Influence of nutritional regime (ryegrass, lucerne, brassica) on sheep meat texture and flavour

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Abstract

Finishing feeds such as lucerne or some brassica crops may have a negative effect on lamb flavour according to some studies and anecdotal evidence. However, few well-designed experiments have been conducted to objectively ascertain to what extent (if any) different finishing feeds affect lamb quality and flavour. In this study we examined the influence of different finishing feeds and sheep genotypes on the sensory and flavour characteristics of grilled lamb. Ewes with known Australian Breeding Values (ABVs) from low IMF (Sires 1 & 6) and high IMF (Sires 3 & 8) genotypes were randomly allocated to four different finishing feeds. Lambs (n = 25 per feed treatment) were finished for 42-days in January/February 2013 on; (1) lucerne, (2) a low glucosinolate brassica cultivar - *brassica napus*, cv. “Titan”, (3) a high glucosinolate brassica *brassica napus*, cv. “Greenland” or (4) a control regime of ryegrass supplemented with grain. Left and right striploins were obtained and cut into standardised 15 mm steaks. High ABV genotypes were initially heavier than low ABV animals; these differences persisted throughout the trial. Significant differences in growth rates were recorded at different periods throughout the trial. Negative growth was recorded in the first 12 days (adjustment period) on all feeds except the ryegrass. In subsequent periods, rapid positive growth was measured for the novel feed treatments compared to the ryegrass; this was especially true for the Titan feed in the early phase of the finishing trial.

Descriptive sensory evaluation was carried out on grilled lamb from right striploins by a trained panel. Consumer acceptance testing using Caucasian and Chinese background Australian consumers was conducted using matching left striploins. The trained sensory panel measured animal, feed and sire related effects. Feed induced flavour differences were minimal, with no evidence of a “taint” present in any of the novel feeds compared to the control. In general, lamb from high ABV Sires had stronger flavour and better texture characteristics. Lamb from the Sire 8 genotype in particular had better sensory characteristics compared to the others.

The consumer study clearly showed that acceptance of grilled lamb was higher for the novel finishing feeds (brassicas & lucerne) compared to the control. Both of the brassica treatments were rated highest by both consumer groups in terms of overall liking and flavour liking. The rye finished lamb was rated the lowest and lamb from sires with high ABVs were clearly preferred by both consumer groups. Further chemical analyses were conducted on samples to characterise differences in free amino acids, branched chain fatty acids, other fatty acids and aroma volatiles according to feed and genotype. Overall, the data provided no evidence that brassica derived taints were present in lamb finished on the experimental feeds. In fact, contrary to our initial hypothesis, the data strongly indicated that lambs finished on the lucerne and brassica feeds had better sensory characteristics than the ryegrass/ grain treatments.

Executive Summary

Summer-active finishing forages such as lucerne and brassicas are undoubtedly useful for Australian lamb producers; however their wider deployment may be being held back by negative perceptions, especially regarding potential flavour issues. Research published in the past has suggested that lucerne may be responsible for undesirable flavours in lamb. Anecdotally, brassica forages have been associated with negative flavours or taints in lamb. Current recommendations are to have a “washout” period on grain after being on brassica crops (AgFacts NSW Report). In this study we aimed to find objective evidence of any negative (or positive) flavour or sensory effects from lucerne and brassica feeds compared to a typical control regime of ryegrass supplemented with grain. The breed or genetics of lambs is also known to affect flavour attributes and eating quality.

We investigated the affects of both genotype and finishing feed on lamb flavour and eating quality using a two factorial experimental design. Ewes with known Australian Breeding Values (ABVs) from low IMF (Sires 1 & 6) and high IMF (Sires 3 & 8) genotypes were randomly allocated to four different finishing feeds. Lambs (n = 25 per feed treatment) were finished for 42-days in January/February 2013 on; (1) lucerne, (2) a low glucosinolate brassica cultivar - *brassica napus*, cv. “Titan”, (3) a high glucosinolate brassica *brassica napus*, cv. “Greenland” or (4) a control regime of ryegrass supplemented with grain. Left and right striploins (*M. Longissimus dorsi*) were taken from each carcass, aged for five days and cut into standardised 15 mm steaks.

Descriptive sensory evaluation was carried out on grilled lamb from right striploins by a trained panel. Consumer acceptance testing using Caucasian and Chinese background Australian consumers was conducted using matching left striploins. The trained sensory panel measured animal, feed and sire related effects. Feed induced flavour differences were minimal, with no evidence of a “taint” present in any of the novel feeds compared to the control. In general, lamb from high ABV Sires had stronger flavour and better texture characteristics. Lamb from the Sire 8 genotype in particular had better sensory characteristics compared to the others.

The consumer study clearly showed that acceptance of grilled lamb was higher for the novel finishing feeds (brassicas & lucerne) compared to the control. Both of the brassica treatments were rated highest by both consumer groups in terms of overall liking and flavour liking. The rye finished lamb was rated the lowest and lamb from sires with high ABVs were clearly preferred by both consumer groups. Further chemical analyses were conducted on samples to characterise differences in free amino acids, branched chain fatty acids, other fatty acids and aroma volatiles according to feed and genotype. Overall, the data provided no evidence that brassica derived taints were present in lamb finished on the experimental feeds. In fact, contrary to our initial hypothesis, the data strongly indicated that lambs finished on the lucerne and brassica feeds had better sensory characteristics than the ryegrass/ grain treatments.

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1 Background

1.1 Research Context

1.1.1 *Lamb Flavour*

Flavour is an important driver of lamb consumer acceptance, closely followed by texture and juiciness (Pleasants, Thompson & Pethick 2005; Pethick, Pleasants, Gee, Hopkins & Ross 2006). The degree to which lamb flavour can be influenced by animal feed is an ongoing and important area of investigation (Watkins, Frank, Singh, Owen & Warner 2013). While some studies demonstrate direct impacts of feed regimes on lamb flavour (Almela, *et al.* 2010; Young, Lane, Priolo & Fraser 2003), others do not (Pethick *et. al*. 2005, Hopkins, Holst, Hall & Atkinson 1995, Young, Cruickshank, MacLean & Muir 1994). Factors shown to influence meat flavour include: animal genetics (Hegarty, 2004; Elmore, Mottram, Enser & Wood 2000), maternal nutrition (Rehfeldt *et al.* 2011), weaning and backgrounding diets (Osorio, Zumalacárregui, Cabeza, Figueira & Mateo, 2008), and pre- and post-slaughter conditions (Fergusson & Warner 2010, Braggins 1996). Other lesser-known factors, such as animal age, sex and carcass weight have been described (Rodrigues & Teixeira 2013).

Cooked lamb meat has a distinctive flavour, recognisably different to pork or beef, depending on the age of the animal and whether lean or fatty meat cuts are used (Maughan & Martini 2012, Rødbotten, Kubberød, Lea & Ueland 2004, Duckett & Kuber 2001, Rhee & Ziprin 1996, Pearson *et al.* 1973). In countries with a tradition of ovine meat consumption, the unique sensory attributes and flavour of sheepmeat may be a positive driver for acceptance. However, some studies have indicated that sheepmeat has a ‘stronger’ flavour, compared to other red meats such as beef and this may negatively influence consumer acceptance (Sañudo, Alfonso, San Julián, Thorkelsson *et al.* 2007, Prescott, Owen & Young 2001, Duckett & Kuber 2001). The stronger (often undesirable) flavour is often attributed to the presence of branched chain fatty acids (BCFAs), unique to ovine fat (Watkins *et al.* 2010, 2014). However, not all studies have found lamb flavour to be stronger than other popular red meats. In one study, lamb was barely distinguishable from beef by a trained sensory panel, except for the presence of a higher “barny” note (Rødbotten, Kubberød, Lea & Ueland 2004). Although the authors did not specify, it is likely that the beef used in the comparison was grain fed and the lamb pasture fed, suggesting a “pastoral flavour” (see following section) was being detected rather than a true species specific difference.

1.1.2 *Feed Induced Flavours in Lamb*

So called “pastoral” or “barnyard” flavour notes in sheepmeat, has been associated with elevated levels of 4-methylphenol (*p*-cresol) and 3-methylindole (Young, Lane, Priolo & Fraser 2003). The extent to which these compounds influence lamb flavour is thought to depend on animal diet especially pasture based diets.

Previous research indicated that finishing lambs on lucerne can create undesirable flavours in Corriedale lambs (Park, Corbett & Furnival 1972, Park, Ford, Minson & Baxter 1975). However, in another study (Young, O.A., Cruickshank, G.J., MacLean, K.S. & Muir, P.D 1994), lucerne finished lamb was no different to rye finished samples.

Forage brassicas are members of the brassica family, which include radish, turnip, swedes, broccoli and cabbage. Forage rape is a common feed source for finishing lambs particularly in summer dry environments (Judson *et al.* 2013, Ayres & Clements 2002). Forage brassicas can provide a nutritious feed, high in protein and energy, with good digestibility. Rapes are annual growing crops and have been traditionally used to fill periods of feed deficits in temperate lamb finishing systems (Barry 2013). Forage rapes are generally sown in the

spring, to provide a summer prime lamb finishing forage crop because they are quick, productive, high quality and relatively tolerant of lower rainfall conditions, but can also respond very well to irrigation. Despite the clear usefulness of brassica feeds, anecdotally there is a widespread belief that the meat from animals raised brassicas may have taint or unpleasant flavour. For example, the AGFACT brassica handbook 2002 (Ayres, L. & Clements, B. 2002 NSW Department of Primary Industries) recommends that animals should be "removed from a brassica crop 3-7 days prior to slaughter to minimise the risk of tainting meat". Despite these recommendations, there are few published studies in the Australian context to support this idea.

In many parts of Australia where sheep production occurs, reliance on traditional forages to finish animals can be risky, especially during low rainfall seasons. Common finishing forages used in south-eastern Australia include; dried summer perennial ryegrass (*Lolium perenne)* which may be supplemented with grains such as barley and lupin feed(either produced on farm or purchased off farm), lucerne (*Medicago sativa)* or forage rape (*Brassica napus)*.

Perennial ryegrass is an ideal finishing forage when the season is favourable or under irrigation. Specifically, late-maturing ryegrass can be especially productive as it will take advantage of the late-spring or early summer rains and produce high quality and highly palatable feed. However early types (i.e. Victorian perennial ryegrass) can be detrimental to lamb production due to reduced feed quality since at critical times potential issues from animals grazing on high toxic endophytes which can be impact detrimental to its growth and performance (Fletcher 1993),

Lucerne is a highly desirable finishing forage option because it is perennial, produces high quality summer feed, adaptability to wide-range of environments, persistence under dry conditions and being a legume has the ability to fix atmospheric nitrogen. Despite the advantages of lucerne, it remains underutilized in Australian mixed-farming systems (Humphries 2012).

It has long been recognised that there is significant between-animal variation in digestion, fermentation and rumen turnover rate (Hegarty 2004). Genotype also affects diet selection and rumen-digesta kinetics. Maintenance of a high nutritional plane in the months leading to slaughter is important to facilitate optimal weight gain without imparting undesirable flavour notes in the meat. The purpose of this study was to ascertain whether i) a trained sensory panel and a consumer panel could discern differences in the flavour of lamb finished on the different regimes and ii) sensory/flavour differences could be measured for meat from the high and low ABV sires. In particular, it was hypothesised that if brassicas do in fact cause a flavour problem, the effect would be largest for the high GSL brassica cultivar "Greenland".

Few studies have been carried out to establish the relative impacts of finishing forages on flavour and other sensory properties of Australian lamb. Meat obtained from animals from each finishing diet and genotype was subjected to sensory descriptive profiling using a trained sensory panel as well as consumer acceptance testing, using both a non-Chinese background consumer cohort (n=60) and a Chinese-background consumer group (n=60). Furthermore, a dynamic headspace (DHS) technique was applied to extract volatiles from grilled lamb for analysis by gas chromatography-olfactometry (GC-O) using a panel of trained assessors with simultaneous analysis by gas chromatography mass spectrometry (GC-MS). Targeted analyses for BCFAs, p-cresol and 3-methylindole were performed on corresponding fat samples. The primary aims of the work were to apply both sensory and instrumental techniques to elucidate the effects of finishing diets on lamb flavour, and if possible, relate sensory flavour attributes to specific flavour molecules

1.1.3 *Industry Research Partnership*

The success of the project was dependent on collaboration with industry and research partners, whose efforts are gratefully acknowledged. PGG Wrightson Seeds was a major collaborator in this project, providing industry expertise and hosting the feeding trials. Specifically, PGG Wrightson Seeds and staff were:

- Involved in initial discussions conceptualising the research design and aims
- Selection of the brassica cultivars used in the trials (the Titan cultivar was developed by PGG Wrightson's and Greenland by Seed Force Australia)
- Preparation of the forages for the feeding trials (lucerne, ryegrass, Titan and Greenland brassicas)
- Preparation and execution of the feeding trials at their Leigh Creek facility with agronomic and feed utilisation monitoring
- Assistance with animal transportation and slaughter

In particular, the efforts of Rob Salmon, James Sewell, Derek Mason and Kelvin Henderson are acknowledged. Glenn Judson is thanked for his valuable input into the design of trial at all stages and in interpretation of the data. Duncan Thomas, a Master's Degree student at Melbourne University, was largely responsible for the day-to-day oversight of the forage preparation and execution of the feeding trial at Leigh Creek. His input was invaluable.

The Victorian Department of Environment and Primary Industries (DEPI) provided ewes from a pre-existing trial — "Proof of concept of Lean Meat Yield and Eating Quality Producer Demonstration Sites" — for the current study. Special thanks to Peter Bailey for his input into the trial. Other staff from D-EPI also assisted in the slaughter of sheep and the collection of meat samples and are thanked for their support (Wayne Brown, Matthew Kerr, Maria Crawford).

Tony Fleetwood (Wexford Pastoral Company) is acknowledged and thanked for his interest and support for the study and for raising the lambs prior to the feeding trial.

The animals were slaughtered during a commercial Coles kill at JBS Swift (Brooklyn). Mark Ingliss is thanked for his assistance and the contribution of Coles is acknowledged for allowing us to remove striploins from sheep for this experiment.

2 Feeding Trials

2.1 Animal Ethics

Approval to conduct the research was obtained from the CSIRO Animal Ethics Committee (CSIRO AEC 2012-6) on December 13 2012.

2.2 Sourcing of Animals

2.2.1 *Selection of Animals & Design*

After initial feedback and consultation with the MLA, lambs (ewes, *n* =125) were sourced from an existing lamb quality trial ("Proof of concept of Lean Meat Yield and Eating Quality Producer Demonstration Sites"), being conducted by the Victorian Department of Environment and Primary Industries (DEPI-V). The project provides objective measurements of lamb carcasses for eating quality from seven Victorian lean meat yield and eating quality producer demonstration sites. These measurements will be used in part to determine the value new research Australian Breeding Values (ABVs) on Dressing %, Lean Meat Yield (LMY) and Eating Quality – Intramuscular Fat (IMF) and Shear Force (SF5) for ram breeders, lamb producers and processors. Details of sire characteristics are shown in Table 1.

Table 1: Australian Breeding Values (ABVs) and research breeding values (RBVs) for the sires used in this trial

Sire	Wwt (kg)	Pwwt (kg)	HCWT (kg)	CEMD (mm)	CCFAT (mm)	IMF (%)	SF5 (kgF)	LMY (%)	Dressing (%)
	ABV's					RBV's			
1	9.7	14.2	0.3	0.4	-0.1	-0.3	5.5	0.4	0.5
3	8.6	14.7	0.6	0.1	-0.8	0.5	-5.3	0.5	-0.2
6	8.3	12.8	0.9	1.5	-0.2	-0.8	2.5	1.4	0.5
8	8.4	13.1	0.7	1	-0.6	0.3	-2.7	0.9	0.3

In this study, progeny from four sires with known breeding values (high and low ABVs), were selected and randomly allocated to each of the four finishing feeds. Progeny from Sires 1 & 6 were low IMF or low ABV animals. Ewes from Sires 3 & 8 were high IMF/high or high ABV animals. Peter Bailey, the principal investigator of the DEPI-V study, worked closely with CSIRO and the commercial partner, PGG Wrightson Seeds, with respect to the design of experiments and preparation of the finishing feed experiments. Peter Bailey also facilitated negotiations with the sheep producer Tony Fleetwood (Wexford Pastoral Company). The experimental design was discussed with MLA and approved by an experienced biostatistician (Gavin Kearney). The primary focus of this study was the influence of the finishing feeds on lamb flavour and quality rather than the effect of ABVs and specific sires. Because of the relatively low numbers of animals in each feed treatment, the experimental design was sufficiently statistically powered to measure effects of ABVs (e.g. comparing low ABVs (pooled 1 & 6) to high ABVs (pooled 3 & 8), but not sufficiently powered to compare individual sire effects. Hence, sire comparisons reported in this study should be considered with this in mind.

2.3 Preparation of the Experimental Plots

The experiment was conducted at the PGG Wrightson Leigh Creek Research Station in the Central Highlands region of south-western Victoria, near Ballarat, Victoria (-37^{o}56'S, 143^{o}95'E). Working closely with plant breeders and the research and development team at PGG Wrightson Seeds, two brassica cultivars of commercial importance with known differences in glucosinolate content were selected. Leaf and stem samples from each cultivar were collected and freeze dried at CSIRO (North Ryde). Dried plant material was sent to the Australian Oils Research Laboratory at the New South Wales Department of Primary Industries for independent chemical measurement of total glucosinolates. The total GSL content of Greenland stems (12 µmol/g) and leaves (15 µmol/g) were considerably higher than for Titan stems (6 µmol/g) and leaves (4 µmol/g). Further analysis of the brassica cultivars is presented later in the report.

Samples of the feed material were collected throughout the feeding trial and stored at -80 ^{o}C, for later analysis. Lucerne and ryegrass paddocks were also prepared. **Figure 1** shows images of the prepared plots in early December 2012. It was determined that the Titan (low GSL), Greenland (high GSL) and Ryegrass (control) plots would be able to support at least 25 animals for a six to eight week period; in contrast, at least 50 animals could be sustained on the lucerne feed (high nitrogen).

Figure 1: Preparation of experimental plots at the Leigh Creek site (Ballarat, Vic.) in early December 2012. (Top left) "Titan" low glucosinolate brassica, (top right) lucerne and (bottom) ryegrass. Greenland is not shown, but appeared similar to Titan.

Available animals were screened by reading their radio frequency identification (RFID) tags on-farm in Warrnambool, Victoria. Considerable time and energy was expended to identify

and separate groups of animals from sires that would allow a balanced allocation of low and high ABV animals across the four treatments according to the target experimental design (**Figure 2**). As different numbers of animals from each sire were available, final allocations were not exactly even for each sire group. Within each sire group, there was a considerable range of initial live weights. Apart from the weight variation within each sire group, animals from sire 3 were significantly heavier than those from other sires.

After final selection, ewes were transported to Leigh Creek on January 15th 2013 and held in a common area. Working within sire groups, animals were allocated such that the distribution of weights was similar for each feed allocation. **Figure 3** shows drafting of sheep at Leigh Creek in January 2013.

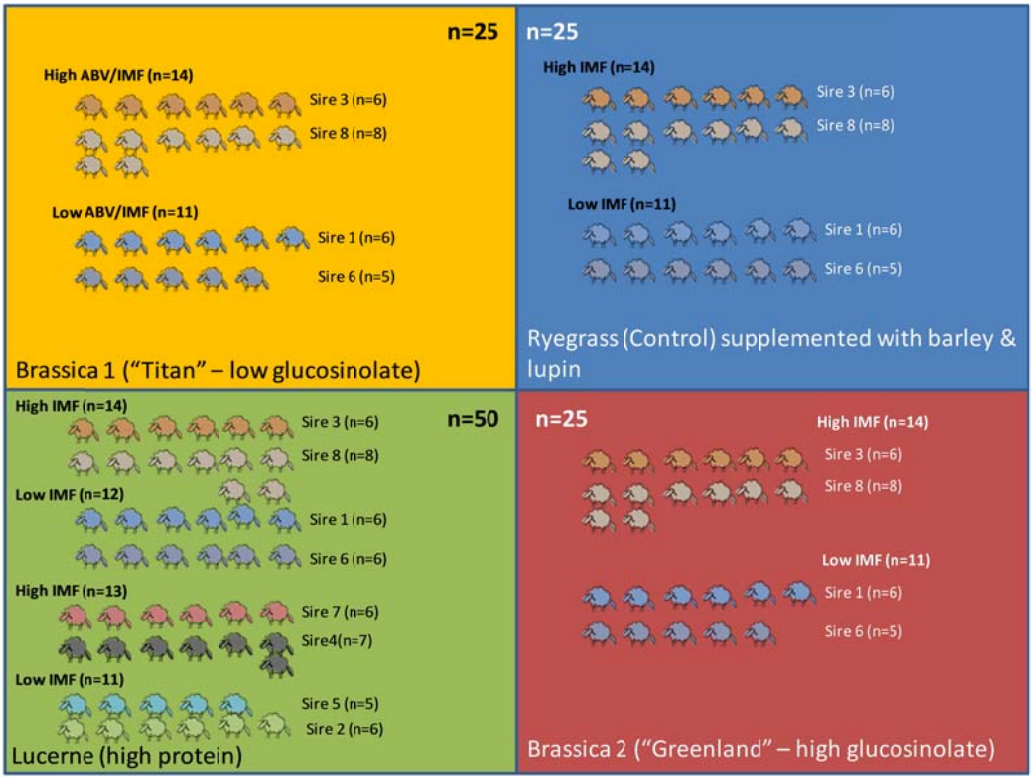

Figure 2: Diagram of lamb allocation to finishing feed regimes balanced with respect to low and high ABVs. Note: different numbers of ewes per sire were available. The central comparison for feed effects were made across animals from sires 3 & 8 (high ABVs) and 5 & 6 (low ABVs). Additional animals were allocated to the lucerne treatment for further comparisons of sire effects.

Figure 3: Drafting of lambs at Leigh Creek to each of the feed treatments using temporary pens and the EID reader.

2.3.1 *Animal Welfare Monitoring*

Animal welfare monitoring was carried out every day for the duration of the 42-day trial. One lamb developed a slight leg injury requiring inspection and treatment from a qualified veterinarian. The animal was successfully treated, recovered rapidly and remained in the trial. No other major animal health issues were observed. The initial target feeding duration was to be for 8 weeks but, due to lack of rainfall and high average temperatures during January and February, the feeding experiment was ended earlier (6 weeks).

Shade cloths were used to provide partial shading within each plot. Water was available to animals *ad libitum* using water dispensers. A CSIRO Animal Ethics Committee representative inspected the experimental site in January 2013 and deemed it to be more than adequate in terms of animal welfare and scientific requirements. Breaks were put in place within each plot to manage crop growth and to control feed intake throughout the trial. Tony Fleetwood, the owner of the sheep, also visited the Leigh Creek site and was satisfied with the arrangement.

2.3.2 *Weather Conditions*

The temperatures for the period leading up to the finishing feed trial were higher and the rainfall was lower in December/January compared to the average. Considering the dry conditions, the 2 brassica rape crops, ‘Greenland’ and ‘Titan’, sown in October managed to produce 5.2 t DM/ha and 4.6 t DM/ha pre-grazing yields respectively at the first break. Lucerne yields or availability was not adversely affected due to the area that was sown. However, the ryegrass treatment could not retain green feed for the grazing period and once completely dried off the diet was supplemented with barley and lupin grain to achieve targeted growth rates of the lambs. This feeding regime closely reflected common practice for supplementary feed program used in the region by sheep farmers in south-eastern Australia.

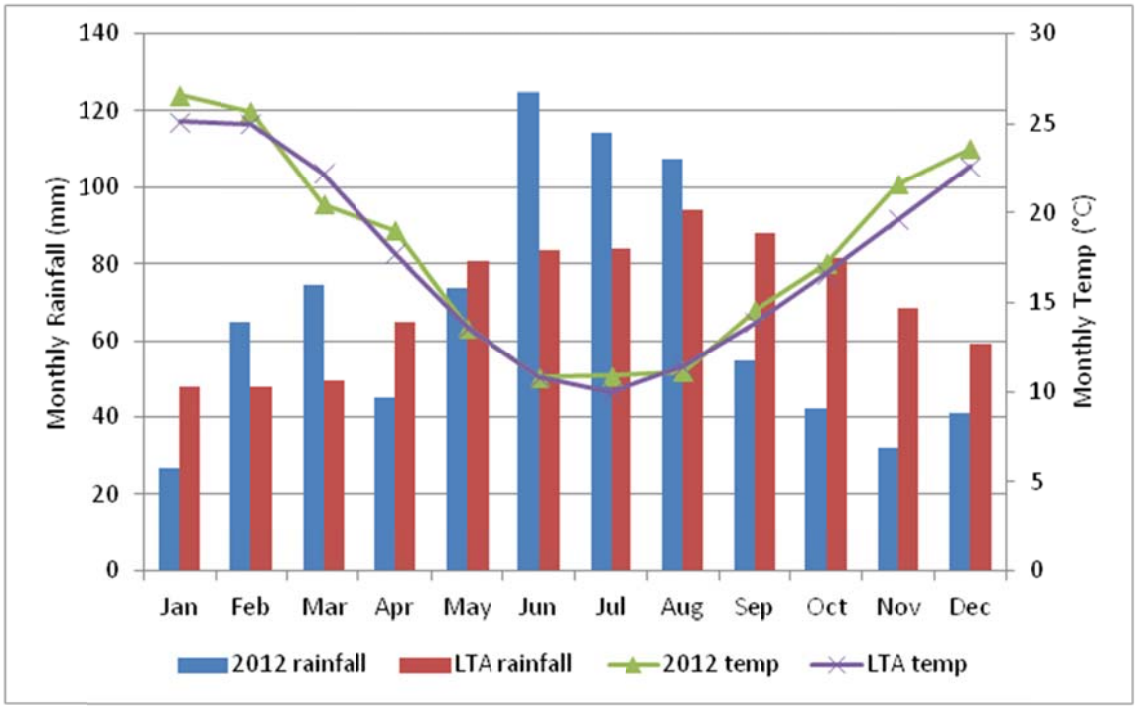

Figure 4: Monthly rainfall totals and monthly mean temperatures for 2012 against the respective long-term averages (LTA) the period, 1908 – 2013).)

