Effect of branched-chain fatty acids, 3-methylindole and 4-methylphenol on consumer sensory scores of grilled lamb meat

P.J. Watkins, G. Kearney, G. Rose, D. Allen, A.J. Ball, D.W. Pethick, R.D. Warner

PII: S0309-1740(12)00278-1
DOI: doi: 10.1016/j.meatsci.2012.08.011
Reference: MESC 5807

To appear in: *Meat Science*

Received date: 30 April 2012
Revised date: 10 August 2012
Accepted date: 11 August 2012

Please cite this article as: Watkins, P.J., Kearney, G., Rose, G., Allen, D., Ball, A.J., Pethick, D.W. & Warner, R.D., Effect of branched-chain fatty acids, 3-methylindole and 4-methylphenol on consumer sensory scores of grilled lamb meat, *Meat Science* (2012), doi: 10.1016/j.meatsci.2012.08.011

Effect of branched-chain fatty acids, 3-methylindole and 4-methylphenol on consumer sensory scores of grilled lamb meat

P.J. Watkins[1]*, G. Kearney[3], G. Rose[4], D. Allen[4], A.J. Ball[2,5], D.W. Pethick[2,6] and R.D. Warner[1,2].

[1]CSIRO Animal Food and Health Sciences, 671 Sneydes Road, Werribee, 3030**, Australia**

[2]Co-operative Research Centre for Sheep Industry Innovation, CJ Hawkins Homestead, University of New England, Armidale, NSW, 2351**, Australia**

[3]36 Paynes Road, Hamilton, Vic. 3300**, Australia**

[4]Future Farming Systems Research Division, Department of Primary Industries, Ernest Jones Drive, Macleod, Vic. 3085**, Australia**

[5]Meat & Livestock Australia, CJ Hawkins Building, University of New England, Armidale, NSW 2351**, Australia**

[6]School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch WA 6150**, Australia**

*Corresponding author: e-mail address: Peter.Watkins@csiro.au.

Abstract

Tenderness, flavour, overall liking and odour are important components of sheepmeat eating quality. Consumer assessment of these attributes has been made for carcasses from the Information Nucleus Flock (INF) of the Cooperative Research Centre for Sheep Industry Innovation. **The concentrations of three branched chain fatty acids, 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA) and 4-methylnonanoic acids (compounds related to 'mutton flavour' in cooked sheepmeat) and 3-methylindole and 4-methylphenol (compounds related to 'pastoral' flavour) were determined for 178 fat samples taken from INF carcasses. Statistical modelling revealed that both MOA and EOA impacted on the 'Like Smell' consumer sensory score of the cooked meat product ($P < 0.05$), with increasing concentration causing lower consumer acceptance of the product. None of the compounds though had an effect on the liking of flavour. Obviously, reducing the effect of MOA and EOA on the odour of grilled lamb will improve consumer acceptance of the cooked product but other factors affecting the eating quality also need to be considered.**

Keywords: sheepmeat, BCFA, 3-methylindole, meat quality, consumer sensory, lamb

1 Introduction

Tenderness, sheep meat flavour, overall liking and cooking odour are regarded as important components of the eating quality of sheep meat (Pleasants et al., 2005**;** Pethick et al., 2005b). For odour, two aromas have often been associated with cooked sheepmeat. The first aroma, generally labelled 'mutton' flavour, is usually associated with an animal's age while the second, generally described as 'pastoral' flavour, is associated with an animal's diet (Young and Braggins, 1998).

Mutton flavour, regarded as the characteristic flavour associated with the cooked meat of older sheep, 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 (Young and Braggins, 1998). Branched chain fatty acids (BCFAs; 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA) and 4-methylnonanoic acids) are the chemical compounds that are accepted as the main contributors for this flavour and research continues to explore the role of these compounds and their contribution to 'mutton' odour (Young et al., 1997).

'Pastoral' flavour becomes evident as a result of cooking the meat of pasture fed ruminants **(Young et al., 1997)**. In Australia, the feed for the domestic flock is **pasture-based** with grain feeding used in summer and autumn, depending on the availability of pasture from irrigation and the length of the dry period (**Rowe, 1986; Wales et al., 1990; McFarland et al., 2006).** Untrained taste panels of Australian consumers are not able to distinguish between meat product obtained from lambs finished on pasture and grain-based diets (Pethick et al., 2005a). This verifies that although pasture is the main feed material for sheep in Australia, Australian consumers are habituated to the presence of pastoral flavour in locally produced sheepmeat. 3-Methylindole, also involved with 'boar' taint in pigs, and to a lesser extent 4-methylphenol (*p*-cresol) are the main compounds implicated as contributors to 'pastoral' flavour (Young et al., 2003).

