.559	0.700			0.312		0.398
0.024	0.573		**0.011**	0.158		0.411
0.069	0.533	**0.002**		**0.050**		0.398
0.017	0.491	**0.045**	**0.046**			0.394
0.017	0.370	*0.056*	*0.073*		0.522	
0.025	0.468		**0.014**	0.216	0.504	
0.067	0.756		**0.019**		0.432	0.381
0.017	0.491	*0.064*	**0.045**			0.394
0.110	0.480	**0.008**			0.567	0.200
0.529	0.682			0.304	0.493	0.286
0.027	0.571		**0.015**	0.159	0.553	0.319
0.058	0.520	**0.002**		**0.047**	0.480	0.263
0.019	0.477	**0.037**	*0.092*		0.571	0.279
0.034	0.508	*0.099*	0.318	0.416		0.415
0.037	0.391	*0.072*	0.426	0.288	0.474	
0.040	0.497	*0.069*	0.444	0.325	0.526	0.289