2.4 Animal Growth Rates

At the time of allocation (11 January 2013), significant differences in initial live weights (LWs) were measured between the sire types. Both high ABV animals were heavier than the low ABV animals (**Table 2**). On average the greater weight of the high ABV animals compared to the low ABV animals persisted throughout the 42 day finishing period (**Table 2**, **Figure 5**). In contrast, the average weight gains (grams/head/day) for different periods was significantly affected by feed type (**Table 3**). Negative growth was recorded in the first 12-day period for all finishing feeds except for the ryegrass. The greatest initial weight loss was recorded for the high GSL Greenland cultivar > low GSL Titan > Lucerne. Positive growth was measured over the next 8-day period for all feed types, with lowest gains recorded for the animals finished on the Greenland once again.

Average lamb growth rates for the grazing period showed the Brassica (average of two treatments) > Lucerne > ryegrass (data not shown). Throughout the first grazing period, there was a negative growth rate in the rape and lucerne crops, but was not observed in the ryegrass treatment, but only very low rates measured with only 29 g/head/day. This is referred to as the 'adjustment period' for stock adjusting to new feeds. Following the 12 day adjustment period, there was a significant increase in growth rates of the rape treatments, especially those animals raised on 'Titan' which reached 328 g/head/day, 197 g/head/day greater than 'Greenland'. Both the lucerne and ryegrass treatment growth rates increased dramatically through this period.

By the third weighing period, the 'Greenland' and 'Titan' growth rates were similar, with 'Titan' achieving 396 g/head/day. The lucerne treatment remained stable and ryegrass declined considerably. The fourth weighing period showed that both rape treatments declined, with 'Greenland' retaining a slightly higher growth rate than 'Titan' by 35 g/head/day, the lucerne growth rate remained consistent and the ryegrass increased slightly. It should be noted that significant differences were also observed in nutritive value of the Brassica treatments and utilisation of the two crops with Titan > Greenland – these data will be reported in a later separate report.

The heaviest final hot carcass weight (HCW) was achieved on the 'Titan' feed, which was 1.01 kg greater than 'Greenland' and 1.70 kg greater than the lucerne treatment. The ryegrass (and supplement) treatment was significantly less than all other treatments.

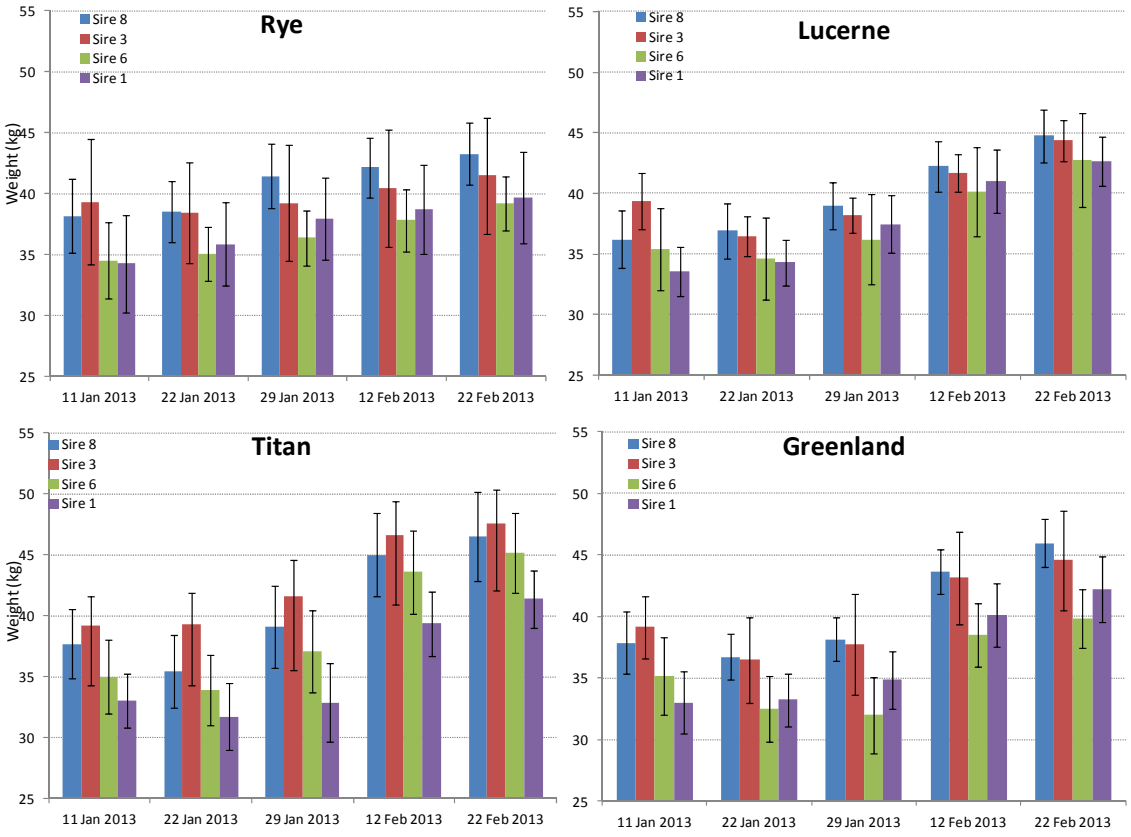

Figure 5: Average weight gains for the four sires on the different finishing feeds. Sire 1 & 6 low ABVs and sires 3 & 8 high ABVs.

Table 2: Animal weights at different times during the 42 day finishing feed trial. The effect of feed was not significant at any date. The effect of sire was significant or approached significance at most time points. The pooled effect of high and low ABV sires was significant, with high ABV animals attaining a greater liveweight at all sampling times.

	FEED effect				SIRE effect						
	RYE kg	LUCERNE kg	TITAN kg	GREENLAND kg	1	6	3	8	Feed	Sire	ABV
11 Jan	36.8	36.2	36.4	36.5	33.5	35	39.3	37.5	—	0.006	<0.001
22-Jan	37.2	35.7	35.2	35	33.8	34	37.7	36.9	—	0.03	0.002
29-Jan	39.06	37.81	37.8	36.03	35.8	35.4	39.2	39.4	—	0.06	0.002
12 Feb	40.1	41.4	43.7	41.6	39.8	40.0	42.97	43.3	—	0.07	0.006
22-Feb	41.2	43.7	45.3	43.53	41.5	41.7	44.5	45.2	—	0.066	0.006

Table 3: Average daily weight gains during different time periods over the 42 day finishing feed trial.

	RYE	LUCERNE	TITAN	GREENLAND	1	6	3	8	Feed	Sire
Gain 1 (12 days) 22nd Jan	0.40	-0.50	-1.20	-1.50	0.31	-0.98	-1.57	-0.62	0.04	0.07
Gain 2 (8 days) 29th Jan	1.9	2.1	2.6	1.0	2.0	1.4	1.5	2.5	0.02	0.09
Gain 3 (15 days) 12th Feb	1.0	3.54	5.94	5.62	4.03	4.59	3.79	3.84	<0.001	ns
Gain 4 (10 days) 22nd Feb	1.11	2.35	1.53	1.87	1.67	1.73	1.53	1.87	0.002	ns

2.5 Animal Slaughter

All animals were killed as part of the routine commercial Coles kill on February 26th at JBS-Swift (Brooklyn). DEPI-V staff assisted CSIRO staff in carcass labelling and collection of samples. Abattoir animal data were recorded. Although animals could only be identified by hook number in the chiller-room, staff removing the striploins commented that some carcasses had a discernible and "unfamiliar" odour. It was also noted that the consistency of the subcutaneous fat on some samples was different in terms of hardness and structure. Fat samples were removed from carcasses and GR-fat depth measurements were taken in the chiller. In the morning of the following day (February 27th) both full striploins were removed from carcasses in the chillers, labelled and packed in snap-lock bags. Samples were transported to a food-grade refrigerated processing facility at CAFHS Werribee in the early afternoon and stored at 10°C.

Meat colour and pH was measured and recorded in the laboratory. Left and right striploins were separately labelled and vacuum packed and placed in foam containers with temperature loggers (i-Buttons). Samples were aged in Cryovac bags for 5 days at 2 ^{o}C before freezing down at 20 ^{o}C. Frozen samples were transported to the Coopers Plains (Brisbane) laboratories of CAFHS. Striploins were cut frozen to 15 mm steaks using a bandsaw in the meat processing facility and vacuumed packed in bags, in preparation for grilling and sensory and consumer testing. Sensory testing is scheduled to occur in late April/early May 2013. In summary, samples were collected as per the experimental design.

3 Chemical and Physical Properties of Lamb Samples

3.1.1 *Carcass Characteristics*

Average carcass characteristics are summarised in **Table 4**. The effect of "Feed" on hot carcass weight (HCW) was assessed; Titan and Greenland finished carcasses were on average heavier than those from the other finishing feeds. The amount of intramuscular fat (% IMF) was highest in the lucerne finished meat and lowest in the rye finished samples. The effect of "feed and "sire" on other carcass attributes were assessed using the HCW as a covariate term. Carcass pH was higher in the Titan samples compared to the other treatments and the Greenland finished meat was less red (a* value) compared to the other finishing feed treatments. The GR fat depth was significantly lower for Sire 8 compared to the other sires. No other sire-related effects were measured.

3.1.2 *Modified Warner-Bratzler Shear Force & % Cook Loss*

The Warner-Bratzler shear (WBS) force measurement is the most widely used objective measure of meat palatability. WBS was performed on cooked samples according to previously reported CSIRO protocols. Samples were weighed and suspended in plastic bags in a 70 $^{\circ}$C water bath for 60 minutes, cooled in ice slurry for 20 minutes, patted dry and re-weighed to determine % "cook loss", the amount of water lost (% w/w) from meat samples during heating. Samples were stored over night at 4 $^{\circ}$C to set before cutting. The tenderness or toughness of meat samples was determined by using a modification of the Warner-Bratzler shear device (Bratzler, 1932) and a Lloyd Instruments LS 2.5 materials testing machine fitted with a 500N load cell (Lloyd Instruments Ltd., Hampshire, UK). Samples used in the Warner-Bratzler device had a rectangular cross-section 15 mm x 6.7 mm (1 cm² cross-sectional area), and were cut with the fibre orientation parallel to the long axis, and at right angles to the knife blade of the device. The force required to shear through the clamped samples with a triangulated 0.64 mm thick blade pulled upward at a speed of 100 mm/min was measured. Data was collected using the Nexygen-Plus 3 software (Lloyd Instruments Ltd., Hampshire, UK); the parameters measured from the shear-force deformation curves were peak force (PF), initial yield (IY), and peak force minus initial yield (PF-IY). Six determinations were made on each sample and the mean recorded. All analyses were performed at room temperature.

3.1.1 *Warner-Bratzler Results*

The finishing feed did not have a significant effect on the final WBS measurements IY and PF, when analysed either with or without the covariate term HCW. High ABV samples had lower IY values compared to the low ABV samples. The effect of individual sire was also significant; IY for Sire 8 was significantly lower than the others, including Sire 3. The covariate term, HCW, was significant for both texture measurements. Some small differences in % cook-loss were measured for the different finishing feeds. The relationships between, IY, PF and GR-fat score and HCW are shown graphically in **Figure 6**. As expected GR fat depth score increased with HCW. As the HCW increased both the IY (r = -0.55, $P < 0.001$) and the PF (r = -0.53, $P < 0.001$) decreased.

Table 4: Carcass parameters and Warner-Bratzler texture measurements.

	RYE	LUCERN	TITAN	GREEN	1	6	3	8	Feed	Sire	ABV	Feed * Sire	HCW
HCW	18.27[b]	19.61[b]	20.82[a]	20.27[a]	19.38	18.38	20.51	20.7	0.002	—	—	—	—
GR	8.3	8.6	9.5	9.31	9.31[a]	9.29[a]	9.56[a]	7.85[b]	—	0.03	—	—	<0.001
pH	5.52[a]	5.51[b]	5.53[a]	5.51[b]	5.51	5.52	5.52	5.52	0.003	—	—	—	—
L*	36.41	37.74	36.84	37.42	37.53	36.87	37.42	36.6	—	—	—	—	—
a*	18.96[a]	18.15[a]	18.53[a]	17.05[c]	17.92	18.57	18.23	17.97	<0.001	—	—	—	—
b*	1.98	1.99	1.72	1.67	1.85	2.21	1.81	1.49	—	—	—	—	—
% IMF	6.8[c]	8.3[ab]	9.6[a]	7.5[b]	7.8[b]	6.8[b]	8.5[a]	9.1[a]	0.03	—	0.03	—	—
% Cook loss	16.3[a]	14.52[b]	14.43[b]	15.7[ab]	15.62	14.67	15.28	15.41	0.02	—	—	—	—
Initial Yield	19.62	18.46	20.39	19.46	21.19[b]	20.08[b]	19.25[ab]	17.42[a]	—	0.03	<0.001	—	<0.001
Peak Force	24.12	22.05	23.5	22.62	24	23.5	22.6	22.3	—	—	0.01	—	0.004

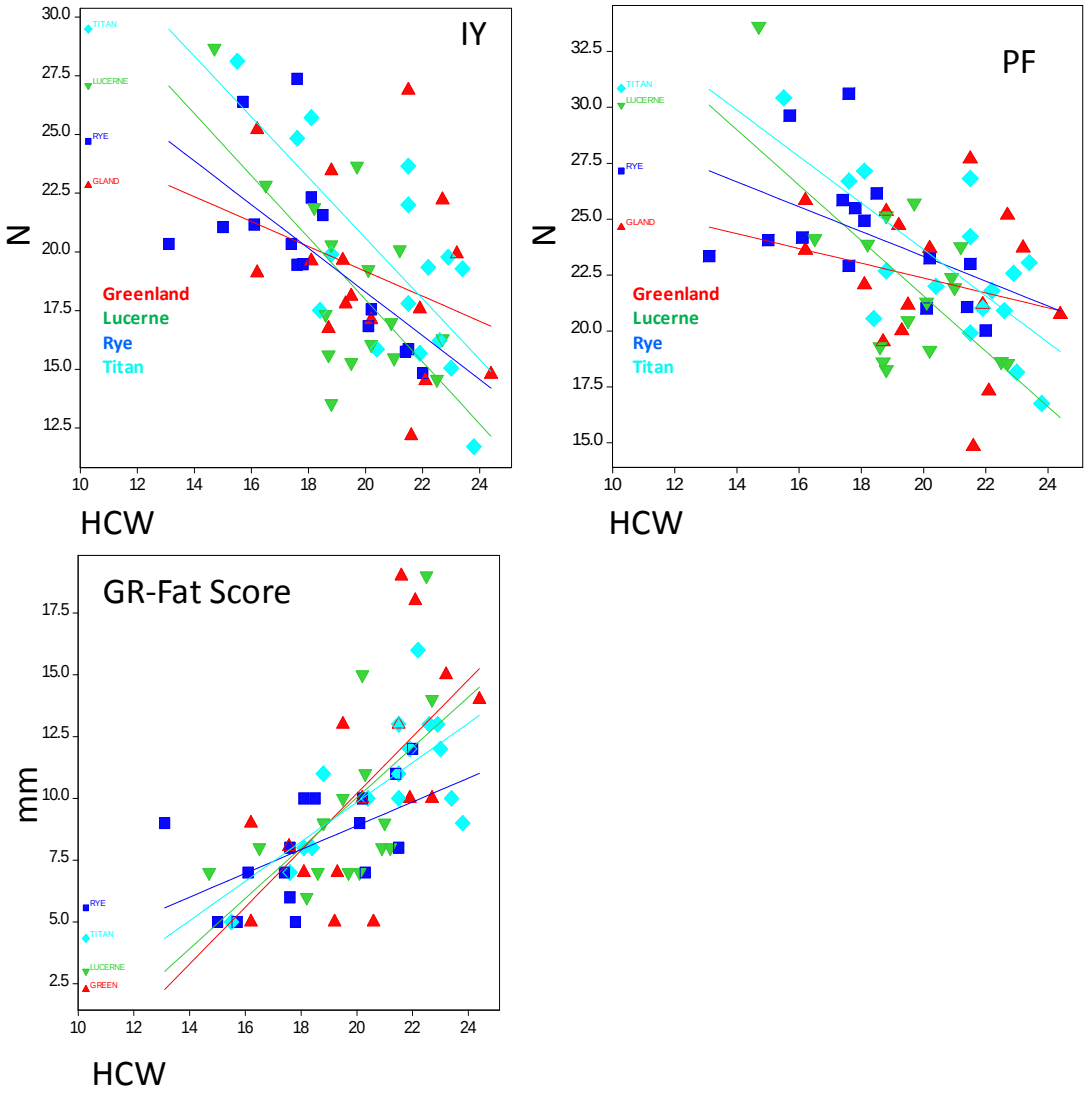

Figure 6: Scatterplots and fitted regression lines showing the relationship between HCW and the Warner-Bratzler parameters Initial Yield (IY) and Peak Force (PF) and GR fat score.

3.2 Summary of Meat Chemical & Physical Properties

- *Differences in carcass characteristics were minimal, regardless of feed or sire*
- *Sire 8 had the highest % IMF, the lowest GR-fat score and the lowest IY score compared to the other samples, suggesting some unique characteristics for meat from ewes from this sire lineage*
- *The % IMF was greater for the high ABV samples (Sire 3 & Sire 8) compared to the low ABV samples (Sire 1 & Sire 6) as expected*
- *IY, PF and GR-fat scores were correlated with HCW measurements*

4 Trained Sensory Panel Assessment of Lamb

4.1.1 *Standardised Grilling Protocol*

As discussed in the previous sections, the final LWs and HCWs varied considerably within feed and sire groups. Hence, the HCW was used to guide selection of samples for sensory assessment so that they were matched as far as was possible. In the design, right striploins were used for the trained sensory panel experiments and the corresponding left striploins were later used in the consumer sensory testing. For the larger animals, more than 15 X 15 mm steaks were obtained. Each steak was sufficient to serve 2 panellists; hence 5 steaks provided 10 servings. As each lamb sample was to be tested in triplicate by 10 panellists, at least 15 steaks were required from each striploin. For each feed X sire combination (e.g.; Greenland X Sire 8), the samples were ranked in order of increasing HCW. As far as possible, samples for sensory testing were selected on the basis of proximity to the target HCW of ~20 kg. For each feed X sire combination, four replicate animals were selected, resulting in a total of 32 animals being assessed. The list of animals used in the sensory evaluation are detailed in (XXX) in the Appendix. Remaining samples were used for the panel sensory training phase.

The same grilling protocol was used throughout this study unless otherwise indicated. Frozen lamb steaks were laid out on aluminium foil on trays according to the randomised presentation order and covered with cling film to defrost overnight in a temperature-controlled chamber (4 °C) prior to cooking. Samples were grilled on a SILEX-grill, pre-heated to 220 °C at least 45 min prior to cooking. A hand-held infrared thermometer was used to periodically measure the grill surface temperatures. Both grill surfaces were sprayed with light olive oil. A thermocouple probe was inserted into the middle of the first steak (five steaks of the same type were cooked simultaneously) and the samples were placed in the middle of the grill. After closing the lid, the steaks were cooked until a final internal temperature of 60 ^{o}C was attained. After grilling, samples were covered loosely with aluminium foil and left to rest for 3 minutes. Samples were immediately cut into four equal pieces. Two pieces were placed into a standard wine glass and covered. Randomly coded samples were then placed on a tray and served to each panellist in the sensory booths.

4.2 Panel Training and Vocabulary

Permission to conduct the sensory evaluation was granted by the CSIRO low risk ethics committee (application LR-15/2012 E). A ten-member flavour sensory panel was recruited from the pool of regular, dedicated CSIRO panellists in February 2013. The panel was composed of nine females and one male; average age = 51± 6 years. Panellists were remunerated for their time. All assessors had been screened for sensory acuity and had extensive prior experience in participating in descriptive sensory analysis of meat and other

products. All samples were prepared and assessed at the sensory facility located in CAFHS, North Ryde, Sydney. Five two-hour training sessions were held to generate and define the sensory vocabulary that best described the differences in odour, flavour, taste, texture, aftertaste and after-feel attributes. During the training period, assessors were equally exposed to samples representing experimental design variables; i.e. grilled meat from each of the four feeds and four sires.

More than 30 consensus attributes were developed for final application to assess grilled lamb odour, flavour, taste and texture attributes. The final list of attributes applied in the descriptive profiling are shown in the Appendix (**Table 14** & **Table 15**). Samples were rated using a 100 mm line scale on a computer screen. Panel performance was monitored using Panel Check Software (Nofima Mat, Norway) with regular feedback until every panellist had a clear understanding of the sensory attributes.

During panel training, the sensory panel:

- Determined the order of evaluation of sensory modalities (i.e. odour, flavour, texture, taste and aftertaste/afterfeel) as well as the order of attributes within each sensory modality.
- Selected reference standards and used selected pure chemical compounds where appropriate to clarify, improve understanding and define sensory attributes.
- Developed the final consensus sensory vocabulary used in evaluations consisted of 29 attributes (9 odour, 9 flavour, 3 taste, 6 texture and 5 aftertaste/after-feel attributes).

The list of attributes, definitions, related terms and reference standards is provided in the Appendix (**Table 14**).

4.3 Descriptive sensory analysis

Evaluations were carried out in individual sensory booths under white light. Each booth had a computer screen and sensory attributes were rated using a 100 mm line scale and a computer mouse. Samples were presented in a randomised order in wine glasses coded with randomised three-digit code. Samples were presented monadically in covered wine glasses to each sensory booth. The trained sensory panel carried out descriptive sensory analysis in triplicate using the agreed method of assessment and the consensus sensory vocabulary. Sensory evaluation of samples was performed in triplicate over a three-week period, resulting in a total of 30 sensory assessments per sample (n=10 panellists x 3 replicates) x 32 samples x 32 attributes.

For evaluation, a blocked design with 12 samples per session was used. Within blocks, order of sample assessment was randomised. A one-minute inter-stimulus interval was

imposed between samples and a five-minute break was imposed after every six samples to reduce assessor fatigue. Plain water and cucumber slices were used as palate cleansers. For each sample, after completing the evaluation of odour and flavour, the assessors were asked to take a drink of water or a bite of cucumber before proceeding to texture, taste, aftertaste and after-feel attributes. Between samples, the assessors were asked to drink water as well as have cucumber slices to cleanse their palate to prevent any carryover.

The samples were blind-coded with random 3-digit codes. The experimental design was produced using the design generation package – CycDesigN. The order of presentation was randomised, with balanced numbers of low, medium and high IMF samples for each sample type included on each day. Attributes were rated on 100 mm unstructured line scales anchored at 5% and 95% for each descriptive attribute. Data were recorded and stored using the Compusense sensory data acquisition software (Version 5.2, 2004; Compusense Inc., Guelph, Ontario, Canada).

4.3.1 *Method of assessment*

Samples were served in a randomised order to assessors in separate booths with individual computer terminals. Assessors were served two pieces of warm grilled lamb weighing approximately 2 g each in a covered wine glass. The panellists were asked to assess odour first, by removing the lid and sniffing the sample (orthonasal evaluation). Assessors were asked to assess *overall odour impact* and other odour attributes using 100 mm line scales on a computer screen. Assessors were allowed to re-sniff the sample headspace as many times they required. For assessment of flavour attributes, panellists were instructed to take one whole piece using a skewer, place in the mouth and evaluate flavour attributes (retronasal evaluation) during eating. Attributes were rated on the computer screen in the order of their perception determined according to the panel consensus.

Prior to assessing texture, taste, aftertaste and after-feel attributes, the assessors were told to cleanse their palate by drinking plain water or eating a piece of cut cucumber. For assessing texture, taste, aftertaste and after-feel attributes, they were instructed to place the second piece in the mouth and start chewing using molars. *Juiciness, Tenderness* and other attributes were assessed. Panellists were instructed to continue chewing and counting chews until the point of swallow and to rate the amount of *Connective Tissue* just before swallowing and to enter the *Number of Chews* to swallow. Aftertaste and afterfeel attributes were assessed 30 seconds after swallowing the sample.

4.3.2 *Statistical Analysis*

Unless otherwise indicated, all statistical analyses were performed using GENSTAT 15th Edition (VSN International Ltd, Hemel Hempstead, United Kingdom). Replicate sensory data were subjected to multivariate analysis of variance (MANOVA). Appropriate post-hoc multiple comparison tests (Fisher's least significant difference; LSD) were carried out where significant differences were found.

For assessment of animal effects, replicate sensory data for individual animals were assessed using and 'animal x panellist' fixed factor design. Sample type differences were assessed by MANOVA by comparing main effects of Feed (rye, lucerne, Titan, Greenland)

ABV (high/ low) or Sire (1, 3, 6, 8). Hot carcass weight (HCW) was used as a covariate term in some analyses, as animal weight is known to affect flavour characteristics.

Principal component analysis (PCA) was conducted using Genstat to summarise the similarities and differences between the samples and to visualise the relationships between all the samples and the sensory attributes. PCA bi-plots (PC1 and PC2) were generated by Genstat and used without modification in the report. Partial least squares (PLS-1) regression analysis (Unscrambler Version XX) was used to better characterise the relationship between sensory attributes and other parameters.

4.4 Sensory Results

4.4.1 *Odour Differences*

The most important odour attributes were *Overall Odour Intensity, Grilled Odour* and *Lamb/Mutton Odour*. *Livery* and *Oxidised Fat* odour were rated as moderate impact odour attributes (**Table 5**). The trained panel were able to discriminate differences between animals for all odour attributes except for *Hay /Grain* Odour. The animal-to-animal differences in odour attributes were similar to previous sensory data for sheep (in preparation) and beef (AMQA.0001). Despite the significant animal-to-animal variability, a number of feed and sire effects were measured. *Overall Odour impact* was rated higher for animals on the Titan and Greenland feeds, compared to the Rye and Lucerne. *Odour Impact* was rated significantly higher for ewes from Sire 3 & 8, and animals with high ABVs compared to low ABVs. *Grilled Odour* was rated highest in Greenland finished animals. Although *Barnyard Odour* was not detected to a great extent in any of the samples, the Rye finished animals were rated higher on average in this attribute. The variability in odour attributes across all samples is summarised in the PCA plots in **Figure 7**. Samples are colour-coded according to finishing feed (top) and according to sire (bottom). The coloured line encloses the limits of sample within a grouping. The size of the space enclosed by the bounding lines is a measure of the variability within the sample set. On the basis of the size of the bounding boxes, the odour of the Greenland finished samples was less variable and more consistent than for the other finishing feeds. *Grilled* odour was positively correlated with the HCW. *Oxidised fat* odour decreased with increasing HCW. The odour variability of lamb from Sire-8 was less than for Sire-3 or the low ABV sires.