The Co-operative Research 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. This work included evaluating cooked meat products, using consumer sensory panels according to Meat Standards Australia (MSA) protocols (Thompson et al., 2005a). As far as we are aware, no study has been performed which examines whether there is a relationship between the compounds responsible for 'pastoral' and 'mutton' flavours in sheepmeat **(in either lamb or older animals)** and consumer sensory attributes. The aim of this study was therefore to identity the effect of BCFAs (4-methyloctanoic (MOA), 4-ethyloctanoic (EOA) and 4-methylnonanoic (MNA) acids), 3-methylindole and 4-methylphenol **measured in sheep fat** on consumer sensory attributes of grilled lamb meat.

2 Materials and methods

2.1 Fat samples

The samples used were taken from lamb carcasses from the Information Nucleus Flock (INF) of the Cooperative Research Centre for Sheep Industry Innovation (Sheep CRC**, Armidale, New South Wales, NSW**) and the design of the INF has been presented elsewhere (Fogarty et al., 2007). The age of the lambs ranged from 215 to 362 d. The results presented in this paper are based on 178 samples taken from a subset of 760 animals **of the 2009/2010 cohort lamb progeny**, selected from the Katanning (Western Australia, WA) and Kirby (NSW) research flocks for sensory testing. **A summary of the nutritional history of these animals is shown in Table 1 (Ponnampalam et al 2012).** The lambs were slaughtered at two separate abattoirs (Tamworth, NSW and Katanning, WA) on four separate dates at Tamworth and three dates for Katanning.

At 24 hr post-mortem the *longissimus thoracis et lumborum* (LTL) and *semimembranosus* (SM) muscles were excised from the carcase, and were vacuum packed and stored at 2°C to age for 5 days. Subcutaneous fat and silver skin were removed, and 5 steaks from each muscle of 15 mm thick were cut and frozen at -20°C for subsequent sensory testing and chemical analysis. The LTL and SM were assessed by MSA consumer panels, as described by Pannier **et al.** (2012 – this edition). **Briefly, the steaks were cooked by grilling on a Silex S-165 clam shell grill unit (Silex Grills Australia Pty Ltd, Marrickville, NSW, Australia) set at 220 – 230 °C. The cooking was controlled by a timer to produce a constant medium degree of doneness (internal temperature of about 65 degrees) and then rested for 2 min prior to tasting (Thompson et al., 2005b). The MSA testing panels consisted of untrained consumers who were familiar with sheepmeat and consumed a meal of cooked meat at least once per fortnight. Details on recruitment of the consumers are given elsewhere (Thompson et al., 2005b). The u**ntrained consumers were used to assessed the steaks for tenderness, juiciness, liking of the flavour ('**Like Flavour**'), liking of the smell ('Like Smell') and overall liking ('Overall Like') based on a 1 to 100 score. **Consumers also graded the samples into the following categories; unsatisfactory, good every day (3 star), better than every day (4 star), or premium (5 star).** Every muscle was tasted 10 times by **6** different consumers, and the individual consumer scores with the **mean** score of the 10 **consumer score**s per sample were recorded. **There was a total of 43 sampling sessions, with 60 consumers per session, which assessed the grilled meat. The mean of the 'Like Flavour' and 'Like Smell' consumer scores for each sample was used for the subsequent statistical analysis. The associated subcutaneous fat samples** (20 g)**, taken from over the *gluteus medius* muscle site at 1 hr post-slaughter, were stored at -20 °C. The samples were transported respectively from NSW and WA to Werribee (Victoria) at -20 °C for chemical analysis. The**

samples were kept at this temperature until required for analysis. The cohort of 178 fat samples were selected to be representative of the range of the mean consumer flavour scores of LTL, according to sire type (Terminal (*n* = 122), Maternal (*n* = 31) or Merino (*n* = 25)) and **production site (Kirby (*n* = 89)** and Katanning **(*n* = 89)**). **The mean hot carcass weight** was 24.7 ± 0.3 (standard error) kg while the mean G**R** fat depth was 16.5 ± 0.5 mm.

2.2 Chemicals

4-Methyloctanoic (MOA), 4-methylnonanoic (MNA), 4-ethyloctanoic (EOA) and undecanoic acids as well as 4-methylphenol (MP) and 3-methylindole (MI) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia) **and used** without purification. Divinylbenzene/Carboxen®/polydimethylsiloxane (DVB/Car/PDMS) solid phase micro-extraction (SPME) fibres were obtained from Sigma-Aldrich. The SPME fibres were pre-conditioned at 280 °C for 90 min. Solvents used were of pesticide grade quality. Nitrogen and helium were ultra-high purity grade (Coregas, Altona, Vic., Australia). All other reagents were of analytical reagent grade.