4.4.2 *Flavour Differences*

Significant animal-to-animal variation in all flavour attributes (except *Grassy* and *Metallic* flavour) were measured by the panel (**Table 5** and **Figure 8**). Overall *Flavour intensity* and *Lamb/Mutton* Flavour were quantitatively the most important flavour attributes, followed by the moderate intensity attributes; *Metallic, Livery* and *Fatty* flavour. *Vegetable, Grassy* and *Dairy Fat* flavour was rated as very low for all finishing feeds. The *Vegetable* flavour attribute was specifically introduced to quantify any “brassica-like” flavours within the samples. Brassica plant materials were used as references for this attribute – the flavour was

described as cooked cabbage or broccoli. *Vegetable* flavour was not detected to any extent in any of the samples, including the high GSL and low GSL brassica finished samples. This finding strongly demonstrates that a specific brassica related flavour taint was not detected in lambs finished on either of the brassica feeds. *Metallic* flavour was rated slightly higher in the Rye and Titan finished animals and lowest in the Greenland. Some small flavour differences were found between sires and ABV. *Overall Flavour* was higher in the high ABV samples, especially Sire-8. Small sire-related differences were measured for *Metallic* and *Fatty* Flavour. The high ABV samples were rated higher in *Fatty Flavour*, particularly meat samples from Sire-8. It can be seen (**Figure 8**) that once again, overall flavour variability was lowest for the Greenland finished samples.

4.4.1 *Taste & Aftertaste Differences*

As for odour and flavour related attributes, animal-to-animal variation in taste and aftertaste attributes were measured (**Table 6**, **Figure 9**). In general Feed effects were minimal, except for higher *Acidic* aftertaste in the Titan finished sample. Sire-related taste and aftertaste effects were measured for most attributes. The high ABV samples were on average *Sweeter,* more *Salty* and less *Acidic*; this was particularly true for the Sire-8 samples.

4.4.1 *Texture Differences*

Highly significant differences were found between animals for texture attributes; feed and sire effects as well as the feed X sire interaction were also measured (**Table 6**). In general, the Titan finished samples were less *Tender,* less *Juicy* and higher in *Number of Chews* and *Connective Tissue*, although there was considerable variability in these attributes. Animals with high ABVs were more *Tender, Juicy* and had less *Connective Tissue* and required less *Number of Chews.* As the feed X sire interaction was significant for many texture attributes, it was explored further (**Figure 12**). In most cases, the low ABV samples were less *Juicy* and *Tender* and required a greater *Number of Chews.* The variability in *Number of Chews* was greater for the low ABV sires (1 & 6) compared to the high ABV sires (3 & 8). Meat from Sire 6 finished on Titan was significantly less tender than Sire 1 finished on Titan, or any of the other samples. Of note, the growth rates of Sire 6 were faster than for Sire 1 on the Titan finish (**Figure 5**). There was less variability in the texture attributes for the Greenland finished animals regardless of Sire, compared to the others (**Figure 10**).

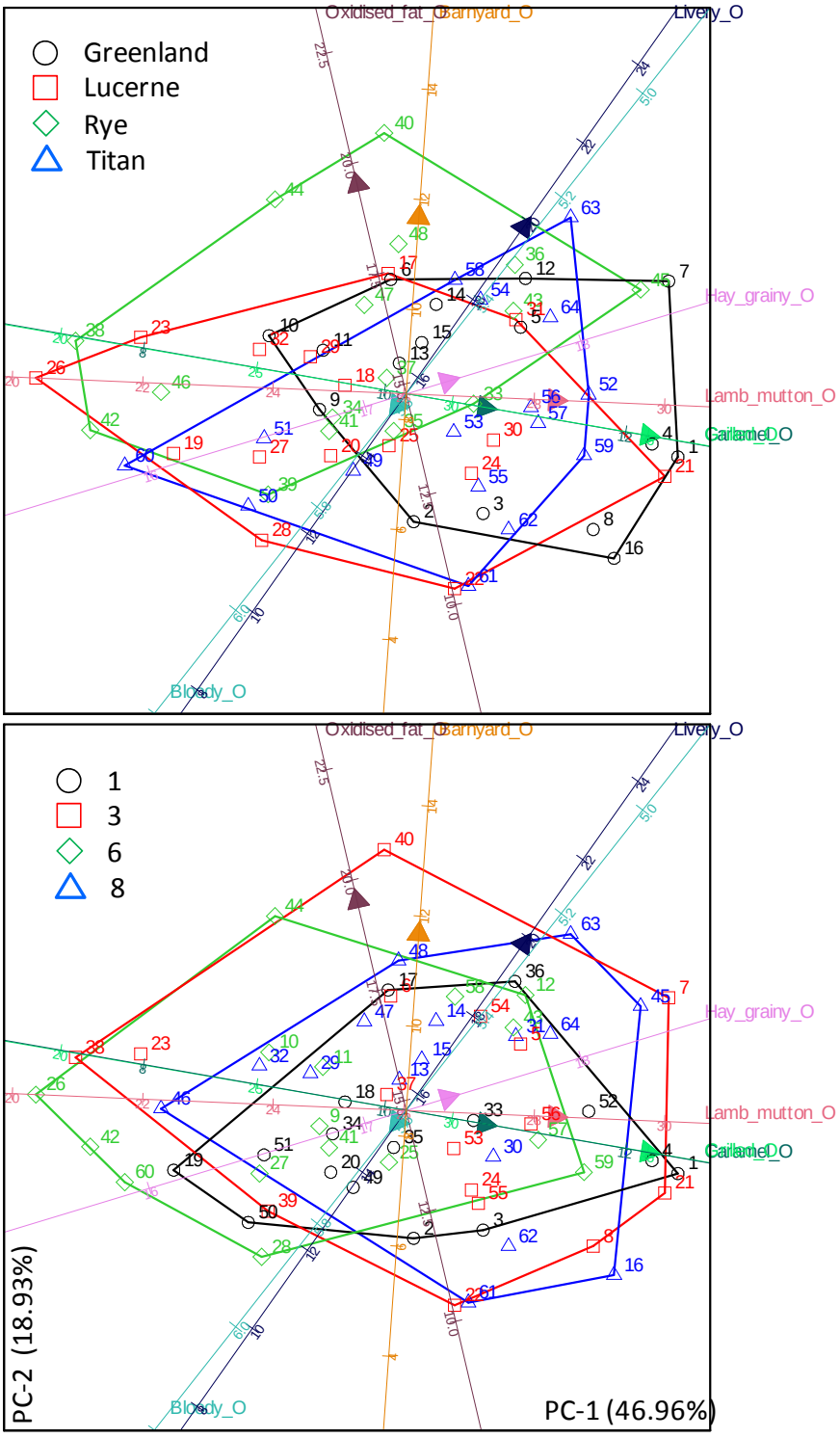

Figure 7: PCA biplots showing the relationship between odour-related attributes and samples; colour-coded according to finishing feed (top) and sire (bottom).

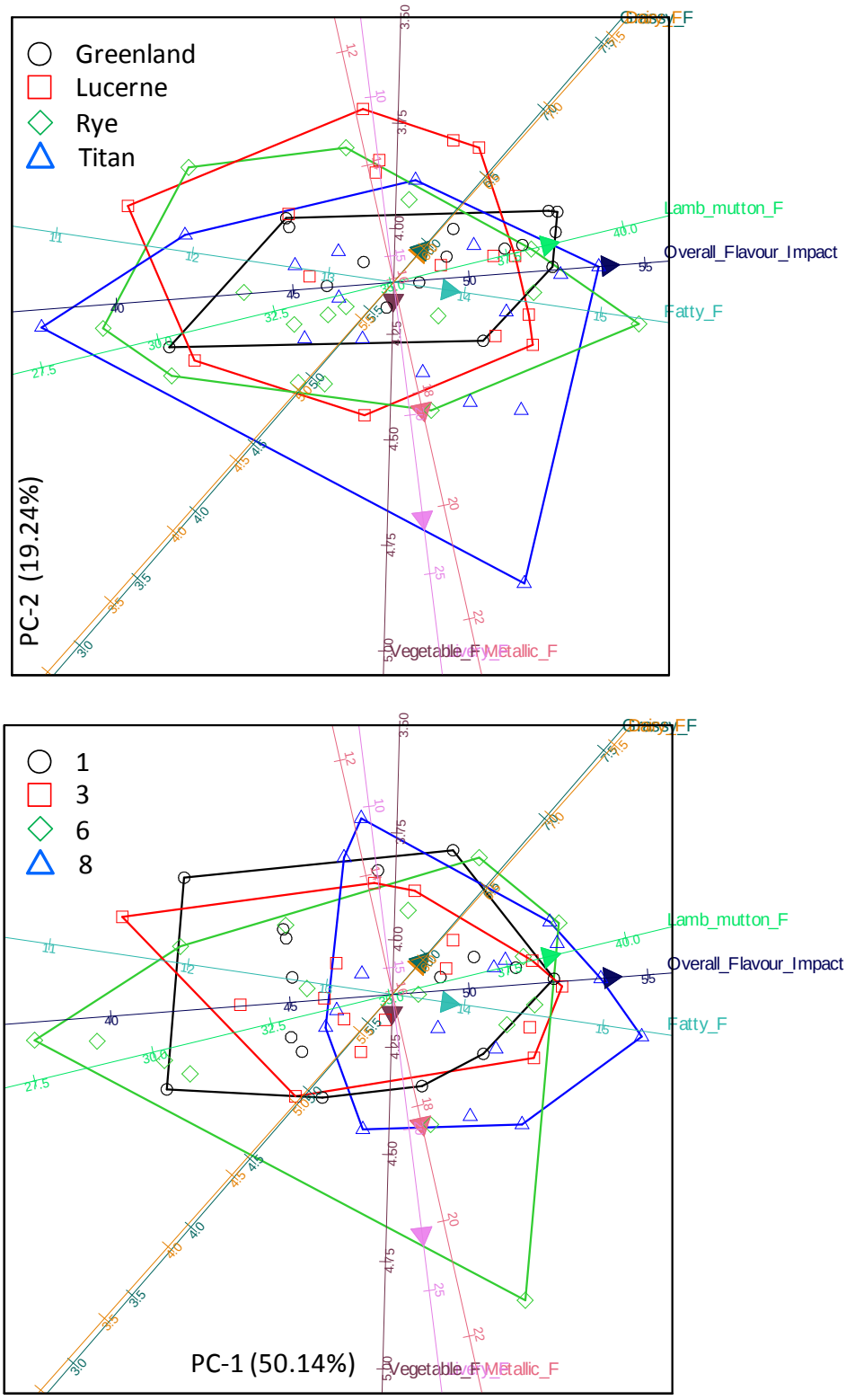

Figure 8: PCA biplots showing the relationship between flavour-related attributes and samples; colour-coded according to finishing feed (top) and sire (bottom).

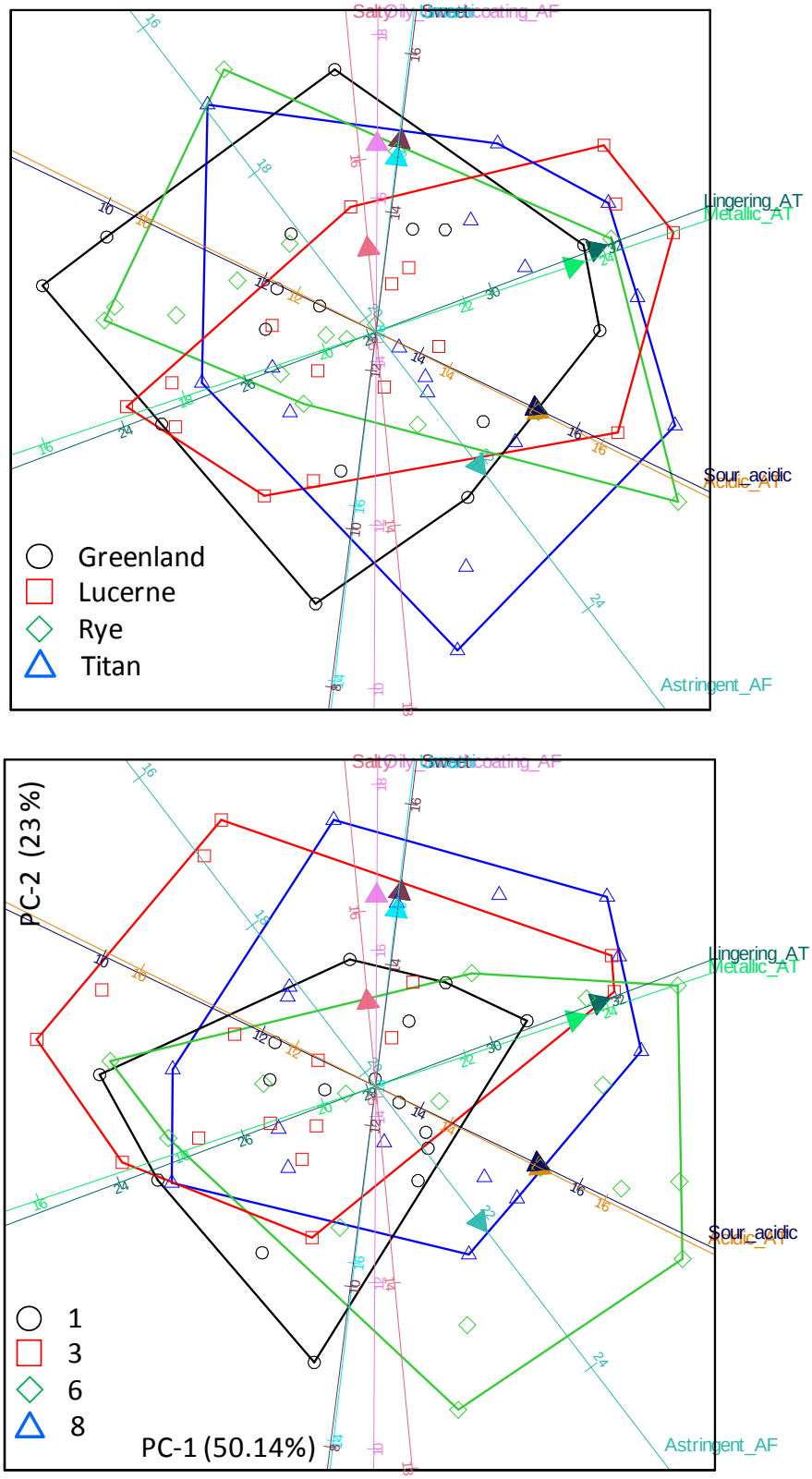

Figure 9: PCA biplots showing the relationship between taste and aftertaste-related attributes and samples; colour-coded according to finishing feed (top) and sire (bottom).

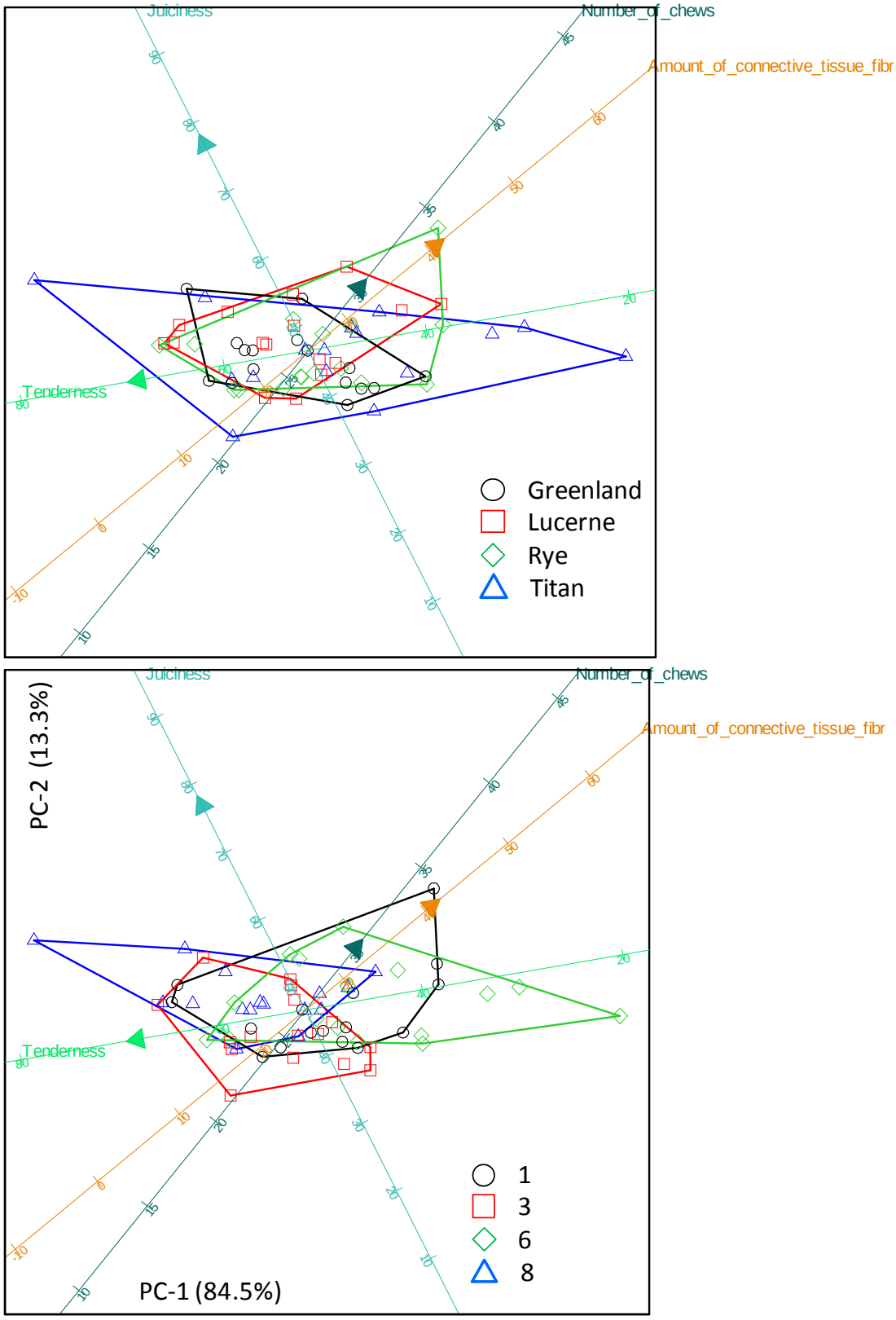

Figure 10: PCA bi-plots showing the relationship between texture-related attributes and samples; colour-coded according to finishing feed (top) and sire (bottom).

4.4.2 *Attributes Correlated to the HCW*

A number of odour, flavour and texture attributes were significantly correlated to the HCW (**Figure 11**). In general, *Tenderness* and *Juiciness* increased and *Number of Chews* decreased with increasing HCW. *Grilled* odour and flavour *Impact* increased, while *Acidic* aftertaste decreased with HCW.

The feed X sire interaction was significant, mainly for texture attributes (**Figure 12**). For example, *Juiciness* and *Tenderness* were generally higher for Sire 8, regardless of the feed. Lamb from Sire 6 was much less *Tender* and *Juicy* compared to Sire 1, but only for the Titan finish.

Table 5: Mean MANOVA sensory scores for odour and flavour attributes of grilled lamb (n=?) for the effects of animal, finishing feed and sire. Hot carcass weight was used as a covariate.

ODOUR	P Animal	RYE	LUCERNE	TITAN	GREEN	P Feed	1.0	6.0	3.0	8.0	P Sire	low	high	P ABV	P Feed * Sire	P Feed * ABV	P Covariate
Overall Impact	<0.001	46.9	47.4	49.2	50.5	0.002	47.9	46.8	49.2	50.1	0.003	47.1	**49.2**	<0.001	0.004	ns	ns
Lamb/mutton	<0.001	25.3	25.4	25.9	26.3	ns	25.5	25.4	25.3	26.6	ns	25.2	26.2	ns	ns	ns	ns
Grilled	<0.001	28.4	26.6	28.8	31.3	<0.001	29.1	27.7	29.2	29.1		27.6	**29.6**	<0.001	<0.001	ns	0.005
Bloody	0.01	4.7	5.7	5.7	5.1	ns	5.2	5.2	5.0	5.7	ns	5.4	5.5	ns	ns	ns	ns
Caramel	<0.001	10.0	9.4	10.0	10.5	ns	10.6	9.0	10.0	10.4	0.01	9.7	10.2	ns	0.003	ns	ns
Barnyard	0.02	9.6	7.4	7.8	8.1	0.01	7.7	7.6	8.3	9.3	ns	7.9	8.7	ns	0.07	ns	ns
Hay/Grain	ns	15.5	16.9	17.5	17.3	ns	16.6	16.5	16.6	17.6	ns	16.8	17.4	ns	ns	ns	ns
Livery	<0.001	14.9	14.3	15.2	15.9	ns	14.4	14.8	14.9	16.2	ns	14.6	**15.8**	0.021	ns	ns	ns
Oxidised Fat	0.03	15.1	14.2	14.3	14.4	ns	13.9	14.2	14.6	15.3	ns	14.7	14.7	ns	ns	ns	0.04

FLAVOUR	P Animal	RYE	LUCERNE	TITAN	GREEN	P Feed	1.0	6.0	3.0	8.0	P Sire	low	high	P ABV	P Feed * Sire	P Feed * ABV	P Covariate
Overall	<0.001	47.7	48.0	47.1	48.2	ns	47.6	46.8	47.1	49.5	0.004	46.4	48.9	<0.001	ns	ns	<0.001
Lamb/mutton	0.003	34.5	34.8	34.5	35.5	ns	35.1	34.5	34.2	35.5	ns	34.4	35.2	ns	ns	ns	0.009
Dairy	0.02	6.0	6.0	5.3	5.5	ns	6.0	5.5	5.3	5.9	ns	5.9	5.9	ns	ns	ns	ns
Grassy	ns	5.6	6.2	5.5	5.4		5.6	6.4	5.4	5.3		5.9	5.8	ns	ns	ns	0.012
Vegetable	<0.001	4.5	4.3	3.7	3.6	0.02	3.7	4.3	3.9	4.3		4.0	4.1	ns	ns	ns	ns
Fatty	<0.001	13.4	13.6	13.0	13.6		13.0	12.6	13.7	14.3	0.04	12.8	13.8	0.02	ns	ns	ns
Livery	<0.001	16.6	14.6	16.4	15.3	0.01	15.3	16.5	15.3	15.6		15.7	15.7	ns	ns	ns	0.03
Metallic	ns	16.1	15.6	16.9	15.1	0.007	15.8	16.6	14.9	16.4	0.027	16.1	16.1	ns	ns	ns	0.03

Table 6: Mean MANOVA sensory scores for odour and flavour attributes of grilled lamb (n=315) for the effects of animal, finishing feed and sire. Hot carcass weight was used as a covariate.

TASTE	P Animal	RYE	LUCERNE	TITAN	GREEN	P Feed	1.0	6.0	3.0	8.0	P Sire	low	high	P ABV	P Feed * Sire	P Feed * ABV	P Covariate
Sweet	<0.001	12.1	12.8	11.7	12.0	ns	12.2	11.4	11.9	12.9	0.03	12.0	12.6	0.089	ns	ns	ns
Salty	ns	14.7	15.2	14.7	15.4	ns	14.7	14.2	15.3	15.7	0.006	14.7	15.5	0.008	ns	ns	ns
Sour Acid	<0.001	12.7	13.7	13.8	13.3	ns	13.2	14.5	12.2	13.6	0.005	13.9	13.2	0.08	ns	ns	ns
Umami	0.006	18.2	16.9	18.0	17.9	ns	17.1	17.5	18.1	18.3		17.1	18.3	0.002	ns	ns	ns

AFTERTASTE	P Animal	RYE	LUCERNE	TITAN	GREEN	P Feed	1.0	6.0	3.0	8.0	P Sire	low	high	P ABV	P Feed * Sire	P Feed * ABV	P Covariate
Acidic	<0.001	12.3	13.1	14.1	12.6	0.002	12.7	14.3	12.3	12.9	0.01	13.6	12.6	0.008	ns	ns	<0.001
Astringent	ns	20.4	20.6	20.8	20.2	ns	20.1	22.1	19.8	20.0	ns	21.0	20.0	ns	ns	ns	ns
Oily Mouthcoating	0.003	14.2	14.3	14.2	14.6	ns	14.2	13.5	14.9	14.7	ns	13.8	14.5	ns	ns	ns	ns
Metallic	<0.001	20.5	20.6	21.0	19.6		19.9	21.2	19.3	21.3	0.01	21.2	20.6	ns	ns	ns	ns
Lingering	0.04	28.3	27.3	28.6	27.0		27.5	29.1	26.3	28.4	0.01	28.8	27.8	ns	ns	ns	ns

TEXTURE	P Animal	RYE	LUCERNE	TITAN	GREEN	P Feed	1.0	6.0	3.0	8.0	P Sire	low	high	P ABV	P Feed * Sire	P Feed * ABV	P Covariate
Tenderness	<0.001	53.5	54	47.3	52.1	<.001	50.3	47	53	56	<.001	48	55	<0.001	<0.001	<0.001	<.001
Juiciness	<0.001	47.5	49.6	43.7	45.7	<0.001	46	45.4	44.9	50.2	<0.001	**45.4**	48.9	<0.001	<0.001	<0.001	<0.001
Number Chews	<0.001	26	28	27.9	26	0.01	26.7	28.1	25.1	26.3	<0.001	28	26	<0.001	0.03	<0.001	0.003
Connective Tissue	<0.001	24.1	23.8	28.6	24.7	<.001	26.0	28.4	23.7	23.1	<.001	28	22	<0.001	ns	ns	ns

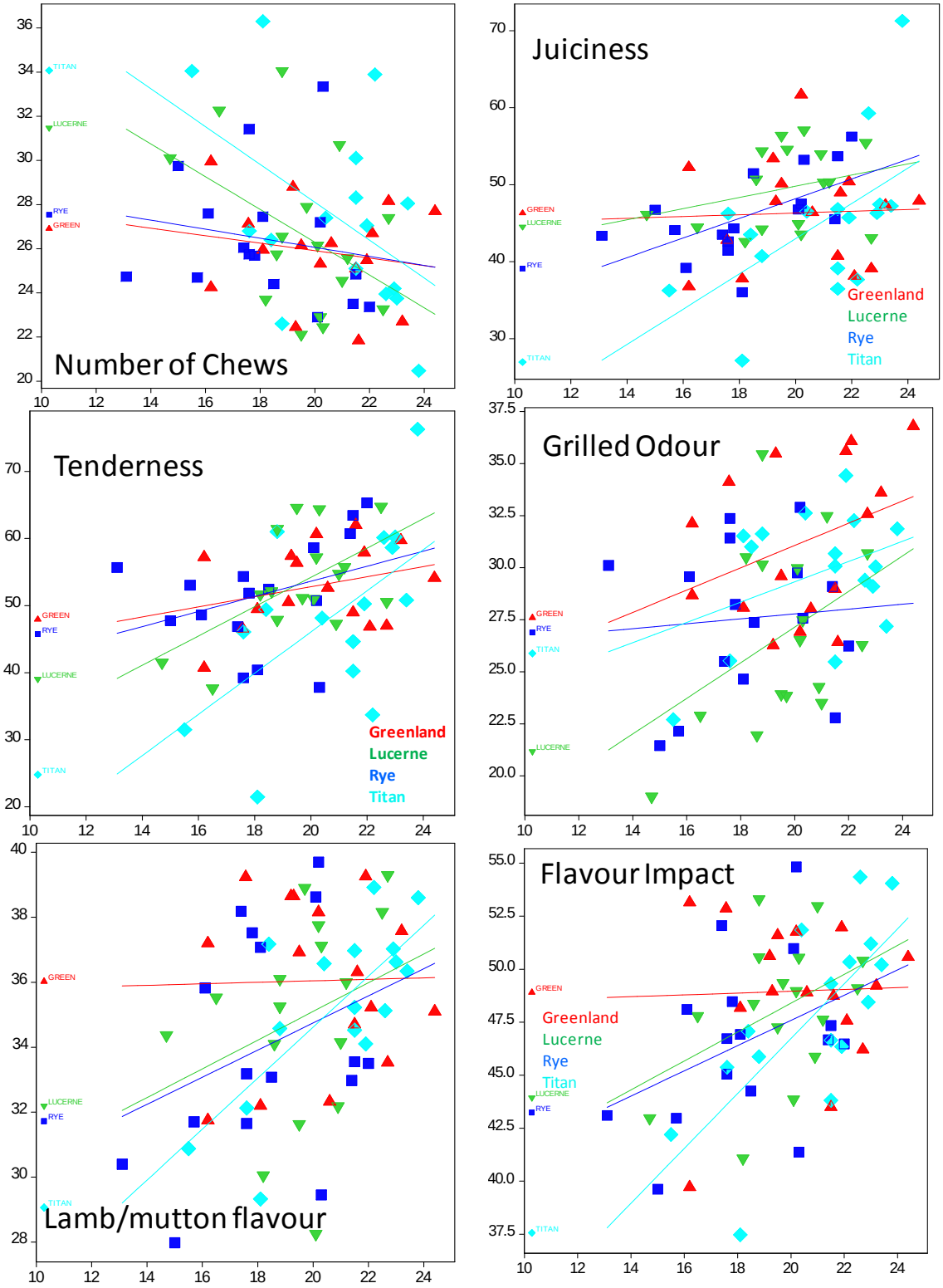

Figure 11: Scatterplot and regression models of the relationship between HCW (kg) and selected sensory attributes

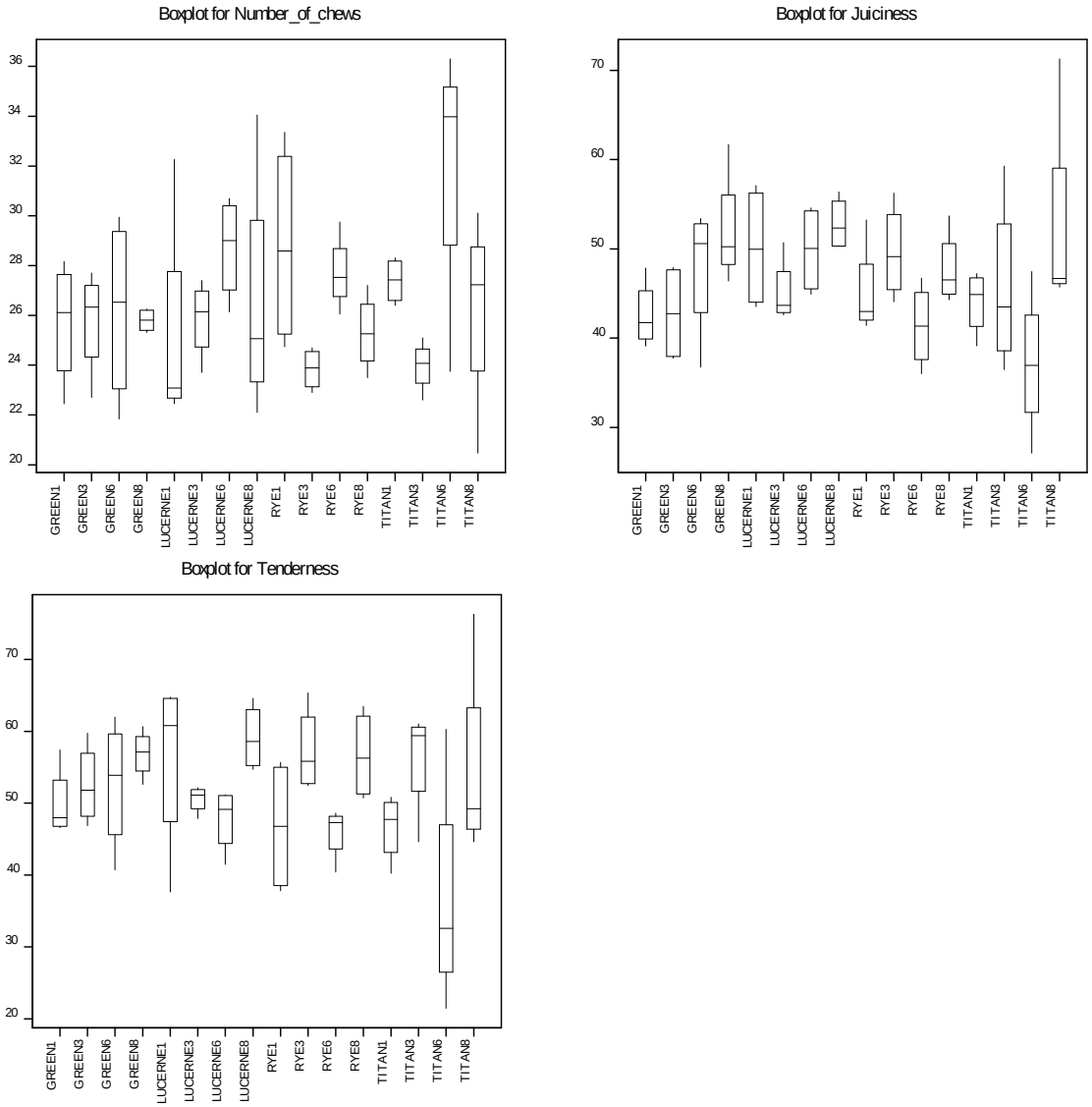

Figure 12: Box plots for texture attributes for which the feed X sire interaction was significant

4.5 Overview of Findings from Sensory Evaluation

- Few significant feed-related odour or flavour differences were measured by the panel. The Rye-finished samples had slightly low overall *Odour Impact* and mild *Barnyard Odour* compared to the other feeds, especially Greenland

- There was no evidence that any unusual flavour taint was detected in either of the brassica finished lamb samples; all samples were rated very low in *"Vegetable"* flavour

- In general, high IMF/ high ABV grilled lamb had higher *Odour* and *Flavour Impact*, higher *Grilled Odour* and more *Fatty Flavour*

- High IMF/ high ABV lamb was *Sweeter*, *Saltier* and less *Acidic* compared to low ABV samples

- *Acidic Aftertaste* was highest in Titan finished lamb and higher in meat from Sire 6

- *Tenderness* and *Juiciness* was highest in Sire 8 and *Number of Chews* and *Amount of Connective Tissue* was higher in the two low ABV sires (1 & 6)

- Meat from Sire 6 finished on Titan was the least *Tender* and *Juicy*

- Overall, the meat from Sire 8 appeared to have the most favourable sensory characteristics

- Notwithstanding the differences measured between Sire 3 and Sire 8, the pooled differences between low and high ABVs were also significant, with high ABV lamb having more favourable sensory characteristics

5 Cross Cultural Consumer Acceptability Study

5.1 Background & Rationale

Previous research has demonstrated that some consumers from Asian backgrounds are sensitive to “lamb flavour” and may have a lower preference for sheepmeat compared to other meats (Prescott *et al.* 2001, 2004, Wong *et al.* 1975). Most of this previous work was conducted with Japanese and Singaporean consumers. China is likely to be a substantial market for Australian lamb in the near future; hence a Chinese consumer panel was used in this study. The location of the sensory testing facility at North Ryde (Sydney) is in close proximity to a large population of recently emigrated Chinese Australians. Many cultural and food consumption habits persist in the local Chinese population, and cultural aversions to particular food flavours (if they exist) are still likely to be present. Conducting cross-cultural acceptance comparisons locally in Australia has obvious advantages in terms of cost and logistics compared to performing testing overseas. There is also the advantage that all of the experimental conditions can be held constant, e.g.; location of testing booths, sample preparation, lighting, serving staff etc. Any unanticipated “culture-specific” factors are also minimised, as the Chinese Australian consumers are already acclimatised to the local culture.

Recent studies show considerable genetic variability in olfactory receptor (OR) genes exist between individuals and between ethnic groups (Olender *et. al.* 2012). It has also been shown that consumer preference can be linked to specific OR genotypes. Although it is unknown how much humans vary in their sensitivity to BCFAs, there is evidence that some Asian consumers may be quite sensitive to them (Prescott *et al.* 2001, 2004). It is unknown whether this is because of a genetic basis (can detect BCFAs at a lower concentration) or simply due to lack of cultural familiarity with these flavours. It has been assumed that lamb consumption is relatively low in Chinese culture compared to other meats, such as pork or chicken. This is however, somewhat at odds with the fact that China is the largest producer of sheepmeat. In this study we hoped to better characterise any potential differences in Chinese consumer liking for Australian lamb produced under traditional and novel feeding systems.

5.1.1 *Recruitment of Consumers*

Approval for conducting the consumer study was granted by the CSIRO low risk ethics committee (LR17/2013). Non-Chinese background (NCB) and Chinese background (CB) Australian consumers were recruited through advertising and telephone by “The Human Network” market research organisation (Terrigal, NSW). Chinese background Australian consumers were recruited according to the following inclusion/exclusion criteria:

Inclusion Criteria:

1. Identifying as Chinese but living in Australia for less than 15 years (Born in China or another Asian country e.g. Singapore or Malaysia) & speaks either Mandarin or Cantonese as the first language.
2. Aged 20-65 years
3. Regular consumer of red meat (at least once a week)
4. Willing to consume Australian lamb grilled to medium or medium rare

Exclusion Criteria:

1. Any known food allergies or intolerances
2. Currently pregnant

Initially, Chinese-background Australians living for 5 or less years in Australia were sought; however recruitment was too slow, so the inclusion criteria were relaxed to 10 years. For the NCB cohort, consumers were selected from an existing database to match as closely as possible the demographics of the CB group. Inclusion and exclusion criteria were the same except that participants were to be of Caucasian background. Sixty NCB and sixty CB consumers (total n = 120) were recruited over a four week period. Each consumer participated in one lamb tasting session of ~ 75 minutes duration. Consumers were remunerated $ 70 in cash for their participation.

5.1.2 *Sample selection and allocation*

Samples (left striploins) used in the consumer testing were a subsample of those assessed by the trained panel (See Appendix). Three samples for each feed X sire combination were selected to be tested by the NCB and the CB cohorts. Before thawing samples, five steaks from each striploin were allocated to each consumer cohort. To minimise steak muscle position effects, adjacent steaks along the muscle were allocated alternately to each group, starting from the thick end. The first or starting steak in the series was alternately allocated to either group to remove bias.

5.1.3 *Design of Electronic Ballot*

Approval to perform the consumer study was granted by the CSIRO low-risk ethics committee (CSIRO LR17/2013). The paper ballot described in Watson *et al.* (2014) was adapted for computer use using the software package Compusense (Version XX). Demographic data were collected for each consumer. *Flavour Intensity, Lamb Intensity, Intensity of Smell, Liking of Flavour, Liking of Smell, Tenderness, Juiciness* and *Overall Liking* were measured on a 100-mm line scale. An overall MQ4 quality score was calculated based on published algorithms (Watson *et al.* 2014).

5.1.4 *Consumer Testing*

Three two hour sessions were performed each day over four consecutive days. CB and NCB consumer groups were allocated evenly into either morning (10-12 am), afternoon (1-3 pm) or evening sessions (6-8 pm). Consumers (10 at a time) were required to meet 15 minutes before testing. Participants were given information sheets describing the purpose of the study – “to evaluate the flavour and texture of lamb samples produced in different ways”. A native Cantonese and Mandarin Chinese speaker was present at all briefing sessions to answer questions and clarify the consumer questions. The consumer questions were written on a whiteboard together with a mock 100-mm line scale. For the question on “lamb flavour

intensity" the Chinese word *"Soo"* (sweaty, mutton-like) was used to help explain the concept. All of the consumers had sufficient understanding of English to perform the assessment tasks. After the briefing session, participants were asked to sign consent forms before assessing samples.

A total of 10 sensory booths with individual computer terminals were available at CSIRO. In this study we wished to capture the data electronically, rather than use the more traditional paper ballot used in MSA testing. Generally it is considered reasonable to expect consumers to be able to assess between 8 and 10 samples within a session, without fatigue becoming an important factor. In our design, each consumer tested 1 of each of the eight main Feed X ABV combinations (e.g. Greenland X high ABV) and a Link sample. The link sample was MSA grade 3 White Pyrenees Lamb obtained from Andrew's Meat. Striploins were cut into 15 mm steaks by hand for testing. Hence each consumer assessed 9 samples. Across the study, even numbers of low and high ABV sires were used, however the consumer study was not sufficiently statistically powered to evaluate individual sire effects, but rather ABV (low or high) effects. A total of 6 X 10 groups of CB and 6 x 10 groups of NCB consumers attended 12 separate sessions.

5.2 Consumer Study Results

5.2.1 *Demographic Data*

As far as was practicable, the recruitment company attempted to match the demographic profile of the CBA and the NCB consumer cohorts. Each of the panels comprised around 70% females (**Figure 13**). The distribution of age bracket and income differed between the two groups. The non-Chinese Australian cohort was slightly older and had a higher income; however the distributions across brackets were similar. Most consumers preferred their lamb rare or medium rare, regardless of ethnic background. The majority of consumers consumed lamb at least 2-3 times per week and enjoyed or liked eating red meat. There were some slight differences in the distribution of consumers in the two groups.

5.2.2 *Chinese background-Australian Consumer Feedback*

During the initial briefing session feedback was given from the Chinese background Australian consumers. Although no official comments were tabulated, a number of items discussed were noteworthy. In the briefing it was explained that the purpose of the study was to evaluate effects of different feeds on lamb flavour and also understand potential barriers to lamb consumption by Chinese consumers in China. Overwhelmingly most of the CBA consumers were adamant that lamb is already currently widely eaten in China and that they did not think that the Chinese had a particular "problem" with lamb flavour. We found this response very interesting in the light of previous consumer studies. Going by the frequency of consumption data discussed later, it appeared that the consumer in our study were mainly regular consumers of lamb. In the debrief session after tasting, the other general feedback from both the CBA and NCB consumers was that none of the samples consumed tasted very strongly of "lamb".

5.2.3 *Consumer Results*

The consumer ratings of samples according to feed, sire and ethnic background are summarised in **Table 7**. The mean consumer scores for the Link sample are also included for reference. In general the consumer scores for the finishing feeds were similar to or better than the Link sample, except for the Rye samples. Numerous feed and sire related differences were found for the consumer sensory attributes. Both NCB and CB Australians rated the rye finished samples as lower in *Flavour Intensity* and *Lamb Flavour* intensity. The CB consumers rated the *Lamb Flavour* intensity consistently lower than the NCB group. Both consumer groups rated *Flavour Liking* lowest for the rye finished samples compared to the other finishing feeds. The CB consumers rated the *Flavour Liking* highest for Sire 3, whereas the NCB consumers rated lamb from Sire 8 highest, however both consumer groups clearly rated the high ABV samples higher than the low ABVs No differences in *Liking of Smell* were measured for any fixed effects. No feed related differences were measured for Juiciness or Tenderness, however both CB and NCB rated the high ABV Sires (3 & 8) as more *Tender* and *Juicy*, compared to the low ABV Sires (1 & 6). Overall Liking did not differ between ethnic groups; the rye finished samples were least liked compared to the other finishing diets. The high ABV sires 3 & 8 were more liked than the low ABV sires (1 & 6). The composite scores MQ4 and the overall Quality Rating were generally lower for the CB compared to the NCB, although directionally similar for both groups. According to MQ4 scores, the Link and Rye samples were rated “3-Star”, whereas lamb from the other finishes were rated “4-Star”. Similarly the low ABV samples were “3-Star” and the high ABVs were “4-Star”.

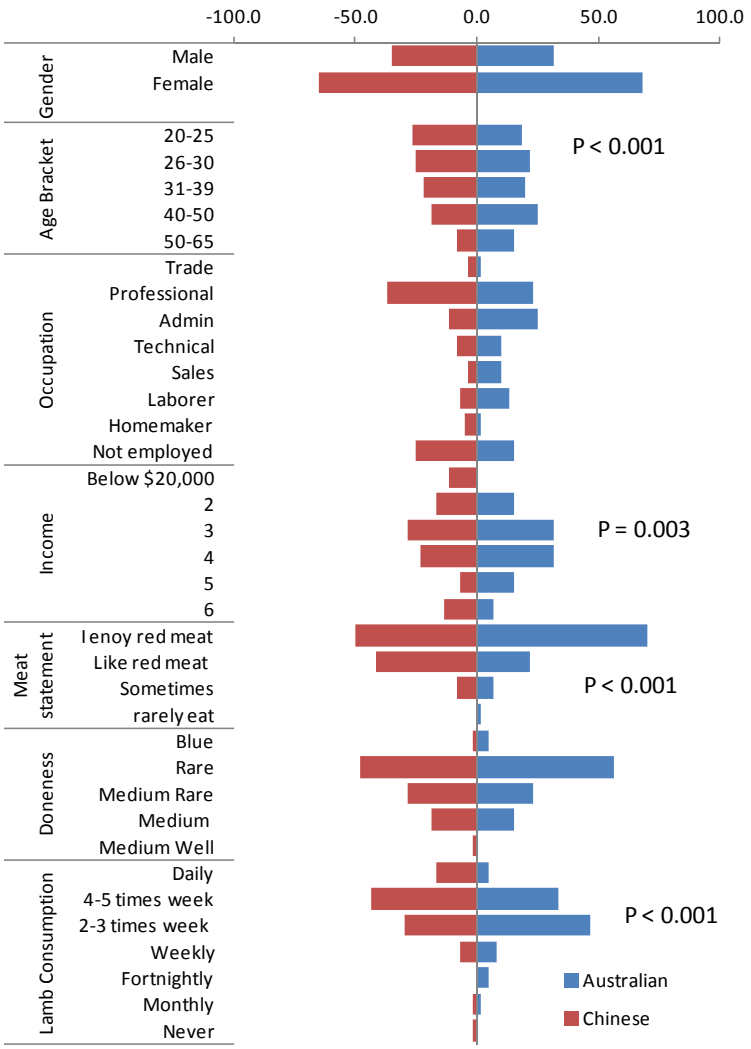

Figure 13: Summary of demographic data for the two consumer cohorts (non-Chinese Australian and Chinese Australian). The effect of ethnicity (Chinese vs non-Chinese background) was significant for some categories.

Table 7: Summary of mean consumer sensory scores for Chinese background (CH) Australian and non-Chinese (AU) background Australian consumers

		Finishing Feed Effect					Sire Effect				ABV							
Sensory Attribute	Ethn	Link*	Rye	Lucerne	Titan	Green	1	6	3	8	Low	High	P Feed	P Sire	P ABV	P Ethnic	P Feed* Sire	P Feed* Eth	P Sire * Eth
Flavour	**AU**	54	55^{b}	60^{a}	62^{a}	63^{a}	56	57	60	66	57	63	0.006				ns	ns	ns
Intensity	**CH**	57	59	63	61	64	63	60	63	61	62	62					ns	ns	ns
Lamb	**AU**	57	55^{b}	61^{a}	62^{a}	62^{a}	58	57	60	64	58	62	0.007		0.03	<0.001	ns	ns	ns
Intensity	**CH**	57	49^{b}	54^{a}	57^{a}	55^{a}	53	55	54	53	54	53					ns	ns	ns
Intensity	**AU**	62	58	60	60	58	60	59	60	58	59	59		0.03			ns	ns	ns
Smell	**CH**	62	58	62	59	62	66^{a}	59^{b}	61^{b}	55^{c}	62	58					ns	ns	ns
Juiciness	**AU**	72	67^{x}	72^{x}	74^{x}	72^{x}	68^{bx}	69^{bx}	75^{ax}	74^{ax}	68	74		<0.001		0.005	0.002	ns	ns
	CH	68	69^{x}	66^{y}	68^{y}	68^{y}	63^{by}	64^{by}	75^{ay}	69^{ay}	64	72					ns	ns	ns
Liking	**AU**	72	63^{b}	68^{a}	70^{a}	72^{a}	65^{b}	67^{b}	68^{b}	73^{a}	66	70	0.009	0.004	<0.001		ns	ns	0.005
Flavour	**CH**	68	65^{b}	67^{a}	67^{a}	68^{a}	67^{b}	62^{c}	72^{a}	66^{b}	64	69					ns	ns	ns
Liking	**AU**	72	64	66	66	63	63	66	64	65	65	65					ns	ns	ns
Smell	**CH**	66	65	68	69	67	67	66	71	65	66	68					ns	ns	ns
MQ4	**AU**	64.5	62.6	68.4	69.5	70.4	64	63	69	73	66	72	<0.001	<0.001	<0.001		<0.001	ns	0.04
	CH	60.1	65.6	65.9	66.9	67.8	62	62	72	67	63	71					ns	ns	ns
Overall	**AU**	71	61	69	70	71	64^{c}	64^{c}	69^{b}	74^{a}	64	72	0.002	<0.001	<0.001		<0.001	ns	ns
Liking	**CH**	68	65	66	67	68	62^{b}	64^{b}	72^{a}	68^{a}	63	70					ns	ns	ns
Quality	**AU**	2.3	2.3^{c}	2.6^{b}	2.7^{ab}	2.8^{a}	2.5^{bx}	2.4^{b}	2.8^{a}	2.8^{ax}	2.4	2.8	0.002	<0.001	<0.001	0.02	<0.001	ns	ns
Rating	**CH**	2.0	2.3^{b}	2.5^{a}	2.5^{a}	2.5^{a}	2.3^{by}	2.3^{b}	2.7^{a}	2.5^{ay}	2.3	2.6					ns	ns	ns
Tenderness	**AU**	65	62	67	68	69	63^{b}	59^{b}	70^{a}	73^{a}	61	71		<0.001	<0.001		<0.001	ns	ns
	CH	65	65	65	67	68	61^{b}	63^{b}	74^{a}	67^{a}	62	70					ns	ns	ns

*Link sample not included in MANOVA analysis for Feed and Sire effects

5.2.4 *Segmentation of Non Chinese Australian Consumer Data*

As there were only 60 consumers within each ethnic group, a formal segmentation analysis was not considered feasible. MANOVA analysis was performed however, using *Overall Liking* scores and testing for the effect of gender, meat statement, age bracket and doneness. Although the *Overall Liking* did not change according to gender, meat statement or meat doneness, there was a significant age bracket effect for the NCB consumers. *Overall Liking* was significantly higher (P = 0.001) for NCB consumers in the 40-50 age bracket compared to the others. For the CB consumers, no differences in *Overall Liking* due to any of these consumer attributes were measured and were hence not explored further.

5.2.1 *External Preference Mapping NCB & CB Consumers*

Since the same lamb samples were evaluated by both trained sensory and consumer panels, it was possible to use an external preference mapping approach, to understand which trained panel attributes best explained difference in consumer *Overall Liking* and *Flavour Liking*. The mean sensory and consumer scores for the feed and sire combinations (4 feeds x two levels of ABVs (low and high) = 8 data points) were used in the modelling.

External preference mapping was conducted using partial least squares regression (PLS) approach, where the consumer liking scores were used as the dependent variable and the trained panel sensory descriptive attribute scores were used as the independent variables. As there were no differences between the CB and NCB consumers in their *Overall Liking* and *Flavour Liking* scores, the data from both consumer groups were initially pooled for modelling. The optimised two factor PLS-1 model for prediction of *Overall Liking* (pooled NCB and CB consumers) from mean trained panel attributes is summarised in **Figure 14**.