2.3 Measurement of branched chain fatty acids

The **fat** samples were wholly melted by heating **4 X 5 g portions between 3 to 5 min (sufficient to melt but not cook the fat)** in a **domestic** microwave oven, ensuring homogeneity of the sample. A sample of the liquid fat (1 g) was injected into a Unitrex sweep co-distillation unit (SGE, Ringwood**, Vic.**) and heated at 200 °C for 1 hr under a flow (200 mL min^{-1}) of nitrogen. Each batch of ten samples included one spiked recovery fat sample containing the internal standard, undecanoic acid (C_{11} FA, 1.00 µg mL^{-1}). 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^{-1}), the sample was treated with (*N*,*O*)-bisilyltrifluoroacetamide at 60 °C for 30 min and **the free fatty acids (including BCFAs)** were derivatised as the trimethylsilyl (TMS) esters.

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 (MS) operating in full scan mode. The septumless programmable injector (SPI) was programmed starting at 45 °C and increased to 325 °C at a rate of 180 °C min^{-1}. The GC oven was held at 75 °C for 2 min then increased to 300 °C at a rate of 10 °C min^{-1} and held at this temperature for 8 min. Helium was used as the carrier gas at a constant pressure of 105 kPa. The MS transfer line was **held at** 280 °C.

Mass spectra were acquired using an ion source temperature of 220 °C and an electron multiplier voltage of 2400 V. The MS was calibrated using FC43 (Varian, Inc., Springvale**, Vic.**).

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^{-1} (or mg kg^{-1} effective concentration in sheep fat) and the standard solutions were **similarly** 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^{-1}. Calculation of the concentration for a given BCFA was made using: [BCFA] (µg kg^{-1}) = $k.\ A_{BCFAsample}/A_{ISsample}$, where k is the slope of a linear calibration curve with intercept set to zero, $A_{BCFAsample}$ 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.

2.4 Measurement of 4-methylphenol and 3-methylindole

After heating at 60 °C for 30 min, 1 g 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 using a CombiPAL SPME autosampler (CTC, Switzerland). The DVB/Car/PDMS fibre was inserted into the headspace above the sample and held for 30 min. Subsequently, the autosampler withdrew the fibre and inserted it into the injector of a Model 6890 gas chromatograph (GC, Agilent, Palo Alto, CA, US**A**) where the adsorbed compounds were desorbed for transfer to the analytical column. The fibre was held in the injector (230 °**C**) 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.

The volatile compounds were separated using a HP-VOC column (Agilent, 60m X 0.32 mm i.d. X 1.8 µm film thickness) in the Model 6890 GC. The oven temperature was initially held at 100 °C and then increased to a final temperature of 280 °C at a rate of 6 °C min^{-1}. Helium was used as the carrier gas with a constant **flow rate** of 1.2 mL min^{-1}. The transfer line was heated at 280 °C. The mass selective detector (Model 5973) 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 4-methylphenol, *m*/*z* = 107 and 108, and 3-methylindole, *m*/*z* = 130 and 131.

orking calibration standard solutions were prepared by spiking hydrogenated coconut oil with 4-methylphenol and 3-methylindole. The standard concentration range for 4-methylphenol was 0 to *ca* 300 μg kg^{-1} (= ng g^{-1}) while, for 3-methylindole, it was 0 to *ca* 250 μg kg^{-1} which spanned the expected range of these compounds in sheep fat. Quantification was performed using the external standard technique.

2.5 Statistical analysis of consumer sensory attributes

Initial models tested the significance of each chemical compound in relation to the three consumer attributes 'Overall Like', '**Like Flavour**' and 'Like Smell', using models with fixed and random terms similar to those used previously (Warner et al., 2010). The restricted maximum likelihood method (REML) was used for all data analyses with abattoir site, slaughter date nested within abattoir site (Site.DATE; May 27, June 21, July 26 and August 23, 2010 for the **Kirby** samples, and February 21, March 16 and May 25, 2010 for the Katanning samples), 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. For convenience, **s**ire 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, MP and MI. The most parsimonious model for each variate was chosen using Wald tests **and** approximate *F* statistics (Kenward and Roger 1997). All statistical analyses were performed using GENSTAT software (12th Edition, VSN International Ltd, Hemel Hempstead, UK).

3 Results and discussion

3.1 Sample description

In total, a subset of **178** fat samples was selected from the CRC cohort and chosen to be representative of the range of the mean '**Like Flavour**' consumer sensory scores. Figure 1 shows a histogram for the distribution of '**Like Flavour**', 'Like Smell' **and 'Overall Like'** values for samples of 'Terminal' sire type and approximately normal (Gaussian) shaped curves can be observed for each attribute. This indicated that no bias had been introduced in selection of the samples and so would be representative of the larger cohort.

MOA was the most abundant BCFA in the samples (Table 2). For example, the mean MOA concentration for sheep of Terminal sire type of the samples taken at Katanning was 230 μg kg^{-1} while, for EOA and MNA, the mean concentrations were 51 and 50 μg kg^{-1}, respectively. This result is comparable to the range of BCFAs reported for a survey of the Australian meat sheep flock (Watkins et al., 2010) where, as in this study, MOA was the most abundant of the BCFAs while MNA was the least abundant and EOA intermediate between these two compounds. The **MP and MI** content of the samples were also measured for these samples (Table 2). These results are comparable to those reported by other workers where **MP** has been found to lie between 5 and 246 μg kg^{-1} (Ha and Lindsay, 1991) while MI has spanned the range of 31 to 154 μg kg^{-1} (Schreurs et al., 2007).

The odour sensory threshold is used to define the minimal quantity detectable by nasal perception (Brennand et al., 1989). In the case of the BCFAs, the odour thresholds for MOA, EOA and MNA are reported (in water) as 20, 6 and 650 μg kg^{-1} respectively (Brennand et al., 1989) while, for MI and MP, these have been reported (in synthetic butter) as 50 and 0.2 μg kg^{-1} respectively (Urbach et al., 1972). The measured concentrations for MOA, EOA and MP were above their respective sensory threshold values for most samples (Figure 2). The odour activity value (OAV), defined as the ratio of concentration of an odourant to its odour sensory threshold (Chaintreau, 2002), is used to quantify a compound's importance to the odour of a food material. **Using threshold values for water and synthetic butter represents a compromise for calculating the OAVs of these compounds in sheep fat since, in food, the support media (water, fat, etc.) has an influence on the odour threshold value. Thus, it needs to be noted the OAVs can be viewed as approximations but still indicative of the impact of each compound on the overall odour.** Based on the mean concentrations shown in Table 1, the odour activities were calculated for each BCFA as well as **MP** and MI. MOA and EOA were the most significant BCFAs with comparable ranges of odour activities (OAV = 4.7 to 26.5 and 3.1 to 28.3, respectively) while MNA was the least active (OAV = 0.0 to 0.1). MP had the highest potential odour activity (OAV = 172.5 to 857.5) while MI was of the same order of magnitude as MNA (0.4 to 0.7). Thus, MOA, EOA and **MP** would be expected to be significant contributors to the odour of the grilled meat.

Each BCFA (MOA, EOA and MNA), along with octanoic acid, have goat- and mutton-like odours (Brennand et al., 1989) and so, when present in sufficient concentration, each of these compounds will contribute to the 'mutton' odour produced during the cooking process. Four other fatty acids may also contribute to the final odour; namely, 3-methylpentanoic, 6-methylheptanoic, 6-methyloctanoic and 8-methylnonanoic acids. These compounds have been reported by Brennand et al. (1989) to have sheepy and wool-like odours, and also have low odour threshold values which suggest that these compounds would be significant odourants. 6-Methyloctanoic and 8-methylnonanoic acids were reported by Wong et al. (1975) to be present in the odour resulting from cooking minced mutton meat. If present in sufficient concentration, these fatty acids would also make contributions to the odour. Considerable attention has been given to the contribution that MOA, EOA and MNA make to 'mutton' flavour and yet the work of Brennand and co-workers suggests that 6-methyloctanoic and 8-methylnonanoic acids, also BCFAs, may contribute to the odour as well. Of course, this is speculative and does need confirmation but nevertheless it suggests that other BCFAs, that have not been measured in this study nor previously investigated, may also contribute to 'mutton' flavour.

3.2 Factors affecting consumer sensory scores

Statistical analysis, with restricted maximum likelihood (REML) models, was used to determine how the BCFA concentrations as well as those of MP and MI influenced the consumer sensory scores; 'Like Smell', '**Like Flavour**' and 'Overall Like'. The first set of models examined the influence of each compound, as a single term, on the consumer scores. Of the BCFAs, MOA and EOA were statistically significant covariates for the modelling of the 'Like Smell' and 'Overall Like' consumer sensory scores ($P < 0.05$, Table 3) but MNA was not ($P > 0.05$). No significant relationship ($P > 0.05$) was found between any BCFA and the '**Like Flavour**' consumer score. While there was a trend to significance for **MP** with '**Like Flavour**' ($P = 0.085$, Table 3), further modelling showed that this was not significant. No significant **relationship** was found between MI concentration and the sensory scores. 'Like Smell' was highly correlated with 'Overall Like' and, given the effect of MOA and EOA on 'Like Smell', it was reasoned that this would be similar for 'Overall Like' and so further modelling of this attribute was discontinued.