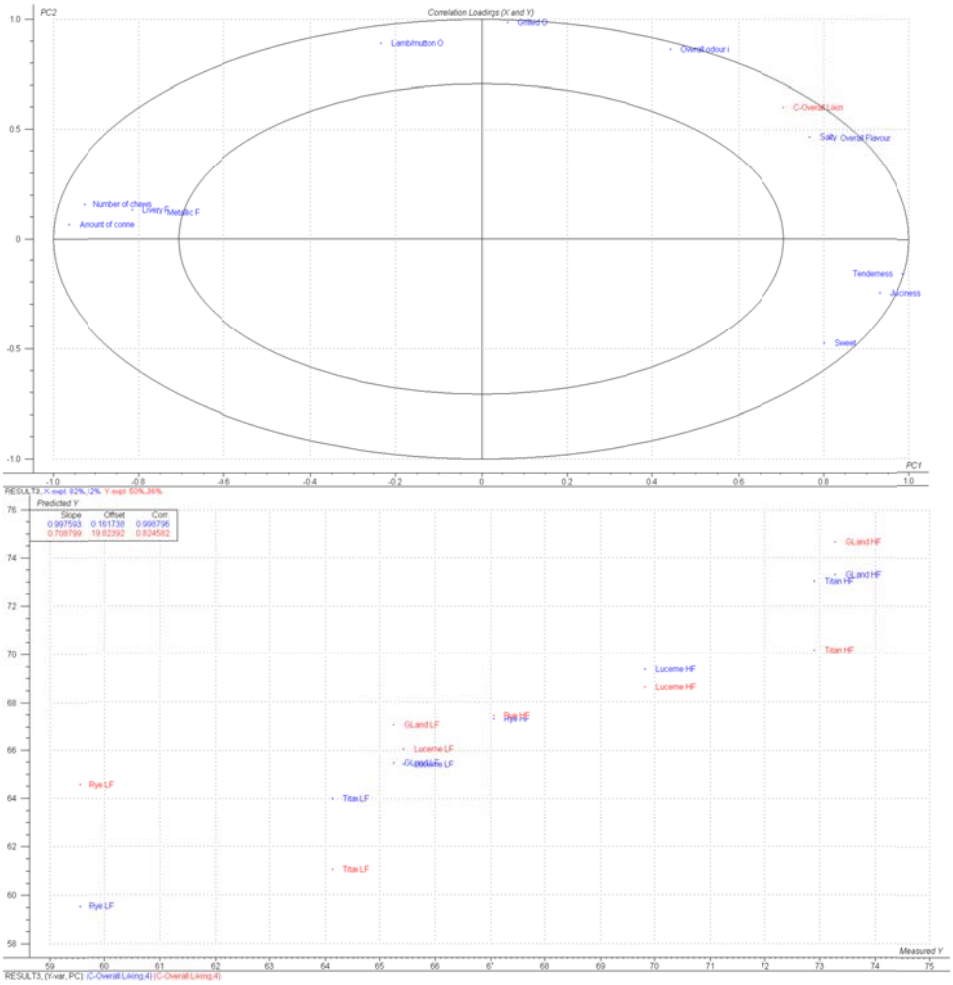

Figure 14: Two factor PLS-1 prediction model for *Overall Liking* of samples based on pooled NCB and CB Australian consumer results. Top – bi-plot of PC1 and PC2, showing the relationship between sensory attributes (blue) and consumer *Overall Liking.* Bottom –model of *Overall Liking* based on sensory attributes (blue) and cross-validation prediction model (red).

A robust prediction model was obtained (bottom graph, blue samples); 94% of the variance in sample *Overall Liking* was explained by the first two PCs, *r* = 0.99. Similarly a good validation model (bottom graph, red samples, 86% variance explained, *r* = 0.82) was obtained. The centred bi-plot (top) shows the relationship between selected sensory attributes (blue) and the *Overall Liking* (red). The line of the outer semicircle denotes a perfect correlation (e.g. either 1.0 or – 1.0) of a given sensory attribute (blue) with *Overall Liking* – the inner circle denotes the limit for a correlation coefficient corresponding to either 0.5 or -0.5. Sensory attributes with correlations less than 0.5 (inside the inner semicircle) are not considered strong predictors and are generally not used in building models. It can be clearly seen that the trained panel texture attributes (*Tenderness & Juiciness*, were major positive drivers of *Overall Liking*, whereas *Amount of Connective Tissue* and *Number of Chews* were strong negative drivers. In terms of flavour attributes, *Overall Impact, Salty* and *Sweet* were positive drivers, whereas *Livery* and *Metallic* flavours were negative drivers. A number of odour related attributes were mainly responsible for explaining the second dimension (PC2), or more subtle drivers of *Overall Liking*. Overall *odour Impact*, *Grilled* odour and *Lamb/mutton* odour were positively associated with *Overall Liking*.

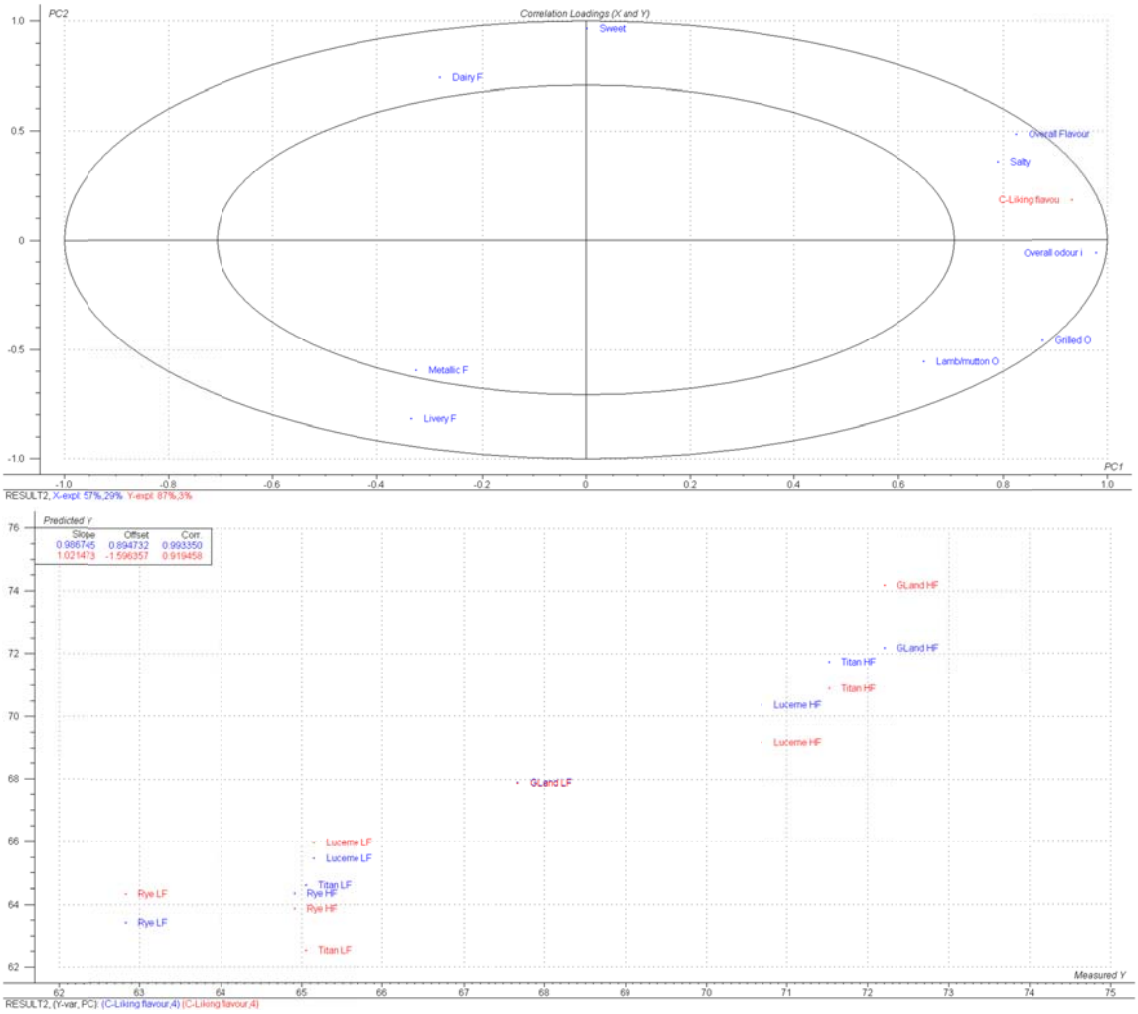

Figure 15: PLS-1 prediction model for *Flavour Liking* of samples based on pooled NCB and CB Australian consumer results. Top – bi-plot of PC1 and PC2, showing the relationship between sensory attributes (blue) and consumer *Flavour Liking.* Bottom –model of Flavour Liking based on sensory attributes (blue) and cross-validation prediction model (red).

Flavour Liking and *Overall Liking* were strongly positively correlated. In the consumer testing *Flavour Liking* was a slightly different question to *Overall Liking*. A PLS-1 model based on only flavour related sensory attributes (e.g. no texture attributes used in the model) was optimised (**Figure 15**). In terms of *Flavour Liking*, it was clear that the high fat ABV samples generally were more liked (at least for the Greenland Titan and Lucerne finished samples) compared to the low ABV samples. When the texture variables were removed from the model, both of the Rye samples were rated as having the lowest *Flavour Liking*, regardless of their ABVs. In the bi-plot (top) the main positive drivers were *Overall Flavour Impact*, *Overall Odour Impact*, *Grilled Odour* and *Saltiness. Livery* and *Metallic* Flavours were negative drivers of *Flavour Liking*. *Dairy Fat* and *Sweet* were positive drivers of *Flavour Liking,* applying mainly to the high ABV samples (PC2).

5.2.2 *Chinese Background Australian Consumer Preference Maps*

The PLS-1 *Overall Liking* preference model for the CB consumer cohort is shown in the Appendix (**Figure 25**). The drivers of *Overall Liking* were essentially the same as for the combined CB and NCB consumer model (**Figure 14**). *Tenderness* and *Juiciness* were positive texture drivers, whereas *Connective Tissue* and *Number of Chews* were negative. *Sweetness, Saltiness*, *Overall Odour Impact* and *Overall Flavour Impact* were positive drivers and *Livery* and *Acidic Flavours* were not. The high ABV samples were clearly most liked and associated with higher *Grilled Odour* and *Lamb/Mutton* odour as well as positive texture attributes. The ranking of samples, based on *Overall Liking* was identical for the CB consumers compared to the combined model.

Flavour Liking was modelled for the CB consumers (**Error! Reference source not found.**). The model was almost identical to the combined model (**Figure 15**), with the same ranking of samples. In contrast to the overall liking models, The Greenland low ABV sample was more liked than the Rye finished high ABV sample, suggesting that the high GSL Greenland brassica finished samples had better flavour than the control treatment. Overall Flavour, saltiness, Overall Odour Impact, Grilled Odour and Lamb/mutton odour were positively associated with Flavour Liking, whereas *Metallic* aftertaste and *Livery* Flavour were negatively associated.

5.2.3 *Non-Chinese Background Australian Consumers*

The *Overall Liking* model for the NCB Australian consumer cohort (**Figure 27**), indicated that the ranking of samples was slightly different compared to the combined and CB model. The Greenland low ABV sample was ranked higher than the Rye finished high ABV sample, similar to the *Flavour Liking* models for the combined CB and NCB. This finding suggests that the influence of flavour related sensory attributes was relatively larger for the NCB consumers, compared to the CB consumers. The Titan low fat low ABV sample was rated relatively low for Overall Liking, reflecting the lower Tenderness and Juiciness scores given by the trained panel for the Titan finished meat from Sire-6 (see **Figure 12**). Finally a very clear predictive and validation PLS-1 model for *Flavour Liking* was achieved for the NCB-Australian consumers (**Error! Reference source not found.**) (r = 0.99, 87% variance described, r = 0.98, 93% explained). Similar to the previous *Flavour Liking* models, the Greenland low ABV samples were ranked higher compared to the Rye high ABV. In terms of flavour, both the low and high ABV rye finished samples were rated lowest in flavour, ranked lower than all brassica and lucerne finished samples.

Taken as a whole, the consumer data was very similar based on either the separate CB or NCB-Australian cohorts. Both consumer groups rated the Greenland high ABV (high IMF) samples as highest in *Overall Liking* and *Flavour,* followed closely by the Titan high ABV and then the Lucerne high ABV samples. In general, the Rye finished samples, regardless of their ABVs were ranked lowest in *Flavour Liking* – it can also be seen that the Rye finished samples were rated lowest in their *Flavour Intensity* by both consumer groups. Both Flavour Intensity and Flavour Liking were strongly correlated, suggesting that a lack of flavour intensity rather than a distinct off-flavour was the main reason for the lower *Flavour Liking*. The sensory data (**Table 5**) indicated that the Rye finished samples were lowest in *Overall Odour Impact*; this sensory attribute was a strong positive driver of consumer *Overall Liking.*

5.3 Summary of Consumer Study

- Contrary to our initial hypothesis, the brassica finished samples, especially the Greenland finished were most liked by consumers regardless of ethnic background
- In general the high ABV samples were clearly more liked than low ABV counterparts
- Few differences in liking scores were found between CB and NCB consumer groups.
- The CB consumers generally rated *Lamb Intensity* and *Juiciness* lower than NCB consumers
- *Overall Liking* was highest for high ABV/high fat Greenland and Titan finished samples more than the other samples
- The main positive drivers of Overall Liking were: *Tenderness, Juiciness, Overall Flavour Impact, Saltiness* and *Sweetness*
- *Flavour Liking* was highest for high ABV Greenland and Titan finished and lowest for Rye finished samples regardless of ABV
- The main positive drivers of *Flavour Liking* were *Grilled odour, Lamb/mutton odour*, *Overall odour impact, Overall flavour impact* and *Saltiness*

6 Volatile and odour characterisation of the brassica feeds

6.1.1 *Flavour Characterisation of Brassicas*

Cruciferous or brassica crops are unique in the plant world for their sulphur containing secondary plant metabolites known as glucosinolates (GSL). A diverse range of over 200 different GSLs have been described across different brassica cultivars (Tripathi & Mishra 2007, Fenwick *et al*. 1982, Halkier *et al.* 2006). Different GSLs share the same basic 6-sinapoylthioglucose structure differing in the nature of the side chain moiety (Fenwick *et al.* 1982). The GSLs present in intact plant tissue are non-volatile. When tissues are disrupted, the GSLs are quickly degraded by the enzyme myrosinase, to form mainly volatile isothiocyanates (ITCs), and nitriles and a number of other volatile compounds. ITCs and other GSL degradation products can be readily extracted from the headspace of slurries of macerated plant material and analysed by GC-MS.

GSLs themselves are biologically inactive molecules and have no taste or flavour. It is not until the GSLs are degraded by enzymes that flavour and biologically active compounds are formed. Depending on the absolute concentration of the breakdown products (e.g. ITCs and nitriles) and their chemical nature, a range of anti-nutritional effects on animals may occur. Some ITCs are known to have bitter flavour properties, pungent flavours and lachrymatory activity. Depending on the brassica cultivar, type and concentration of GSLs, the breakdown products can disrupt iodine availability in the thyroid, and in turn affecting animal growth and production (Tripathi & Mishra 2007).

In general ruminants are considered to be more tolerant to GSL than other animals. A number of published trials have shown reduced growth rates in lambs at high concentration of total GSLs (> 15 μmoles/g feed) and other have shown no growth effects at lower concentrations (< 4.22 μmoles/g feed) —in these studies some histological evidence of thyroid disturbance was measured however. It should be noted that based on the total GSL measurements in the brassica forages in this study, the Titan brassica (~5 μmol/g) was considered unlikely to affect animal growth rates, whereas the Greenland brassica (~13 μmol/g) may have been expected to have an effect. There is also some evidence that rates of animal forage intake of high GSL brassicas may be lower than low GSL cultivars. Previous research has shown that elevated levels of alkenyl-ITCs in brassica forages are related to low palatability and low animal acceptability scores (Sarwar *et al.* 20XX). High levels of propenyl-, butenyl- and pentenyl-ITCs in brassica cultivars rendered forages “unpalatable” according to Sarwar *et al.* 1997.

The ITCs and other breakdown products are known to have strong flavours (Table). Anecdotally, there has been a belief that the ITCs and other breakdown products can remain in the animal meat causing a flavour taint (Watkins, Frank *et al.* 2012). In this section we analyse brassica plant material for the main odour-active volatiles and volatile ITCs.

6.1.1 *Volatile and odour characterisation of brassica feeds*

Samples of Greenland and Titan stem and leaf material were collected throughout the trial and frozen at -80 ^{o}C until required for analysis. Samples were collected at three time points; 22 January (day 11), 30 January (day 20) and 13 February 2013 (day 34). Frozen leaf samples were macerated in ice cold Milli-Q water and transferred into gas tight headspace sampling vessels. After equilibration at 37 ^{o}C for 30 minutes, the headspace volatiles from slurries of brassica samples were concentrated by DHS Tenax and subjected to GC-MS and olfactometry. Duplicate leaf and stem volatile data were subjected to MANOVA to test for the effects of cultivar (Greenland vs Titan), time-point (22 Jan, 30 Jan, 13 Feb) and plant part (leaf or stem). There were large differences in the ITC concentration measured between the two cultivars and between leaves and stems (**Table 8**). The dominant GSL derived volatiles were identified as 4-methylpentyl-ITC, isobutyl-ITC and isopropyl-ITC; all were present at higher concentration in the Greenland cultivar. A number of nitriles and sulphide compounds were also present at relatively high concentration. Leaf samples collected on February 13 were subjected to gas chromatography-olfactometry (GC-O) analysis, to determine the main odour-active compounds (described in following sections).

Volatile compounds identified and corresponding odour character perceived during sniffing experiments are summarised in **Table 8** and **Figure 16**. The major impact compounds included dimethyl disulphide and dimethyltrisulphide, the latter mainly responsible for a very strong *garlicy sulphur rotten* odour. An unidentified volatile (MW 127) was extremely pungent in the Greenland sample. Most of the ITCs increased in the samples over the feeding trial. 4-isothiocyanato-butene (*pungent, mustard oil*) was a major impact odour. Octanenitrile was associated with a *Chinese broccoli-like* odour. Hexanal (*grassy, green*) and 1-penten-3-one (*grassy, herbal*) were also important odour-impact volatiles. 2-Methylbutanal was also identified in the headspace of both cultivars with a characteristic odour (*malty, meaty*). This compound is fairly ubiquitous in many foods and is a known important meat flavour volatile.

Summary

- The Greenland brassica cultivar had higher amounts of a number of isothiocyantes compared to the Titan
- An unidentified odour with a very strong *mustard oil, nasturtium-like* odour was present mainly in the Greenland brassica
- The main odour-active volatiles present in the headspace of the samples were elucidated by olfactometry
- Both dimethyl disulphide and dimethyl trisulphide were responsible for strong rotten sulphur odours in both cultivars; these compounds are likely mainly responsible for the negative attitude towards brassica odour

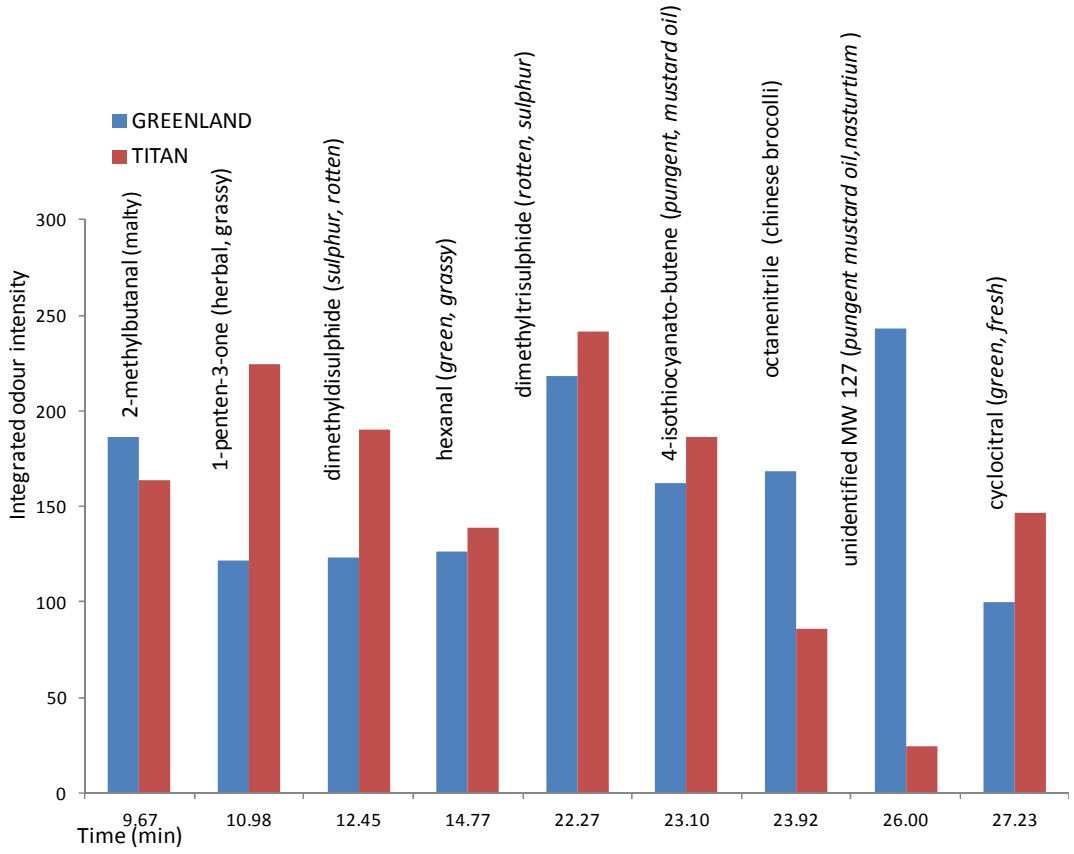

Figure 16: Aromagrams for headspace extracts of Greenland (blue) and Titan (red) leaf samples summarising the relative impact of the main odour-active volatiles

Table 8: Major volatile compounds identified by GC-MS in the Greenland and Titan leaves and stems on three different collection dates. Compounds that were odour active as determined by olfactometry are described by associated odour.

RT	Volatile Compound	*Odour Description at odour port*	Green n=12	Titan n=12	P_{Type}	Leaf n=12	Stem n=12	P_{Part}	22-Jan n=8	30-Jan n=8	13-Feb n=8	P_{Time}
9.003	2-methylbutanal	*sap, vomit, strong*	253	152		293	99	0.04	70	118	403	0.012
12.33	1-pentene-3-one	*pungent, green, herbal*	84	166		177	71		84	48	238	0.013
13.68	dimethyl disulphide	*mild sulphur*	1385	2566		27	4155	<0.001	617	1815	3549	
16.75	isopropyl-isothiocyanate (MW 102)	—	425	221		82	576	0.012	498	229	217	
17.60	(E)-2-hexenal (MW 98)	—	162	**1204**	0.002	824	578		483	1125	563	0.04
18.86	isothiocyanato-butane (MW 115)	—	**1654**	521	0.003	431	**1752**	<0.001	1124	781	1248	
20.04	isobutyl-isothiocyanate (MW 115)	—	**107**	53	0.02	55	**105**	0.03	53	56	124	0.01
20.69	4-methylpentyl-isothiocyanate (MW 143)	—	**1062**	422	0.03	530	944		840	395	908	
20.97	allyl-isothiocyanate (MW 99)	*sulphur, metallic*	**73**	24	0.03	59	36		31	32	78	
21.20	nonanal		56	124	0.02	134	46	0.004	76	89	109	
21.66	dimethyl trisulphide	*rotten fish, sulphur, metallic*	107	74		26	159	<0.001	44	68	154	0.002
21.93	heptanenitrile (MW 111)	*wooden metallic*	42	79.7		23.6	103	<0.001	52	30	100	0.006
22.56	1-octen-3-ol	—	24	20.1		32.1	10.9	0.015	10	12.4	42.3	0.006
23.11	4-isothiocyanato-butene (MW 113)	*extremely pungent, mustard*	229	179		171	238	0.03	149	160	294	<0.001
23.54	octanenitrile	*Chinese broccoli, mustard*	26	15		3.4	38.6	0.003	26.1	10	23.4	
24.93	unidentified (MW 127)	*mustard oil, nasturtium-like, nauseating, peppery*	**3368**	53	0.009	2232	991		351	512	3911	0.02
27.01	isothiocyanato-heptane (MW 157)	—	211	97		14	**302**	<0.001	176	10	251	0.008
34.22	benzenepropanenitrile (MW 131)	—	**460**	235		70	**640**	<0.001	430	21	537	0.007
34.26	benzylnitrile (MW 117)	—	3.31	6.74		1.82	**8.68**	0.002	4.5	0.2	10	<0.001

7 Fatty Acid Composition of Intramuscular Fat

7.1.1 *Percent Intramuscular Fat in Meat*

The percent fat contained within the lamb samples was determined using the oven moisture method described in a previous AMPC report (AMQA.001). The fat content (% Fat) was calculated, according to the validated algorithm published by Thornton *et al* (1981), using the equation: % Fat = 95.6 - %H_2O x 1.24.

Table 9: Mean % fat in the raw lamb according to feed, sire and ABV.

Feed				Sire				ABV				
Rye	Lucerne	Titan	Green	1	3	6	8	low	high	P_{Feed}	P_{Sire}	P_{ABV}
6.97^{b}	8.28^{ab}	9.59^{a}	7.52^{b}	7.97	8.43	6.85	9.12	7.4	8.8	0.03	0.09	0.03

The average % fat varied between ~4 to 11 % in the raw lamb meat. The mean % fat according to feed, sire and ABV are shown in **Table 9**. Significant differences in the % fat were found after correcting for HCW. On average the Titan-finished samples had the highest % fat in the meat samples > Lucerne, Greenland and Rye. The effect of Sire approached significance; the low ABV sires (1 & 6) had lower % fat on average compared to the high ABV sires (3 & 8). When the pooled high and low ABV sires were compared the effect was significant.

The relationship between the % fat and the HCW are summarised in **Figure 17** according to Finishing feed (left) and Sire (right). As expected, a positive relationship between HCW and % fat was measured (P = 0.02). It can also be seen that there was a high degree of variability in the measured % fat at a given HCW. The final % fat was more variable (and lower on average) for the high ABV Sire 3 compared to Sire 8. The sensory panel found meat from Sire 3 to be less *Juicy* and less *Tender* than Sire 8 on average, consistent with this finding. It is also of note that the Warner-Bratzler measurements indicated that the IY was significantly higher for Sire 3 samples compared to Sire 8 (**Figure 6**).