More complex modelling of the impact on MOA and EOA on 'Like Smell' was done with the inclusion of other covariates (eg MP or MI) as terms or other parameters (eg sex, sire type, etc). With almost all models, both MOA and EOA were either significant ($P < 0.05$) or close to significance ($P < 0.1$, results not shown) for the 'Like Smell' sensory score. Of the other parameters tested none were significant ($P > 0.05$) except for the kill date (site.DATE). The final modelling of 'Like Smell' included MOA and EOA as covariates with the inclusion of kill

date (Site.DATE), site, and sire type as fixed effects, **with the inclusion of the last two terms as blocking treatments and so not described any further.** Both MOA and EOA made significant impacts on the 'Like Smell' consumer sensory score ($P < 0.05$**, Models 1 and 2, Table 4**) **and, in assessing the combined impact of the two BCFAs, MOA was significant ($P < 0.05$) and EOA was close to significance ($P < 0.1$, Model 3, Table 4).** With increasing BCFA concentration, consumer acceptance of the odour ('Like Smell') resulting from the grilled meat product decreased. This can be clearly seen in Figure 3, which shows a plot of the predicted 'Like Smell' score against the concentration range for MOA and EOA. This means, of course, that consumers preferred lamb meat with low concentrations of these compounds. This is not surprising given the high OAVs for MOA and EOA as reported above indicating that these compounds do contribute to the overall liking of aroma. **It is important to note that these compounds were measured in sheep fat rather than the associated meat which was grilled and then used for sensory evaluation. These compounds will probably be present at lower concentrations in the meat compared to fat indicating the significance of MOA and EOA as odourants.** MP would have also been expected to contribute to the cooked meat odour because of the high OAV associated with this compound. **It is possible that the MP levels in the cooked meat might have been low enough not to contribute to the overall odour but this is speculative and would need confirmation.**

BCFAs are regarded as the main contributors to the 'mutton' aroma found in the cooked meat of older sheep (Young et al., 1997). It is commonly accepted that these compounds increase with animal age, and are more associated with the odour from cooked mutton (> 2 yrs) rather than that resulting from lamb (< 1 yr). As far as we aware, the impact of these compounds on the consumer scores for odour of cooked lamb meat has not been reported. An extension of this present study would be to augment the data set with fat samples taken from animals classified as hogget and mutton, which would provide further information on the roles that MOA and EOA have on sheepmeat aroma. EOA is known to increase with age with higher amounts present in mutton than in lamb and hogget (Watkins et al., 2010). With higher concentrations in meat from older animals, the associated OAVs would also increase and so reduce consumer acceptance of the meat product. For this study, the effect of age on the levels of MOA and EOA was tested but nothing significant was observed ($P > 0.30$ for both compounds). However, this may be due to the comparatively narrow range in the age of animals used for the study (215 to 362 d) in contrast to those of the animals where 'mutton' flavour is usually found (> 2 yr) as in the case of the earlier study (Watkins et al., 2010).

Nutrition **can have** an influence on the BCFA content of sheep fat (Watkins et al., 2010) and so, by extension, **can also** impact on the aroma of the associated grilled meat product. Elevated MOA levels have been found in sheep fat taken from animals fed on mixed lucerne, native pasture and saltbush diets prior to slaughter,

compared to those that had received diets based on grain, lucerne and pasture (Watkins et al., 2010). **In this present study, higher BCFA concentrations were found in the fat taken from the Kirby samples compared to those found in the Katanning samples (Table 2). There are no significant differences though between nutritional histories for each site (Table 1) which might explain the differences in the BCFA concentrations between the two sample sets; for example, the animals at both sites, during early post weaning, were fed on green forage with lupin supplementation or a mixture thereof. However, the use of concentrates might provide a clue for this observed difference of BCFA levels in sheep fat. The use of grains for feeding has been associated with higher BCFA concentrations in sheep meat (Wong et al., 1975; Duncan and Garton, 1978; Young et al., 2003), and has been attributed to the higher availability of carbohydrate from grains and concentrates to the animals compared to that available in pasture (Young and Braggins, 1998). However, cereal grains do differ in their propensity to generate BCFAs (Young and Braggins 1998) and so without more detailed knowledge about the feed, it is difficult to make specific conclusions on the relationship between diet and BCFA concentrations. For MP and MI, there appears to be no trend evident between the concentration in the fat and the nutritional history, in spite of the fact that MP and MI are respectively formed from tyrosine and tryptophan present in the pasture (Ha and Lindsay, 1991; Tavendale et al., 2005). The reason for this remains unclear.**

Fat levels and other fatty acids have been previously reported to be affected by the nutritional history prior to slaughter; e.g. total fat, total ω-6 fatty acids and ω-6/ω-3 are higher for meat from short-term grain-fed lambs compared to that obtained from low quality pasture-fed lambs (Ponnampalam et al., 2010). Variation in pre-slaughter nutrition has been reported for 2007 progeny for the Sheep **INF** which has resulted in variations in the polyunsaturated fatty acid (PUFA) content of lamb meat (Pannier et al., 2010). These authors found that higher concentration of ω-3 PUFAs were associated with pasture consumption while low ω-3 PUFA levels were related to feeding regimes of grain and low quality hay. **During cooking, the PUFAs will be subject to oxidation causing the formation of aldehydes and related oxidation products. These compounds will impact on the volatile profile produced during grilling and ultimately smelt by the consumers.** Thus, while feed base measures were in place for the INF animals, some local variation in dietary supplementation could impact on the sensory scores.

Obviously, ameliorating the impact of MOA and EOA on the sensory component of grilled lamb meat will result in higher acceptance of the final product by Australian consumers. However, the presence of these BCFAs is not the only factor which will influence consumer acceptance; tenderness and juiciness, for example, are also important considerations (Thompson et al., 2005c). Additionally, the overall odour and flavour of

sheepmeat will be affected by other factors as well; e.g. ultimate pH (Braggins, 1996). Thus, while strategies to reduce MOA and EOA in sheepmeat will assist product acceptance by consumers, other factors need to be considered since these will also impact on the overall sheepmeat quality.

4 Conclusion

MOA and EOA, powerful odourants present in sheep fat, impacted on the 'Like Smell' sensory score of grilled lamb meat assessed by Australian consumers. Higher acceptance of the final cooked meat product was found with lower concentrations of MOA and EOA. MP, another significant odourant, was expected to make an impact to the overall aroma but **this** was not the case. None of the odourants contributed to the overall **liking of** flavour of the final meat product. Of course, reducing the impact of MOA and EOA will improve consumer acceptance of the cooked meat product but other factors that contribute to the overall sheepmeat quality also need to be considered.

Acknowledgements

This work was funded by Meat & Livestock Australia which is gratefully acknowledged. We also thank the CRC for Sheep Industry Innovation for making the samples and sensory information available to us. The authors wish to thank Xuemei Han and Geert Geesink (University of New England) and Mal Boyce (Murdoch University) for arranging the transport of the samples as well as Joanne Bui (DPI-V) for preparation of samples for BCFA analysis.

References

Braggins, T.J. (1996). Effect of stress-related changes in sheepmeat ultimate pH on cooked odour and flavour. *Journal of Agricultural and Food Chemistry*, *44*, 2352–2360.

Brennand, C.P., Ha, J.K., & Lindsay, R.C. (1989). Aroma properties and thresholds of some branched-chain and other minor fatty acids occurring in milkfat and meat lipids. *Journal of Sensory Studies, 4*, 105–120.

Chaintreau, A. (2002). Quantitative use of gas-chromatography-olfactometty: The GC-"SNIF" method. In Flavor, Fragrance and Odor Analysis, R. Marsili, ed. (New York: Marcel Dekker, Inc.), pp. 333–348.

Duncan, W., and Garton, G. (1978). Differences in the proportion of branched-chain fatty acids in subcutaneous triacylglycerols of barley-fed ruminants. *British Journal of Nutrition*, *40*, 29–33.

Fogarty, N.M., Banks, R.G., van der Werf, J.J.F., Ball, A.J., & Gibson, J.P. (2007). The information nucleus – a new concept to enhance sheep industry genetic improvement. *Proceedings of the Association for Advancement of Animal Breeding and Genetics*, *17*, 29–32.

Ha, J.K., & Lindsay, R.C. (1991). Volatile alkylphenols and thiophenol in species-related characterising flavors of red meats. *Journal of Food Science*, *55*, 1197–1202.

Kenward, M.G., & Roger, J.H. (1997). Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics*, *53,* 983-997.

McFarland, I., Curnow, M., Hyder, M., Ashton, B., & Roberts, D. (2006). *Feeding and managing sheep in dry times*, Department of Agriculture and Food, Western Australia.

Pannier, L., Ponnampalam, E.N., Gardner, G.E., Hopkins, D.L., Ball, A.J., Jacob, R.H., Pearce, K.L., & Pethick, D.W. (2010). Prime Australian lamb supplies key nutrients for human health. *Animal Production Science*, *50*, 1155–1122.

Pannier, L., Pethick, D.W., Geesink, G.H. , Ball, A.J., Jacob, R.H., &Gardner, G.E. (2012) Intramuscular fat is reduced in lambs from sires selected for leanness, *Meat Science,* (submitted for publication)

Pethick, D.W., Davidson, R., Hopkins, D.L., Jacob, R.H., D'Souza, D.N., Thompson, J.M., & Walker, P.J. (2005a). The effect of dietary treatment on meat quality and on consumer perception of sheep meat eating quality. *Australian Journal of Experimental Agriculture*, *45*, 517–524.