7.2 Branch Chained Fatty Acid (BCFA) Analysis

7.2.1 *Method of Analysis of Total BCFAs*

BCFAs are present in lamb fat as either free acids or esterified within the triacylglycerols (Young *et al.* 2002). In the current method the sum of free and esterified BCFAs was measured as total BCFAs. Note that this method is in contrast to other reported methods where only free BCFAs were measured. An aliquot (114 µL) of molten sheep fat was added to a Kimax tube. After the addition of the internal standard, 2-butyloctanoic acid (10 µL of 5.00 mg mL^{-1}), THF (2 mL) and 5 % (v/v) H_2SO_4/MeOH (1 mL), the tube and its contents were heated at 50 °C for 16 hours (overnight). After cooling, hexane (1.0 mL) was added and the tube and its contents were vortex mixed. Next, brine solution (saturated NaCl solution, 1 mL) was added and then vortex mixed. The organic layer was removed and

washed with 5 % $NaHCO_3$ solution (1 mL). After standing ~ 20 mins, the organic layer was transferred to vials, ready for analysis.

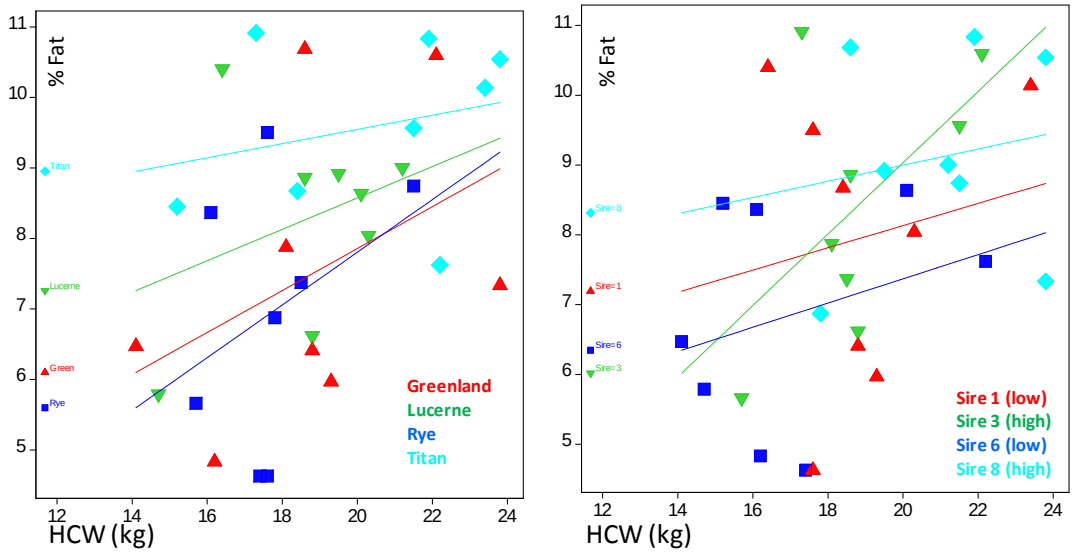

Figure 17: Relationship between animal HCW and the % fat determined grouped according to feed (left) and sire (right)

The analysis was performed using an Agilent Model-6890 GC interfaced to an Agilent Model 5793 Mass Selective Detector. Separations of the branched chain fatty acids (BCFAs) as methyl esters (MEs) were performed using a DB-5 capillary column (J&W, length = 30 m. i.d. = 0.32mm, film thickness = 0.32 µm). The column oven temperature was initially held at 80 °C for 3 min, heated to 160 °C at a rate of 8 °C min^{-1} and then heated to 300 °C where it was held for 2.5 min. The injector was heated at 250 °C and operated in splitless mode. Helium was used as the carrier gas (2.0 mL min^{-1}). The transfer line was held at 280 °C. The mass spectrometer was operated in single ion monitoring (SIM) mode with the detector set to 400 V above the autotune value. The analyte response was quantified using a characteristic target ion using Chemstation software. The same target ion was used for the MEs of the BCFAs as well as 2-BO, *m/z* = 87. Additional qualifying ions were used to confirm identification of the compounds, which were respectively for 4-methyloctanoic (MOA), 4-ethyloctanoic (EOA), 4-methylnonanoic (MNA) and 2- butyloctanoic acids, *m/z* = 115, 113, 129 and 130. Quantification was performed using relative response ratios of the BCFAs to the internal standard. The detector response was not recorded beyond 12 min.

7.2.2 *Feed differences in BCFAs*

After correction for the covariate effect of HCW, finishing feed had a significant effect on the concentration of the BCFAs measured in the subcutaneous fat (**Table 10**). The effect of ABV was not significant, however the covariate term (HCW) was. The fat from the Titan-finished

samples contained the highest concentration of each of the measured BCFAs: Titan > Greenland > Lucerne > Rye.

Table 10: Quantitative data for total branched chain fatty acids in the lamb subcutaneous fat. Means for the effect of feed and ABVs. Cov = HCW covariate.

	Feed					ABV			
$\mu g.g^{-1}$	Rye	Lucerne	Titan	Greenland	P_{Feed}	High	Low	P_{ABV}	Cov
EOA	8.9^{a}	13.1^{a}	17^{a}	16.5^{a}	0.004	14.7	12.7	ns	0.06
MNA	2.1^{bc}	3.1^{b}	8^{a}	5^{b}	<0.001	4.5	4	ns	0.03
MOA	15^{c}	24.4^{bc}	44.9^{a}	31.8^{b}	<0.001	30.6	25.3	ns	0.013

7.2.3 *Relationship between HCW and the concentration of total BCFAs*

The relationship between the HCW and the concentration of BCFAs were explored using regression analysis. Scatterplots of carcass weight *vs* individual BCFAs for each of the finishing feed types are shown in **Figure 18**. Significant overall relationships ($p < 0.001$) between HCW and each individual BCFA were measured, based on HCW + Feed + HCW*Feed regression models (**Figure 18**). The concentration of the three BCFAs increased in the triacylglycerols as the HCW increased especially for the Titan, Greenland and Rye finished animals. The relationship was weaker for the Lucerne finished animals, however one of the data points may have been an outlier. The relationship differed according to feed type and individual BCFAs. The BCFAs were positively correlated with each other: EOA & MNA (r = 0.61, $P < 0.001$), EOA & MOA (0.82, $P < 0.001$) and MOA & MNA (0.90, $P < 0.001$) The ratio of MOA to MNA appeared to be around 5-fold as described previously (Young *et al.* 2010).The average concentrations of MOA and MNA determined were of a similar magnitude to the total BCFA values reported in Young *et al.* 2002 for pasture, maize-fed and lucerne-fed lambs. The concentrations were generally ~ 50% lower in this present study, compared to their data. The trends were, however, directionally similar, with the lowest BCFAs measured in the pasture-fed lambs compared to the lucerne or maize fed. The higher total BCFAs measured in the lucerne and maize fed lamb was associated with higher ”lamb” flavour scores, but importantly not “mutton” flavour. It is important to note that these flavour scores were not related to consumer liking data. In another study, no relationship between the free BCFAs and “sheepmeat odour” were reported (Young *et al.* 2006).

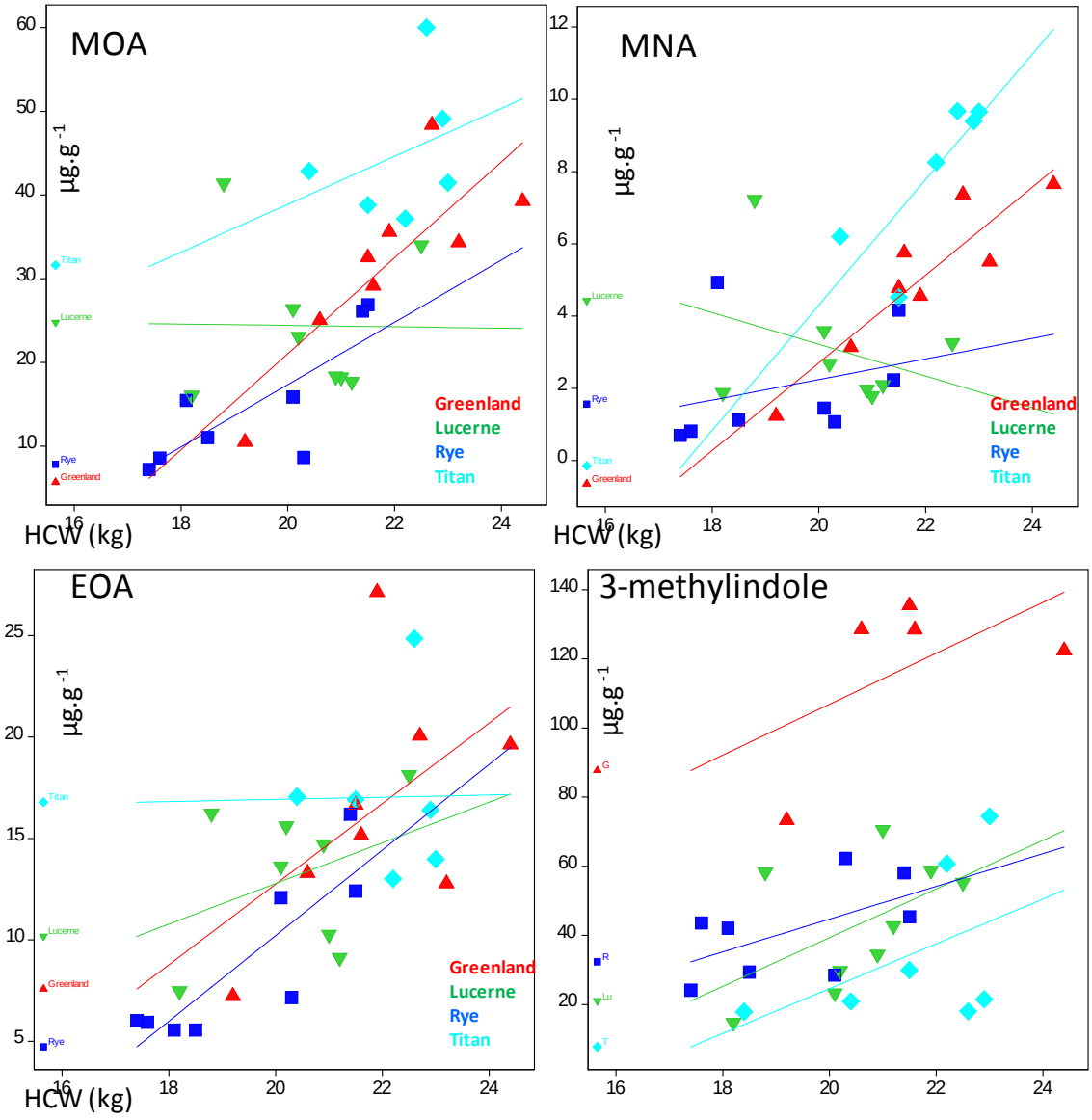

Figure 18: Relationship between HCW and BCFA concentration lamb fat

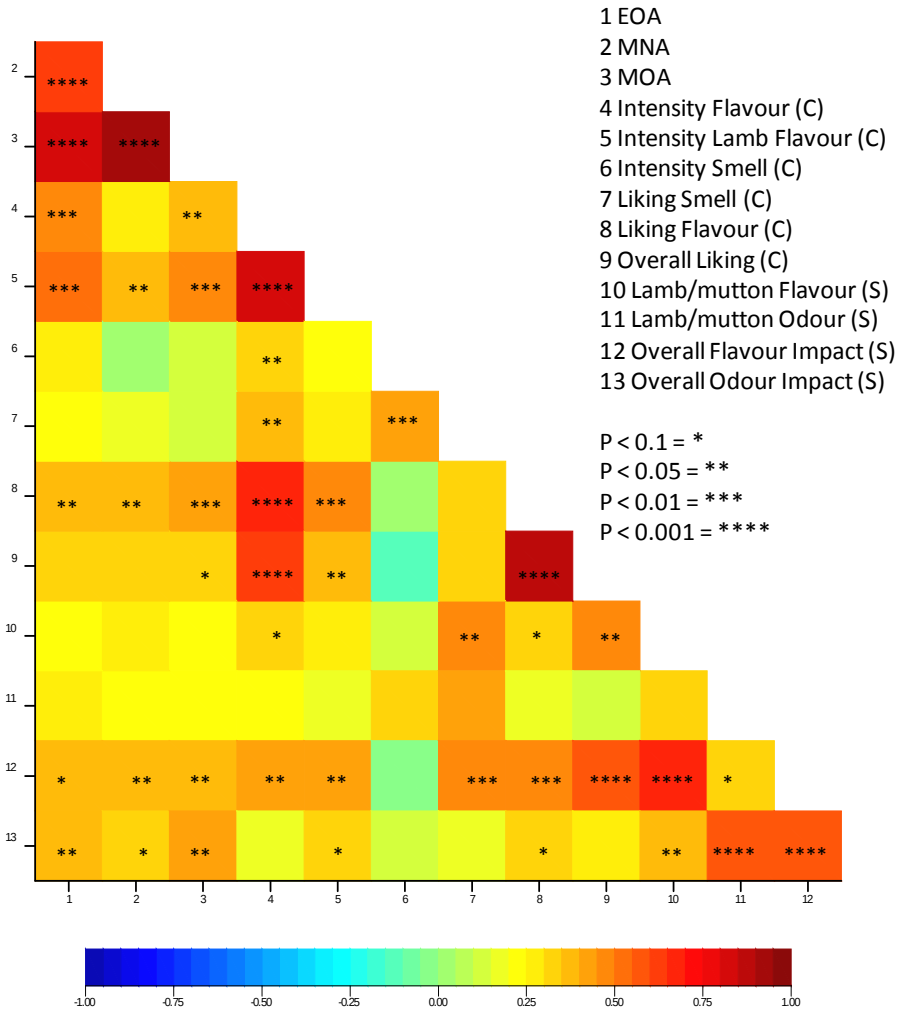

Figure 19: Correlation plot showing the relationship between BCFAs and selected sensory and consumer attributes. P < 0.1 = *, P < 0.05 = **, P < 0.01 = *** and P < 0.001 = ****.

7.2.4 *Relationship between BCFAs and sensory and consumer data*

BCFAs are normally linked to “mutton” flavours - considered undesirable in lamb flavour. BCFA data was available for most of the lamb samples for which sensory and consumer liking data were performed. Mean data (n=30) for trained panel sensory, consumer liking and BCFA measurement were subjected to correlation analysis (**Figure 19**). The strength of the association is shown on the scale at the bottom of the graph. The significance of the correlation is denoted by the asterisks. The BCFA data were positively correlated with each other as expected (*P* < 0.001) and mainly positively correlated with consumer sensory and liking scores - denoted by (C) - as well as trained panel sensory attribute scores – denoted by (S). The consumer *Intensity of Flavour*, *Intensity of Lamb Flavour* and *Liking of Flavour* scores were all positively correlated with the concentration of BCFAs. Although the relationships were not significant, the *Liking of Smell* consumer score was also positively related to the individual BCFAs. The BCFAs were also positively correlated with the trained sensory panel attributes: *Overall Flavour Impact, Odour Impact*, *Lamb/mutton odour* and *Lamb/mutton flavour*.

These positive relationships between total BCFAs and the consumer and sensory scores are somewhat at odds with published literature (Watkins *et al.* 2014, Watkins *et al.* 2010 and Prescott *et al.* 2001). In all cases, these studies found negative relationships between BCFAs and consumer liking. Unfortunately, the relationship between the BCFAs in the free form compared with to the total content, ie comprising of the free and that bound in triacylglycerols, present in sheepmeat is unknown. It is not known whether an increase of total BCFAs also corresponds to a linear increase of BCFAs in the free form. In a paper by Ha & Lindsay (1988), the ratio of free and esterified fatty acids in milk fat was determined and they found on average a 500-2000-fold difference. Working on an assumption of an average 1000-fold difference, the data in **Table 10** can be read in units of µg/kg. Assuming that there is a relationship between total BCFAs and free BCFAs, the current data suggest that the free BCFAs were well below a concentration where pronounced "mutton" flavour became apparent. The data also suggests that up to a certain threshold concentration, the BCFAs may play a positive role in defining lamb flavour and consumer acceptance. Ultimately, the stability of the meat fat, and the degree to which esterase enzymes break down the TAGs to release the free BCFAs, are likely to be important determinants of the degree of mutton flavour.

7.2.5 *3-Methylindole and 4-methylphenol content (ng/g) in Subcutaneous Fat from lamb according to feed and ABVs*

	Feed					ABV			
	Rye	Lucerne	Titan	Green	P_{Feed}	High	Low	P_{ABV}	HCW
3-Methylindole	53b	44bc	27c	110[a]	<0.001	55	63	ns	<0.001
4-Methylphenol	96[a]	36[b]	33[b]	68[ab]	0.04	63	55	ns	ns

Both 3-methylindole and 4-methylphenol are associated with "pastoral" or "barnyard" flavours. The concentrations of 4-methylphenol are comparable to those reported elsewhere (Watkins *et al.* 2014, Young *et al.* 2003). The concentration of 3-methylindole was highest in the Greenland finished fat and lowest in the Titan-finished. 3-Methylindole was not strongly correlated with any of the consumer or sensory scores. The concentration of 4-methylphenol measured in the fat was generally negatively related to the consumer scores for *Liking of Flavour* (r = -0.39, P = 0.05), *Liking of Smell* (r = -0.37, P = 0.06) and *Overall Liking* (r = -0.32, P = 0.11). 4-Methylphenol was positively correlated with *Barnyard Odour* (r = 0.46, P = 0.02). The rye finished samples were highest in 4-methylphenol; the Greenland samples were also high. The trained panel also found the rye finished samples to be highest in Barnyard Odour followed by the Greenland.

7.2.6 *Fatty acid methyl ester analysis*

Fatty acid methyl ester (FAME) solutions prepared for the BCFA analysis were used to characterise the FAME profile of the subcutaneous fat sample. After derivatisation, FAMEs (1 µL) were separated using a Supelco SP-2560 column (l = 100 m, i.d. = 0.25 mm, film thickness = 0.2 µm) in an Agilent model 6890 gas chromatograph (GC) with a flame ionisation detector (FID). The GC oven was isothermally heated at 180 °C. Helium was used

as the carrier gas (flow rate = 1.2 ml min^{-1}).The injector was heated at 250 °C with split injection (50:1) used for the analysis. The FID was heated at 250 °C and the flow rates for H_2 and air were 45 and 450 mL min^{-1}, respectively. Nitrogen was used as the make-up gas (45 mL min^{-1}) for the FID. FAME identification was made using Supelco GLC-20 FAME mix standard, and comparison with FAME solutions prepared from standard anhydrous milkfat.

Table 11: Fatty acid composition of subcutaneous fat according to finishing feed and ABV status

		RYE % total	LUCERNE % total	TITAN % Total	GREEN % total	P Feed	Low	High	P ABV
Saturated									
C10:0	*decanoic*	0.25	0.21	0.21	0.22	—	0.21	0.22	—
C12:0	*dodecanoic*	0.48	0.29	0.28	0.42	0.04	0.30	0.39	—
C14:0	*tetradecanoic*	6.21	4.81	4.72	5.88	0.05	4.89	5.62	—
C15:0	*pentadecanoic*	0.18	0.11	0.09	0.14	0.001	0.10	0.14	0.006
C16:0	*palmitic*	25.8	26.3	26.2	27.2	—	26.45	26.41	—
C17:0	*heptadecanoic*	1.81	2.27	3.04	2.46	<0.001	2.55	2.42	—
C18:0	*stearic*	21.4	24.4	24.5	22.5	0.04	24.42	22.63	0.04
C20:0	*eicosanoic*	0.19	0.15	0.13	0.10	0.002	0.14	0.13	
Σ SFA		**56.4**	**58.5**	**59.1**	**58.9**	—	**59.1**	**57.9**	—
Monounsaturated									
C14:1	*cis-9-tetradecanoic*	0.85	0.86	0.93	0.92	0.03	0.86	0.92	0.01
C16:1,c9	*cis-9-hexadecenoic*	1.50	1.22	1.02	1.28	0.002	1.12	1.30	0.03
C17:1,*9c*	*cis-9-heptadecenoic*	0.62	0.68	0.79	0.75	0.04	0.71	0.73	—
C18:1, *9c*	*oleic*	36.5	33.9	34.2	34.1	—	34.0	34.8	—
Σ MUFA		**39.5**	**36.7**	**36.9**	**37**	—	**36.7**	**37.8**	—
Polyunsaturated									
C18:2, *9c,11t*	*conjugated linoleic acid*	1.68	1.25	1.01	1.37	0.02	1.12	1.41	0.05
C18:2, *9c,12c*	*linoleic*	1.63	1.92	1.44	1.44	0.007	1.63	1.58	—
C18:3	*α-linolenic acid*	0.83	1.62	1.58	1.23	<0.001	1.48	1.29	—
Σ PUFA		4.2	4.8	4	4	0.04	4.2	4.3	—

7.2.7 *FAME Results*

Mean FAMES expressed as % of subcutaneous fat are shown in **Table 11**. The predominant saturated fatty acids present in the subcutaneous fat were palmitic and stearic acids consistent with previous reports (French *et al.* 2000). Oleic acid was the dominant monounsaturated fatty acid present. The PUFAs measured included conjugated linoleic acid. Although traces of omega-3 fatty acids were detected, they were present at very low levels; EPA < 0.1 % and DHA < 0.05 % of total fatty acids (not reported here). Significant feed related differences were measured for specific fatty acids, however overall SFA, MUFA were not affected by feed. The total PUFA was slightly higher in the lucerne finished samples.

7.3 Summary of FAME Results

- The overall FAME composition of the samples were similar
- Minimal sire related differences in the FAME composition were measured
- Feed related differences in individual fatty acids were measured, although total SFA, MUFA and PUFA did not differ significantly due to either feed or ABVs

8 Volatile and Olfactometry Analysis of Grilled Lamb Samples

8.1.1 *Background - Lamb Volatile Analysis*

Significant odour and flavour differences between individual lamb samples were measured by the trained panel. However, both sensory and consumer data indicated that consistent feed-related odour differences between the grilled lamb samples were minimal. Only the overall *Odour Impact* and *Grilled* odour were significantly higher in the Greenland finished samples and lowest in the Rye finished samples. Sire and ABV related differences were clearer; the *odour Impact, Caramel* and *Grilled* odour was higher in the high ABV samples compared to the low ABV samples. In particular, these attributes were highest in meat from Sire 8.

8.1.1 *Dynamic Headspace Method*

Lamb steaks (n = 3 at a time) were grilled according to the same standardised protocol used for sensory analysis. After allowing samples to rest under foil, a middle section of steak was cut and added to Milli-Q water. A 1:1 ratio sample (e.g. 60 g) to Milli-Q water (e.g. 60 g at ~ 40^{o}C) was immediately macerated in a glass beaker with a hand blender. Replicate samples were made from different steaks. Three or more replicates were prepared for each sample type. The final extraction method conditions were selected to reflect the in-mouth conditions of eating. For example, volatiles present in the meat are mixed with saliva (mainly water) in the mouth as the meat structure is broken down into fine particles. Volatile compounds are often “hydrophobic” and are pushed into the headspace in the presence of water. Hence addition of water to samples mimics the release of volatiles during eating conditions.

The macerated meat slurry (30 g) was weighed into a 250mL Schott bottle with an internal standard (50 µL of 4-methylpentanol). The bottle was sealed with a gas tight Teflon closure fitted with custom made connecting gas ports. The whole Schott bottle was placed in a water bath and the internal temperature was equilibrated to 45^{o}C. The headspace was purged with 150 mL/min of high purity nitrogen for 30 minutes at 45°C and volatiles were collected onto Tenax-TR (poly-2,6-diphenyl-p-phenylene oxide, 60/80 mesh size, 100 mg) traps. The traps were desorbed using a short path thermal desorption unit (Scientific Instrument Services, New Jersey, USA) directly into the hot GC injector (250°C). A GC-MS (Varian 4000 ion-trap) and an olfactory port (ODO-II, SGE, Australia) were connected to the GC capillary column via a splitting device; the column effluent was split approximately 1:1 to MS detector and the “sniff-port”.

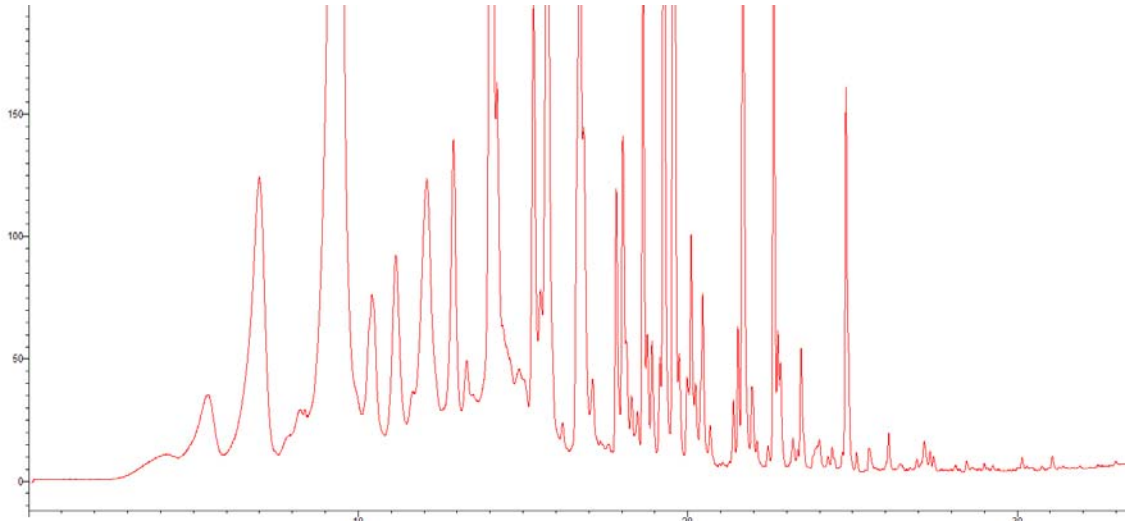

Figure 20: Total ion chromatogram of volatile compounds extracted from the headspace of a grilled lamb sample using dynamic headspace and a Tenax trap

Volatile separation was achieved using a Zebron-WAX column (Phenomenex, 30 m, 0.32 i.d., 0.5 µm film) with the following temperature programming; initial temperature 40 °C (held for 5 minutes) then increased at 6 °C/minute to 245 °C (held for 0 minutes)and finally 30 °C/min at 260°C.(1 min hold) The transfer line to the MS was held at 260 °C and the ion-trap detector was operated at 200 °C, the emission current set at 10 µAmps for electron impact (EI) mass spectra. In addition to EI mass spectrometry, selected samples were also run in methanol chemical ionisation (CI) mode in order to obtain further information regarding the mass of the $M+H^+$ parent ion. Total ion chromatogram data were analysed and integrated by the Varian Star MS-Data Review Software (Vers 6.41). Reference standards were used to confirm the identity of a number of key compounds. Integrated area data were normalised to the IS and expressed as a percentage of the IS. Mass spectral matches were conducted with the NIST Mass Spectral Search database.