Pethick, D.W., Hopkins, D.L., D'Souza, D.N., Thompson, J.M., & Walker, P.J. (2005b). Effects of animal age on the eating quality of sheep meat. *Australian Journal of Experimental Agriculture*, *45*, 491–498.

Pleasants, A.B., Thompson, J.M., & Pethick, D.W. (2005). A model relating a function of tenderness, juiciness, flavour and overall Liking to the eating quality of sheep meat. *Australian Journal of Experimental Agriculture*, *45*, 483–489.

Ponnampalam, E.N., Warner, R.D., Kitessa, S., McDonagh, M.B., Pethick, D.W., Allen, D., & Hopkins, D.L. (2010). Influence of finishing systems and sampling site on fatty acid composition and retail shelf-life of lamb. *Animal Production Science*, *50*, 775–781.

Ponnampalam, E.N., Butler, K.L., Jacob, R.H., Pethick, D.W., Ball, A.J., Hocking Edwards, J.E., Geesink, G. & Hopkins, D.L. (2012). Health beneficial long chain omega-3 fatty acids in Australian lambs managed under extensive finishing systems. *Meat Science* (submitted for publication)

Rowe, J.B. (1986). Supplementary feeds for sheep. *Journal of Agriculture, Western Australia*, *27*, 100–102.

Schreurs, N.M., McNabb, W.C., Tavendale, M.H., Lane, G.A., Barry, T.N., Cummings, T., Fraser, K., López-Villalobos, N., & Ramírez-Restrepo, C.A. (2007). Skatole and indole concentration and the odour of fat from lambs that had grazed perennial ryegrass/white clover pasture or Lotus corniculatus. *Animal Feed Science and Technology*, *138*, 254–271.

Tavendale, M.H., Lane, G.A., Schreurs, N.A., Fraser, K., and Meagher, L.P. (2005). The effects of condensed tannins from *Dorycnium rectum* on skatole and indole ruminal biogenesis for grazing sheep. *Australian Journal of Agricultural Research*, *56*, 1331–1337.

Thompson, J.M., Hopkins, D.L., D'Souza, D.N., Walker, P.J., Baud, S.R., & Pethick, D.W. (2005a). The impact of processing on sensory and objective measurements of sheep meat eating quality. *Australian Journal of Experimental Agriculture*, *45*, 561–573.

Thompson, J.M., Gee, A., Hopkins, D.L., Pethick, D.W., Baud, S.R., & O'Halloran, W.J. (2005b). Development of a sensory protocol for testing palatability of sheep meats. *Australian Journal of Experimental Agriculture*, *45*, 469–476.

Thompson, J.M., Pleasants, A.B., & Pethick, D.W. (2005c). The effect of design and demographic factors on consumer sensory scores. *Animal Production Science*, *45*, 477–182.

Urbach, G., Stark, W., & Forss, D.A. (1972). Volatile compounds in butter oil II. Flavour and flavour thresholds of lactones, fatty acids, phenols, indole and skatole in deodorised synthetic butter. *Journal of Dairy Science*, *39*, 35–47.

Warner, R.D., Jacob, R.H., Edwards, J.E.H., McDonagh, M.B., Pearce, K.L., Geesink, G., Kearney, G., Allingham, P., Hopkins, D.L., & Pethick, D.W. (2010). Quality of lamb meat from the Information Nucleus Flock. *Animal Production Science*, *50*, 1123–1134.

Watkins, P.J., Rose, G., Salvatore, L., Allen, D., Tucman, D., Warner, R.D., Dunshea, F.R., & Pethick, D.W. (2010). Age and nutrition influence the concentrations of three branched chain fatty acids in sheep fat from Australian abattoirs. *Meat Science*, *86*, 594–599.

Wong, E., Nixon, L.N., & Johnson, C.B. (1975). Volatile medium chain fatty acids and mutton flavor. *Journal of Agricultural and Food Chemistry*, *23*, 495–498.

Young, O.A., Berdagué, J.-L., Viallon, C., Rousset-Akrim, S., & Theriez, M. (1997). Fat-borne volatiles and sheepmeat odour. *Meat Science*, *45*, 183–200.

Young, O.A., & Braggins, T.J. (1998) Sheepmeat odour and flavour. In 'Flavor of Meat, Meat Products and Seafoods' (Ed. F. Shahidi) pp. 101-130. (Blackie Academic & Professional, London)

Young, O.A., Lane, G.A., Priolo, A., & Fraser, K. (2003). Pastoral and species flavour in lambs raised on pasture, lucerne or maize. *Journal of the Science of Food and Agriculture*, *83*, 93–104.

Wales, W.J., Doyle, P.T., & Pearce, G.R. (1990). The feeding value of cereal straws for sheep. I. Wheat straws. *Animal Feed Science and Technology*, *29*, 1–14.

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

Figure 2. Box plots of individual **concentrations** of 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA), 4-methylnonanoic (MNA) acids, 3-methylindole (MI) and 4-methylphenol (MP) for different sire types (Maternal, Merino and Terminal)**, measured in fat. The data is taken from Table 1.** The box spans the interquartile range of the values, so that the middle 50% of the data lies within the box, and the line in the middle of the box indicate the median. The perpendicular lines extend to the most extreme data values within the inner "fences", which are at a distance of 1.5 times the interquartile range beyond the quartiles, or the maximum value if that is smaller. The red dashed lines represent the odour sensory threshold values of the respective compounds.

Figure 3. Plot of predicted 'Like Smell' consumer sensory score against 4-methyloctanoic (MOA) and 4-ethyloctanoic (EOA) acid concentration (mg kg^{-1}). The dashed lines indicate $\pm$ 2 times the standard error. **The regression equations for MOA and EOA respectively are *y* = -7.49 x [MOA] + 71.41 and *y* = -45.30 x [EOA] + 72.60 where *y* is the predicted 'Like Smell' consumer score, and [MOA] and [EOA] are the respective concentrations.**

Figure 1

Figure 2

Figure 3

Table 1 Summary of nutritional history of 2009/2010 lamb progeny used in this study[A]

Site	Early post weaning		Late post weaning	
Katanning	**Pasture**	**Concentrate**	**Pasture**	**Concentrate**
	Green annual grass and subclover	**60:40 Lupins and oats supplementary fed in lick feeders**	**Dried senesced pasture, annual grass and subclover**	**60:40 Lupins and oats Supplementary fed in lick feeders**
Kirby	**Improved pasture**	**Lupin**	**Grazing oats**	**Prime lamb finisher**

[A]Ponnampalam et al 2012

Table 2. Mean and standard error (s.e.) values for 4-methyloctanoic acid (MOA), 4-ethyloctanoic acid (EOA), 4-methylnonanoic acid (MNA), 4-methylphenol (MP) and 3-methylindole (MI) concentrations (μg kg^{-1}) in fat taken from sheep of three sire types (Terminal, Maternal, Merino) at two sites (Katanning and Kirby)

		Terminal[A]	Maternal	Merino
Site	Compound	Mean ± s.e.	Mean ± s.e.	Mean ± s.e.
Katanning	MOA	230 ± 20	215 ± 32	93 ± 9
	EOA	51 ± 6	39 ± 7	19 ± 4
	MNA	50 ± 2	43 ± 3	30 ± 3
	MP	121 + 15	89 ± 17	35 ± 6
	MI	26 ± 4	30 ± 10	19 ± 4
Tamworth	MOA	530 ± 4	344 ± 61	172 ± 40
	EOA	170 ± 20	78 ± 18	34 ± 18
	MNA	97 ± 6	104 ± 9	38 ± 6
	MP	172 ± 37	113 ± 18	118 ± 21
	MI	34 ± 8	18 ± 5	30 ± 7

[A]For Katanning, the number of samples (*n*) from Terminal, Maternal and Merino sires was 60, 19 and 10 while, for Tamworth, it was 62, 12 and 15, respectively.

Table 3 *P* values for terms in the models relating the 'Like Smell', 'Like Flavour' and 'Overall Like' consumer sensory scores to the concentrations of 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA), 4-methylnonanoic (MNA) acids, 4-methylphenol (MP) and 3-methylindole (MI) as covariates with Site (Katanning and Kirby) and Siretype (Terminal, Maternal and Merino) included as fixed effects. Significant terms (*P* < 0.05) are shown in bold.

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

Table 4 Coefficients (s.e. in parenthesis) and level of significance (*P*-value) of the coefficient for covariates in models relating 'Like Smell' to site (Katanning and Kirby), kill date (Site.DATE) and siretype (Terminal, Maternal and Merino) with (1) 4-methyloctanoic acid (MOA), (2) 4-ethyloctanoic acid (EOA) and (3) combined terms (MOA+EOA) with adjustment for sensory session fitted as random effect. Significant terms (*P* < 0.05) are shown in bold.

		Model		
Term	Numbers	1	2	3
Site	*P*-value	0.798	0.744	0.866
Site.DATE	*P*-value	0.062	0.116	0.082
siretype	*P*-value	0.591	0.648	0.412
EOA	*P*-value		**0.027**	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)

Highlights

Cooking odour is an important component of sheepmeat eating quality.
BFCAs, 3-methylindole and 4-methylphenol were related to consumer sensory scores of grilled lamb.
Two BCFAs negatively impacted on 'Like Smell' consumer sensory score of cooked meat product.