8.1.2 *Grilled Lamb Volatile Profiles - Results*

Replicate measures for volatiles were subjected to MANOVA analysis. The effects of feed and ABV were examined. A summary of the main volatile compounds identified by their electron impact mass spectra are listed in the Appendix (**Table 16**).

Most of the compounds identified have been reported in lamb previously (Madruga *et al.* 2013). The concentration of many of the volatiles differed significantly between individual samples (animal differences), supporting the sensory data, where greater flavour differences were measured between individual animals. Significant volatile differences due to Feed and ABV were also measured – many of them important flavour volatiles. Quantitatively, hexanal was the most abundant headspace volatile, followed by 2/3-methylbutanal (co-eluting compounds). The concentration of 2/3-methylbutanal was highest in the Greenland samples and lowest in the Rye; this compound was also significantly higher in the high ABV samples. Further feed-related differences in the concentration of various aldehyde compounds were measured; generally the concentration was found to be highest in the Greenland and lowest

in the Rye-finished samples. Feed and ABV volatile differences were found for different chemical classes. For the pyrazine compounds, important volatiles associated with cooked and grilled flavour, no significant feed-related trends were measured. In contrast, most of the pyrazines were present at slightly a higher concentration in the headspace of the high ABV samples; some of these differences were significant.

The concentration of the key sulphur aroma volatile, methional, was significantly higher in the high ABV samples, as was dimethyl disulphide. Methional is also an important odour-impact volatile in meat aroma.

8.2 Gas Chromatography-Olfactometry

A subset of lamb samples (4 high and 4 low ABV samples from each finishing feed treatment, 4 X 4 = 16) were subjected to analysis by gas chromatography olfactometry (GC-MS) and GC-MS/olfactometry. The main odour-active volatile compounds in grilled lamb aroma were elucidated by gas chromatography-olfactometry (GC-O) using dynamic headspace extracts. After desorption of samples into the GC-MS (Varian, ion-trap) the effluent was sniffed by a panel of five “sniffers” or trained assessors. The intensity of the odour was recorded using a computer mouse controlled 10 mm line scale using SensoMaker® data capture software. Time intensity (TI) data were captured at a rate of 1 scan per second. Assessors described out loud the quality of the perceived odours, which were recorded onto a digital audio file. The TI data for each individual was annotated with descriptors before further processing. After aligning data across replicate samples, any odours reported by two or less assessors were considered as noise and deleted. In some cases, odour peaks may have corresponded to multiple very closely eluting or co-eluting compounds – in this case only one integrated odour intensity was calculated. The TI responses had both maximum intensity (height) and duration (width). After aligning data, the integrated area for each odour peak was calculated and used for statistical analysis. Mean data were calculated.

8.2.1 *Olfactometry Results*

More than 30 odour peaks were detected in the headspace of the grilled lamb samples (**Figure 21**). The aromagram shows the average integrated area of odour intensity for different odour peaks in the high ABV (blue) and low ABV (red) samples. The variance in odour intensities across samples and sniffers is indicated by the error bars. In many cases significant differences in the perceived odour intensity were measured, indicated by the asterisks –- according to sample (top), feed (middle) and ABV (bottom). The majority of volatiles were identified on the basis of their electron impact mass spectra (EI) and retention time matches with authentic reference compounds (Ref). Other compounds were identified through electron impact (EI) mass spectra and chemical ionisation mass spectra (CI).

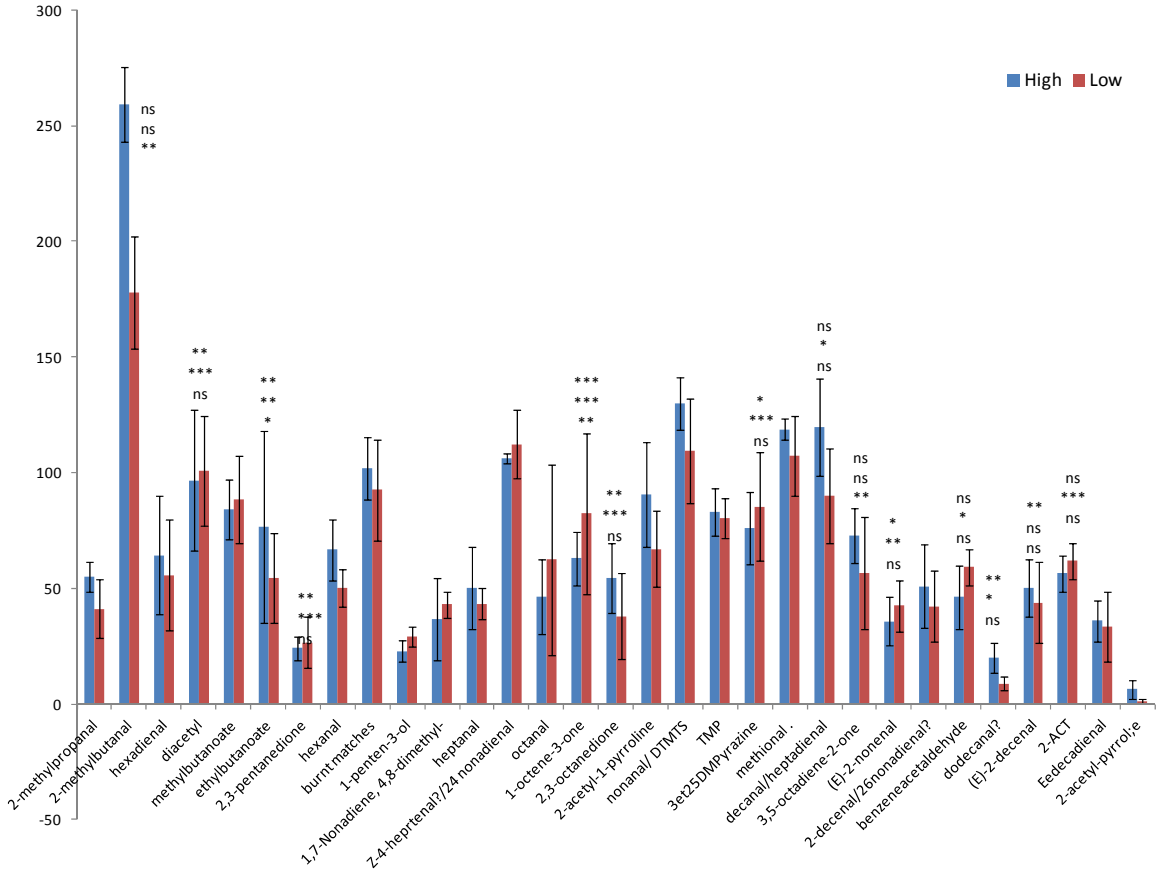

Figure 21: Average grilled lamb aromagrams obtained over all high fat (n=24) and low fat (n=24) samples from DHS-Tenax headspace extracts. The higher the bar the greater the odour-impact, e.g. the odour-impact of 3-methylbutanal was around 4-times higher than (*E*)-2-nonenal. Error bars are a guide to the variability in reported strength of an odour stimulus across samples

In terms of relative odour activity, the top-12 most potent volatiles were, in descending order:

- 2/3-methylbutanal (*savoury, beef, broth*)
- nonanal & dimethyl trisulphide (*green, herbal, grassy, plastic*)
- methional (*baked potato, savoury*)
- decanal & 2,4-(*E,E*)-heptadienal (*earthy, starch, fatty*)
- 4-*(Z)*-heptenal (*mushroom, dried fish, stale*)
- 2-methylthiophene (provisional identification, *garlic, burnt matches)*
- 2,3-butanedione (*toffee, sweet, caramel*)
- 2-acetyl-1-pyrroline (*roast beef, dry hay, popcorn*)
- ethylbutanoate (*fruity, sweet, green*)
- trimethylpyrazine (*baking, fatty, earthy, chocolate*)
- 3-ethyl-2,5-dimethylpyrazine (*roasted, chocolate, musty*)
- 1-octen-3-one (*mushroom*)

Most of these compounds have been reported as odour-active in lamb olfactometry studies previously, with the exception of a volatile tentatively identified as 2-methylthiophene. Further olfactometry peaks with their most frequent odour descriptors given by the panel and likely chemical identity is listed in **Table 12**. In most cases, the compounds have previously been identified in the headspace of cooked lamb. It should be noted that none of the odours associated with the isothiocyantes identified by olfactometry in the brassica plant samples were detected in the grilled lamb aromagrams. Strong odours associated with the presence of 2-methylbutanal and dimethyltrisulphide were identified by olfactometry in the headspace of the brassica plant samples – these compounds were also present in grilled lamb extracts. The headspace concentration of 2/3-methylbutanal in the grilled lamb aroma was higher in the Greenland finished meat, but was also high in the lucerne finished lamb. 2- and 3-methylbutanal are derived from the amino acids isoleucine and leucine respectively; both of these amino acids are compounds are present in raw lamb meat (see later section), and are unlikely to come from the feed.

8.2.1 *Slow Cooked Lamb Olfactometry Experiment*

During the handling and packaging of raw lamb meat throughout experiments, no obvious odour was present in any of the samples, including brassica finished, to suggest a “taint” was present. As a primary goal of this study was to evaluate the potential for a brassica taint to arise in lamb meat, a second olfactometry experiment was performed using Greenland finished raw meat. It was hypothesised that if isothiocyanates (or other plant volatiles) were transferred into the raw meat it may result in a perceptible “taint”. An experiment was designed to extract the headspace of a slurry of Greenland-finished raw lamb meat over a longer period of time (60 minutes) at 50 ^{o}C – “slow cooked”, to enable extraction of any “taint” volatiles to be extracted, if present. The slow-cooked samples were sniffed by the same panel of assessors as the grilled lamb. The average aromagram for the slow-cooked raw lamb (**Figure 22**) did not contain any peaks not previously identified in the aromagrams of the Greenland plant material. Odour peaks corresponding to 2/3-methylbutanal were also absent, confirming that these compounds are mainly formed during grilling, and not transferred from the plant material.

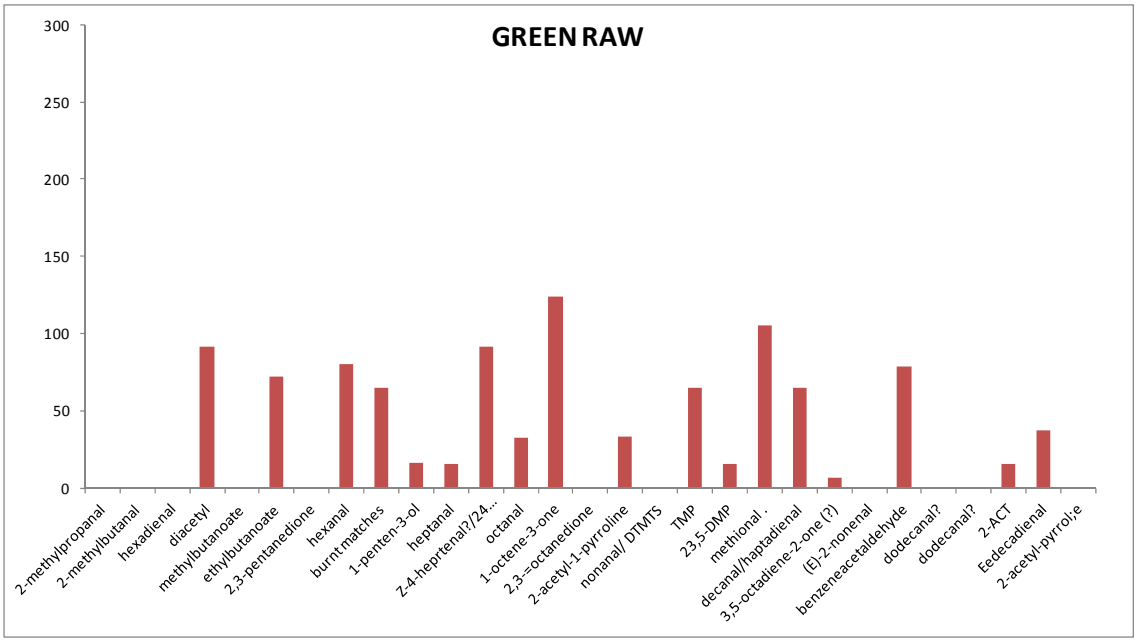

Figure 22: Average aromagram profiles for grilled (blue) and slow cooked raw (red) lamb

Table 12: List of odours detected in the headspace of grilled lamb samples by olfactometry in order of retention time (tR, min). The main volatile compound associated with the odour is listed together with other co-eluting compounds. Identification Method; Ref = matched retention time of authentic reference, EI = electron impact mass spectrum match: C I= chemical ionisation match. *m/z* = main ion or ions used for quantification

Peak No	*tR*	*m/z*	Identification Method	Odour descriptors used by the trained panel	Main compound(s)	Previously Identified in Literature	Co-eluting compounds
1	6.04	*72*	Ref	*Savoury, sap, acetone, barnyard,*	2-methylpropanal	6	
2	8.98	*RIC*	Ref	*Strong, savoury, beefy, broth, sweaty, grainy, green*	2-methylbutanal	1,4,5,6	
3	9.99	*81*		*Grassy, sweet, green, plastic, floral*	2-ethylfuran	2	
4	10.8	*86*	Ref	*Toffee, caramel, sweet, baked, honey*	2,3-butanedione	1,3,4	
5	10.9	*74*	Ref	*Sweet, strawberry, fruity, alcohol, fresh, green*	methyl butanoate		
6	12.6	*88*	Ref	*Mild, green, fruity, sweet*	ethyl butanoate		
7	13.2	*57*	Ref	*Toffee, caramel, hay, sweet, honey*	2,3-pentanedione	5	
8	13.9	*RIC*	Ref	*Green, plant, pasture, starch, fresh-mowed lawn, garlic*	hexanal, dimethyl disulphide	1,4,5,6	
10	14.2	*97*	EI, CI	*Strong, onion, burnt matches, burnt toast, coffee*	2-methylthiophene (TBC)		
11	15.8	*57*	Ref	*Burnt peanut oil, vegetal, plastic, chemical*	1-penten-3-ol	5	
12	16.77	*70*	Ref	*Savoury, cooked potato, baked, brothy*	heptanal		2-heptanone
13	17.6	*81*	Ref	*Sweet, savoury, floral, baking, starch*	2-pentylfuran		
14	18.8	*68*	Ei, ci	*Strong, mushroom, dried fish, wet plastic, stale fish, strange*	(*Z*)-4-heptenal	1,2,4,5	4-methylthiazole
15	19.3		Ref	*Fresh, floral, lemon, melon, fatty*	octanal	1,2,4,5,6	2-octanone
16	20.2	*55+70*	Ref	*Strong, mushroom*	1-octene-3-one	1,2,3,6	
17	20.9	*99*	Ei, ci	*Starch, sweet, caramel*	2,3-octanedione		
18	21.2	*111+83*	EI, CI	*Roast beef, dry hay, popcorn, roast nuts, baked vegetables, grain*	2-acetyl-1-pyrroline	2,3,6	ethylpyrazine
19	21.7	*RIC*	Ref	*Green, herbal, grassy, plastic, sweet, fish oil, seaweed, fishy, garlic metallic*	nonanal	2,4,5,6	dimethyl trisulphide
20	21.9	*122*	Ref	*Baking, fatty, earthy, chocolate*	trimethyl pyrazine	5	
21	23.0	*135*	Ref	*Roast, musty, chocolate, barbequed, grilled meat*	3-ethyl-2,5-dimethylpyrazine	1,2,5,6	1-octen-3-ol
22	23.2	*48+104*	Ref	*Meaty, potato, savoury*	methional	2,3,5	2-ethyl-3,5-dimethylpyrazine
23	24.2	*57+82*	Ref	*Earthy, starch, savoury floral, roasted*	decanal	1,5,6	2,4-(*E,E*)-heptadienal[1,5,6]
24	24.9	*95*	EI, CI	*Floral, plastic, fatty*	(*E,E*)-3,5-octadiene-2-one		2-acetylfuran , pyrrole
25	25.6	*83*	Ref	*Fatty, oxidised, rain, woody, earthy*	*(E)*-2-nonenal	1,2,3,	
26	26.2		EI, CI	*Grassy, dry grass, fatty, lamb, plastic*	*(E)*-2-decenal ?	2,5,6	
27	27.1	*91*	Ref	*Floral, hyacinth, sweet violet, honeysuckle*	phenylacetaldehyde	5	3-isopentyl-2,5-dimethyl pyrazine
28	27.9	*70+82*	EI, CI	*Sweet, green, stale,*	undecanal		
29	28.6	*55+82*	EI, CI	*Green, stale, fatty*	dodecanal		
30	29.4	*60+129*	Ref	*Meaty, popcorn, cooked meat, roasted*	2-acetyl-2-thiazoline	2,3,6	
31	30.2	*81*	EI, CI	*Fatty, floral, cooking oil, roast lamb, sweet*	2,4-(*E,E*)-decadienal	1,2,3,5,6	

Previously reported as odour-active in cooked lamb aroma by: 1 = Resconi *et al.* 2010, 2 = Bueno *et al.* 2011, 3 = Rota & Schieberle 2006, 4 = Young *et al.* 2003, 5 = Elmore *et al.* 2000, 6 = Bueno *et al*. 2013

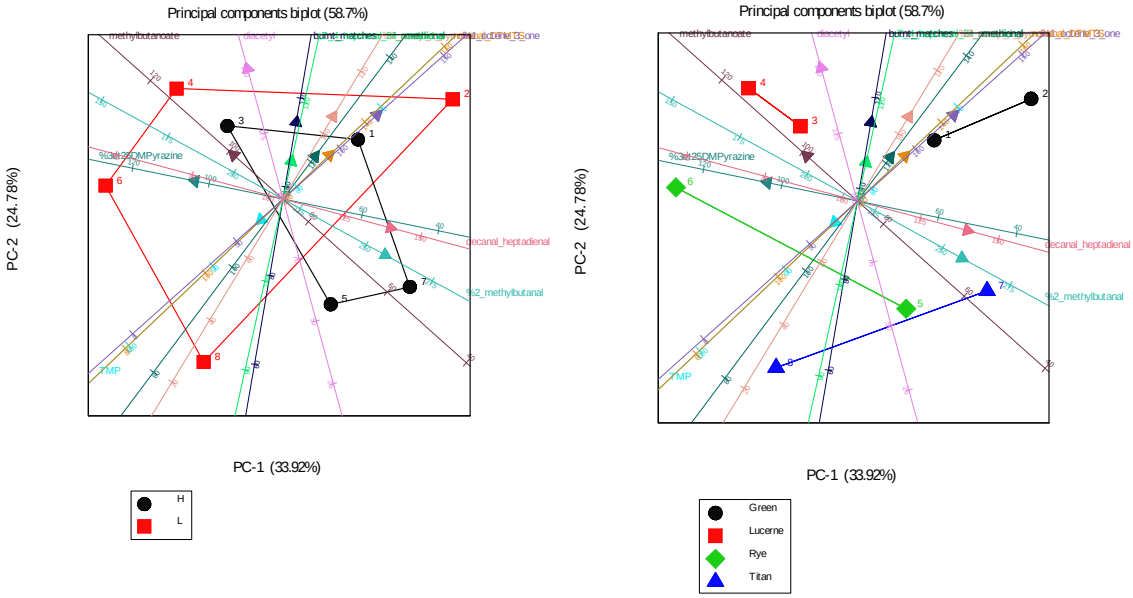

Figure 23: PCA plots based on the mean aromagram peak areas (sensory responses) for the twelve most intense odour peaks. Left – samples are grouped according to high fat ABV (black circle) or low fat ABV (red square). The high fat samples were more similar to each other than the low fat samples. Right – samples grouped according to finishing feed

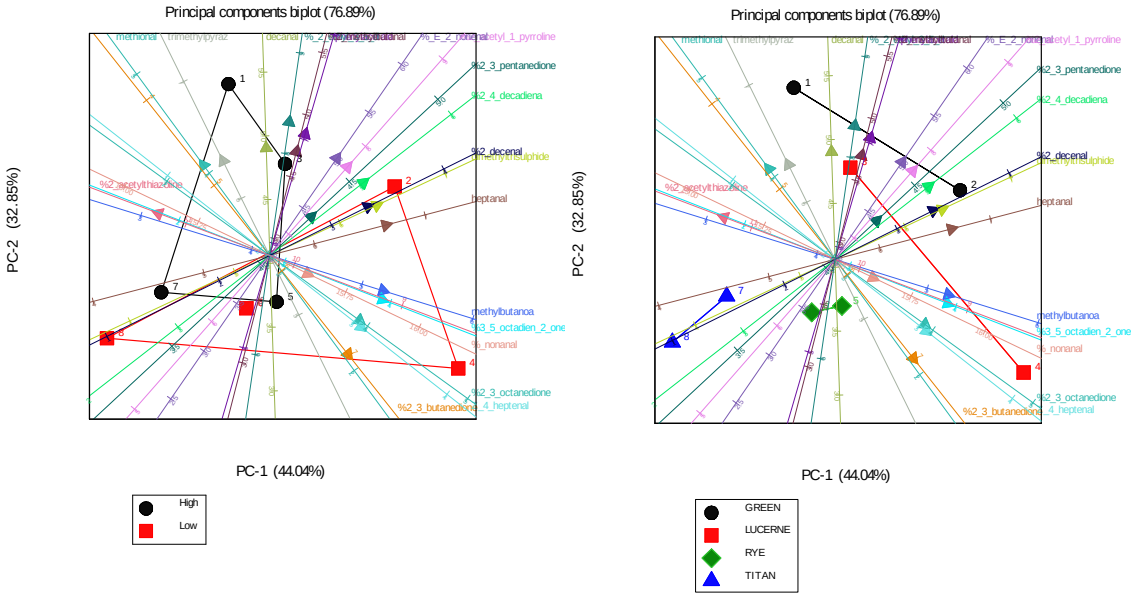

Figure 24: PCA plots based on the mean aromagram peak areas for the twelve most intense odour peaks. Left – samples are grouped according to high fat ABV (black circle) or low fat ABV (red square). The high fat samples were more similar to each other than the low fat samples. Right – samples grouped according to finishing feed

8.2.2 *Olfactometry Sample Differences*

The average integrated odour intensities (sensory responses) as assessed by the trained panel for the top 12 odour impact for each of the feed types are summarised in the PCA plot in **Figure 23**. The right hand figure shows the samples colour-coded according to finishing feed type. Based on odour intensities the samples could be discriminated into groups separated into different quadrants of the PCA map. The odour profiles of the high and low ABV Greenland samples (black circles) were quite similar to each other as were the two lucerne finished samples. The same samples are plotted according to ABV status. Generally the high ABV samples (black circles) had odour profiles more similar to each other than the low ABV samples (red squares), indicating there were discernible odour differences between high and low ABV samples. Similarly, PCA was performed using the integrated volatile peak areas from the GC-MS data for the same samples (**Figure 24**). Samples could be separated into high and low ABV groups and the feed types could also be grouped according to volatiles.

8.3 Summary of olfactometry findings

- *Thirty one odour compounds were detected in Tenax headspace extracts of grilled lamb.*
- *No unique odour active peaks were detected between sample types in the grilled lamb aromagrams. Differences were found mainly in relative odour intensity and ratios*
- *No odour volatiles from the brassica plants (e.g. ITCs or other compounds) were detected in raw or grilled lamb meat. No evidence for a "brassica" taint was found*
- *The odour-active volatiles identified in the current research agree in large part with those reported by other researchers (Bueno et al. 2011, 2013)*
- *Differences in aromagram responses and odour active volatiles could be related to feed and ABVs (IMF level)*

9 Free Amino Acids & other Non-Volatile Flavour Compounds in Raw Lamb

9.1.1 *Contribution of free amino acids to meat flavour*

The sensory panel data indicated minimal feed related flavour and taste differences, except that Titan samples were more *Acidic*. A number of Sire and ABV-related differences in flavour and taste attributes were measured: Overall "*Flavour Impact", Fatty Flavour, Sweet, Salty and Sour.* Unlike the aroma related flavour attributes, these flavour modalities are expected to be perceived primarily through taste receptors on the tongue and in the oral cavity, rather than through olfactory receptors. It is well known that the free amino acids and small peptides contribute considerably to the flavour and orosensory properties of meat (Dunkel & Hofmann 2009, Chen & Zhang 2007, Pereira-Lima *et al.* 2000, Schlichtherle & Grosch 1998). Most free amino acids are flavour-active in their own right, depending on their concentration in the food matrix. Free amino acids are also important precursors to volatiles formed during the Maillard reaction, such as alkylpyrazines and Strecker aldehydes. The volatile aldehyde 3-methylbutanal is formed directly from the Strecker degradation of the amino acid leucine, 2-methylpropanal from valine and methional from methionine. Hence, any differences in the concentrations of free amino acids and other non-volatile sapid compounds in the raw and cooked meat may contribute to the flavour potential either through providing a greater supply of substrates for the formation of aroma volatiles and in their own right as sapid non-volatile components.

9.1.2 *Derivatisation of free amino acids and analysis by GC-MS*

Semi-quantitative measurement of free amino acids and other non-volatiles in raw lamb macerates was achieved by adapting the method described by Smart *et al.* (2010), Leggio *et al.* (2012) as well as previously described (MAQA.00001). Two samples each from each sire and feed combination were analysed, such that a total of 32 raw samples were analysed. MANOVA analysis was conducted to ascertain feed, sire and ABV effects (**Table 13**).

Virtually no feed-related differences in the concentration of free amino acids or other compounds were measured, except for higher α-ketoglutarate measured in the lucerne-finished samples. The effect of ABV (high vs low) was not significant for any of the measured non-volatile compounds. In contrast, for the effect of Sire, various free amino acids were present at significantly higher concentration in the raw meat from Sire 8, compared to meat from the other sires. This finding was not expected, however it provides further evidence that meat derived from Sire 8 was not identical to the other high ABV sample (Sire 3). A higher initial pool of free amino acids may result in cooked meat with more intense flavour. Indeed the trained sensory panel rated the *Overall Odour* and *Flavour Impact* of meat from Sire 8 highest. *Sweet* and *Salty* taste was also rated highest by the trained panel in meat from Sire 8. Differences in rumen microbial flora are thought to affect breakdown of plant protein and release of amino acids (Hegarty 2004).

Table 13: Semi-quantitative data for methylformate derivatives of free amino acids and other non-volatile compounds in raw lamb meat. Data are averaged across n=8 samples. R = rye, L = lucerne, T = Titan, G = Greenland

	Feed					Sire				
	R	L	T	G	P_{Feed}	1	3	6	8	P_{Sire}
Glycine	0.035	0.032	0.031	0.032	—	0.032	0.031	0.033	0.033	—
Alanine	6.5	7.7	5.4	7.7	—	6.9	6.1	7.9	6.9	—
Valine	7.0	8.5	8.9	8.7	—	7.9	6.9	7.1	**12.8**	0.003
Leucine	18.5	23.0	19.1	21.6	—	19.4	18.2	19.4	**27.2**	0.039
Serine	1.2	1.4	1.5	1.5	—	1.2	1.1	1.2	**2.2**	0.002
Isoleucine	3.2	3.8	3.8	3.9	—	3.5	3.0	3.1	**5.9**	0.001
Proline	4.9	5.6	6.7	5.9	—	5.1	4.6	5.9	**8.5**	0.015
Aspartic acid	0.28	0.27	0.40	0.26	—	0.34	0.19	0.16	**0.66**	<0.001
Glutamic acid	0.80	0.67	0.72	0.76	—	0.79	0.67	0.62	0.97	—
Carnosine	2.9	3.3	2.7	2.7	—	2.7	2.3	3.5	3.3	—
Methionine	1.4	1.8	1.8	1.9	—	1.7	1.4	1.5	**2.7**	0.007
Phenylalanine	1.9	2.3	2.8	2.5	—	2.1	1.9	2.0	**4.1**	0.003
Tryptophan	2.0	1.9	1.8	1.9	—	1.9	1.8	2.1	1.8	—
					—					—
Lactic acid	190	212	163	208	—	189	182	200	206	—
α-ketogluarate	0.64	0.95	0.50	0.65	0.02	0.71	0.67	0.61	0.72	—
Fumarate	0.43	0.51	0.35	0.52	—	0.35	0.44	0.54	0.43	—
Palmitic	0.69	0.87	1.57	0.69	—	0.88	0.86	0.63	1.60	—
Succinate	0.97	1.52	1.09	1.12	—	1.22	1.12	1.00	1.40	—
Stearate	0.21	0.32	0.75	0.49	—	0.37	0.41	0.50	0.58	—
Niacinamide	0.17	0.20	0.12	0.20	—	0.17	0.17	0.18	0.17	—

9.2 Summary of Olfactometry Findings

- Virtually no differences in the concentration of amino acids or other non-volatiles in the raw meat were measured due to feed effects

- The free amino acids measured in Sire 8 were higher compared to the other sires, which may relate to the greater *Flavour Impact* measured in these samples

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Appendix 1

Table 14: Final consensus odour and flavour attributes developed by the trained panel to assess grilled lamb

ODOUR	Definition	Anchors		
Overall impact O	The intensity of the overall aroma	low to high		
Lamb / mutton O	Odour associated with cooked lamb	low to high		
Grilled O	Odour generated during grilling	low to high	*barbeque, roasted*	
Bloody O	Odour associated with fresh blood	low to high	*raw meat*	lamb blood juice
Caramel O	Sweet odour associated with burnt sugar	low to high		caramelised sugar solution
Barnyard O	Odour associated with barnyard or stables	low to high	*cow pat, Easter show, urine*	p-cresol (1 ppm)
Hay /grainy O	Odour associated with dry hay or unprocessed grains	low to high	*hay bale, dried grass*	
Livery O	Odour associated with grilled liver	low to high		grilled beef liver
Oxidised fat O	Odour associated with degradation of fat	low to high	*rancid, stale, warmed-over flavour*	
FLAVOUR				
Overall impact F	The intensity of the overall flavour of the sample	low to high		
Lamb / mutton F	Flavour associated with cooked lamb	low to high		
Dairy F	Flavour associated with milk, butter and other dairy products	low to high	*milk, butter, cream*	unsalted butter
Grassy F	Flavour associated with freshly cut grass	low to high	*green, leafy*	hexanal solution (20 ppm)
Vegetable F	Sulphur like flavour associated with cooked cabbage/brassica vegetables	low to high	*broccolini*	Greenland Brassica in water microwaved 1 min and cooled
Fatty F	Flavour associated with oil	low to high		
Livery F	Flavour associated with grilled liver	low to high		
Metallic F	Flavour associated with iron	low to high	*minerals, iron tablets*	iron tablet solution

Table 15: Final consensus odour and flavour attributes developed by the trained panel to assess grilled lamb

TASTE				
Sweet	The perceived intensity of sweet taste	low to high		sugar solution
Salty	The perceived intensity of salty taste	low to high		salt solution
Sour /acidic	The perceived intensity of sour/acidic taste	low to high		citric acid solution
Umami	The perceived intensity of umami taste	low to high		monosodium glutamate (MSG)
AFTERTASTE / AFTERFEEL				
Acidic aftertaste	The residual intensity of acidic / sour taste	low to high		
Astringency afterfeel	Dry sensation on mouth surfaces	low to high		
Oily mouthcoating	Amount of oil left on mouth surfaces	None to much	greasy, fatty	
Metallic aftertaste	The residual intensity of iron taste	low to high		
Lingering aftertaste	Aftertaste 30 seconds after swallowing	low to high		
TEXTURE				
Tenderness	Tenderness of the sample while chewing between molars	tough to tender		
Juiciness	Amount of juice released from the sample	none to a lot		
Number of chews	Number of chews required in order to swallow	Count number		
Connective tissue	Amount of connective tissue/fibrous present in the sample	none to much	sinew	

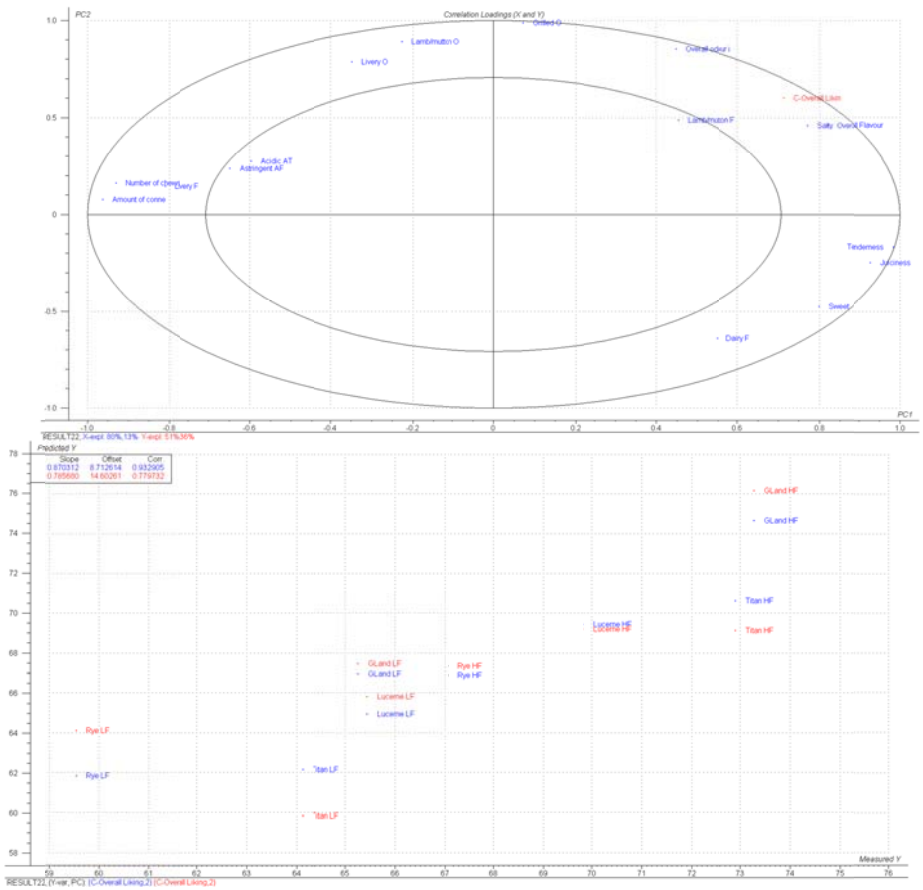

Figure 25: PLS-1 prediction model for *Overall Liking* of samples based on CB Australian consumer data only. Top – bi-plot of PC1 and PC2, showing the relationship between sensory attributes (blue) and consumer *Overall Liking.* Bottom –model of *Overall Liking* based on sensory attributes (blue) and cross-validation prediction model (red).

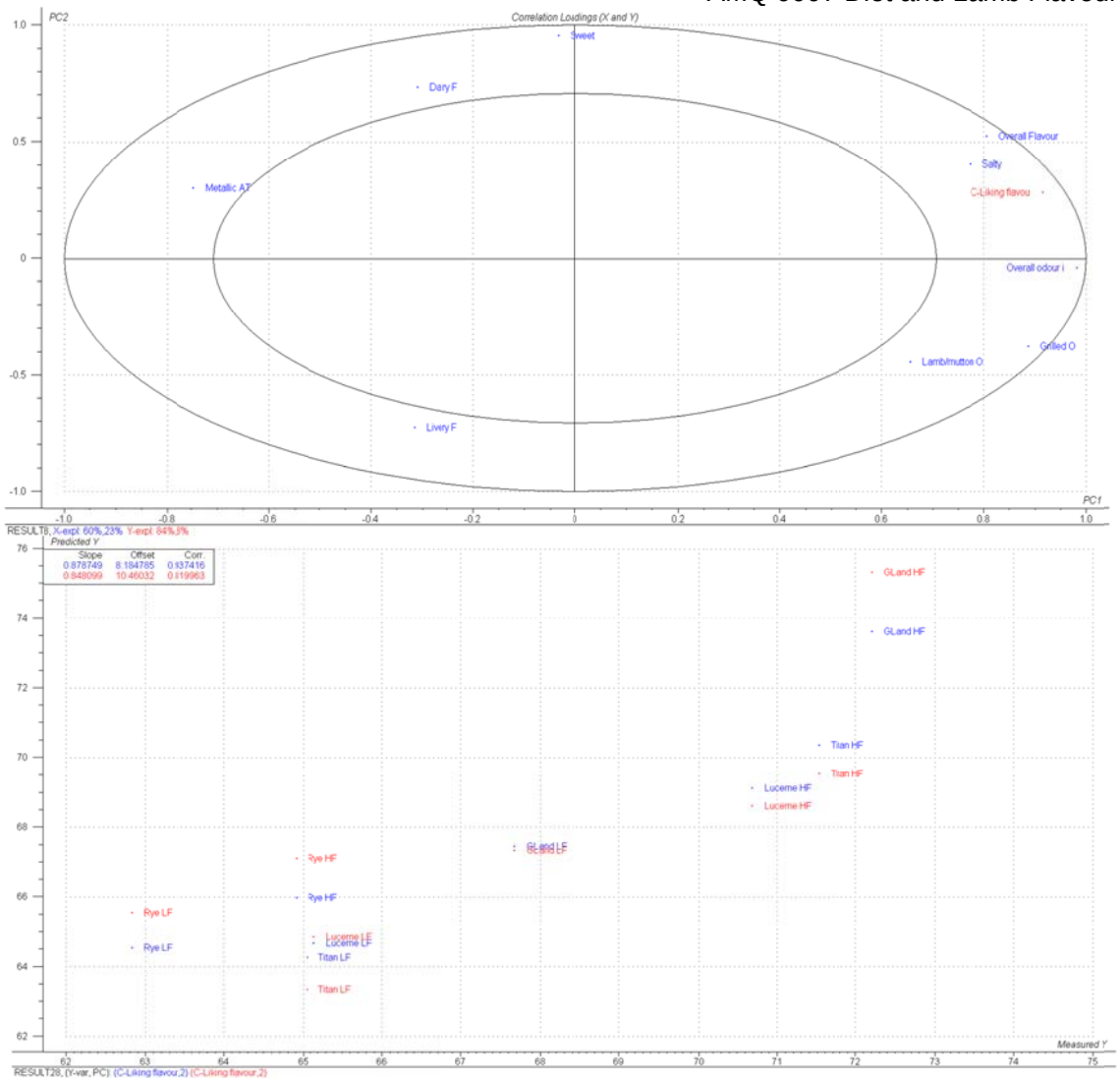

Figure 26: PLS-1 prediction model for *Flavour Liking* of samples based on CB-Australian consumer data only. Top – bi-plot of PC1 and PC2, showing the relationship between sensory attributes (blue) and consumer *Flavour Liking.* Bottom –model of Flavour Liking based on sensory attributes (blue) and cross-validation prediction model (red).

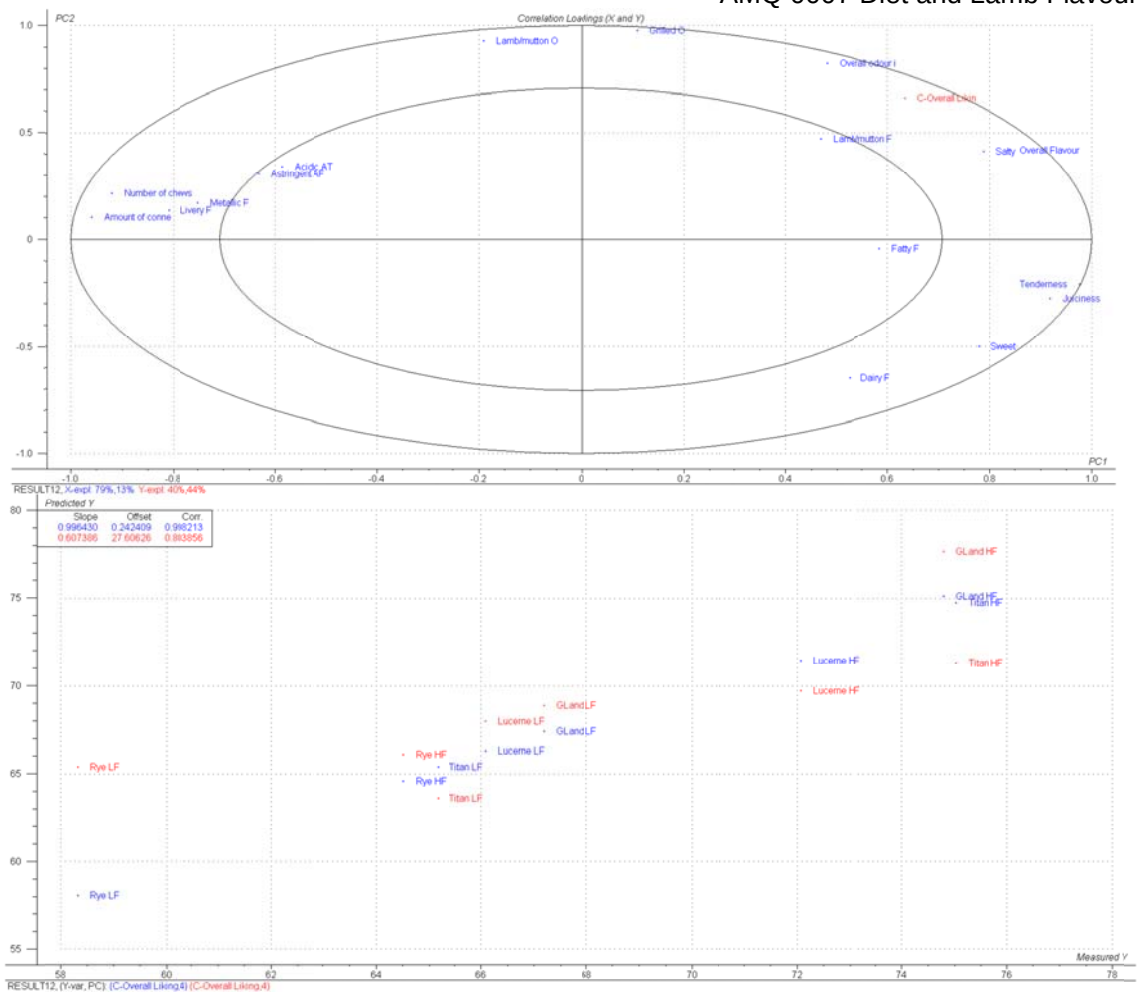

Figure 27: PLS-1 prediction model for *Overall Liking* of samples based on NCB-Australian consumer data only. Top – bi-plot of PC1 and PC2, showing the relationship between sensory attributes (blue) and consumer *Overall Liking.* Bottom –model of *Overall Liking* based on sensory attributes (blue) and cross-validation prediction model (red).

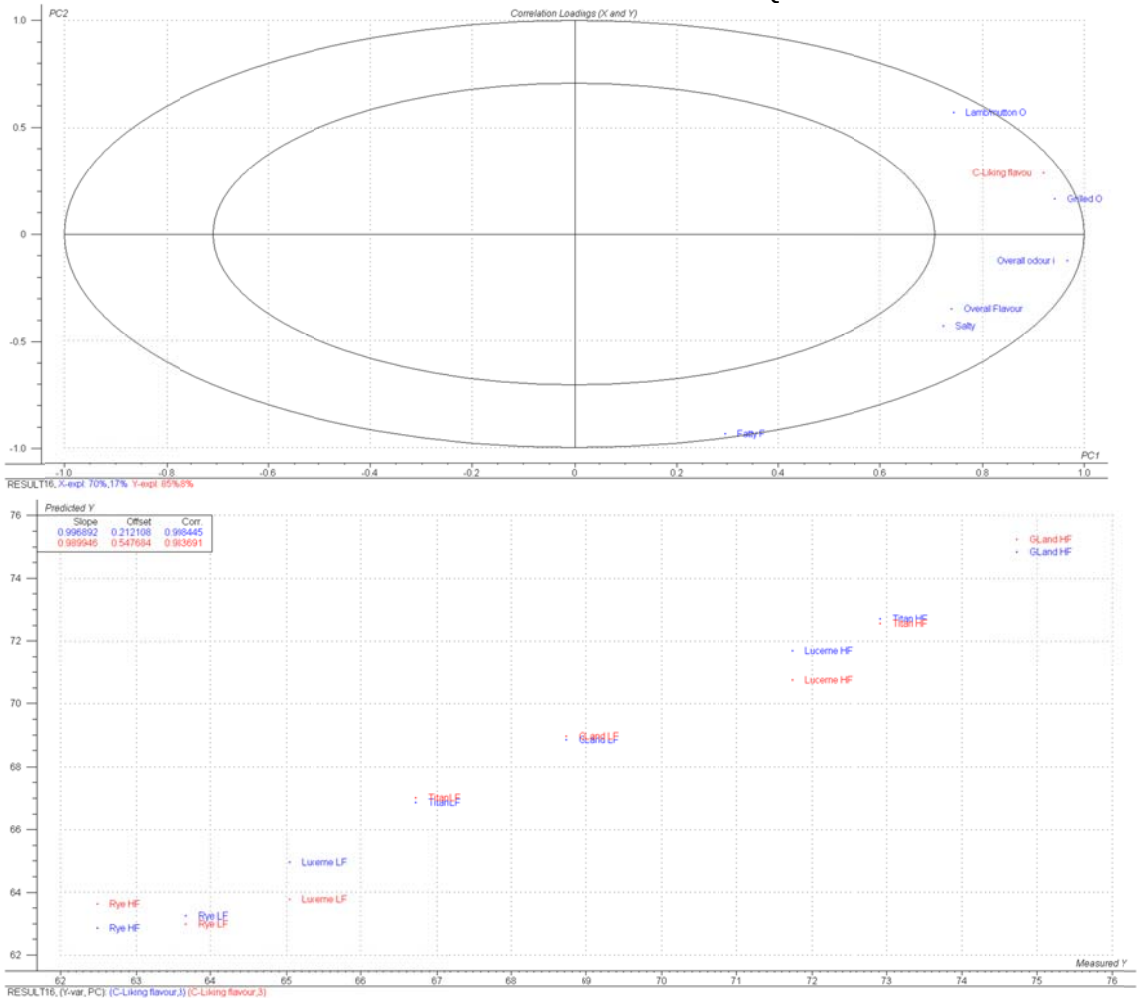

Figure 28: PLS-1 prediction model for Overall Liking of samples based on NCB Australian consumer data only

Table 16: Semi-quantitative data for main volatiles measured in the headspace of grilled lamb samples by Tenax-DHS. Relative concentration units.

Rt	Volatile	RYE	LUCERNE	TITAN	GREEN LAND	P Feed	Low	High	P ABV	Feed * ABV
	Aldehydes									
8.4	2-methylpropanal	3.2	4.1	3.2	4.4	0.016	3.52	4.03		
10.98	2-methylbutanal	18.3	33.0	25.8	38.1	<.001	24.50	34.40	0.005	0.008
13.89	hexanal	61	110	44	72	<0.001	87.20	57.30	0.002	<0.001
16.75	heptanal	2.31	3.57	1.68	3.45	<0.001	1.76	1.47		<0.001
18.13	Z-4-heptenal	0.29	1.48	0.71	0.79	<0.001	0.90	0.76		0.002
19.33	octanal	0.09	0.24	0.09	0.90	0.002	0.25	0.46		
23.47	(E)-2-octenal	1.06	1.47	1.08	1.48		1.30	1.27		
23.95	decanal	0.36	0.15	0.21	0.40	0.014	0.02	0.05		
24.83	benzaldehyde	3.89	4.32	2.44	4.15	0.043	3.64	3.83		
24.9	(E)-2-nonenal	0.49	0.44	0.26	0.71	0.004	0.53	0.44		
26.9	(E)-2-decenal	0.09	0.21	0.09	0.27	0.05	0.20	0.14		
27.2	Phenyl-acetaldehyde	0.45	0.53	0.38	0.59		0.44	0.55		
28.47	4-ethyl-benzaldehyde	0.03	0.05	0.02	0.04		0.04	0.03		
28.68	nonanal	3.76	2.89	3.05	3.59		3.67	2.96	0.103	
29.09	dodecanal	0.01	0.02	0.01	0.03		0.02	0.02		
	Alcohols									
18.02	3-methylpentanol	3.39	5.71	3.25	5.52	<0.001	4.26	4.85		0.006
22.63	1-octen-3-ol	5.70	7.63	4.46	6.92	0.013	6.11	6.39		
27.06	guaiacol	0.025	0.016	0.021	0.023		0.02	0.03	0.032	0.024
34.82	4-methylphenol	0.010	0.023	0.010	0.028	<0.001	0.02	0.02		
		9.13	13.38	7.74	12.49		10.40	11.29		
	Esters									
10.955	methylbutanoate	0.27	0.59	0.41	0.48		0.51	0.37		
12.65	ettylbutanoate	0.03	0.01	0.02	0.04		0.02	0.03		
	Ketones									
10.78	2,3-butanedione	1.58	2.49	1.94	1.78	0.004	1.98	1.91		
13.178	2,3-pentanedione	1.33	1.99	1.54	2.19	0.08	1.69	1.89		<0.001
16.599	2-heptanone	1.01	1.67	1.29	1.84		1.10	1.88	0.009	
19.19	2-octanone	0.63	0.76	0.58	1.18		0.63	0.99		
19.98	2,3-octanediuone	1.04	2.36	0.80	1.37	<0.001	1.46	1.36		
20.38	6-methyl-5-hepten-3-one	5.98	8.84	4.22	8.16		6.46	7.38		
24.27	3,5-octadien-2-one	0.07	0.18	0.06	0.13	0.006	0.13	0.10		0.08

Table 17 Contuinued: Semi-quantitative data for volatiles measured in the headspace of grilled lamb samples measured by Tenax-DHS. Relative concentration units.

Rt	Volatile	RYE	LUCERNE	TITAN	GREEN LAND	P Feed	Low	High	P ABV	Feed * ABV
	Maillard									
10.24	2-ethylfuran	2.54	6.76	3.43	4.03	<0.001	4.09	4.40	0.015	
12.55	2-acetylfuran	0.08	0.06	0.11	0.11		0.07	0.11	0.097	
18.75	2-pentylfuran	4.56	4.58	1.62	4.41	0.047	4.00	3.67		
20.4	2-acetyl-1-pyrroline	0.07	0.10	0.06	0.09	0.057	0.07	0.09		
23.35	2,5-dimethylfuran	0.13	0.39	0.12	0.18	0.016	0.26	0.15		0.018
23.38	furfural	0.06	0.07	0.07	0.10		0.02	0.03	0.09	0.02
24.41	pyrrole	0.50	0.39	0.63	0.44		0.40	0.57	0.03	<0.001
24.47	2-acetylfuran	0.15	0.31	0.16	0.16	0.02	0.17	0.22		
25.11	3-methylpyrrole	0.18	0.12	0.22	0.16		0.12	0.22	0.014	0.026
25.48	2-methylpyrrole	0.26	0.17	0.27	0.19		0.17	0.27	0.04	0.008
26.45	2-ethylpyrrole	0.03	0.04	0.04	0.04		0.03	0.04	0.085	0.085
33	2-acetylpyrrole	0.05	0.06	0.07	0.07		0.06	0.06		
4.7	heptane	0.78	2.60	1.11	1.83	<0.001	1.76	1.47		<0.001
17.08	limonene	0.18	0.04	0.17	0.20		0.15	0.15		
17.81	2,4-nonadienal	4.57	4.92	2.36	4.40		4.27	3.92		
19.9	p-cymene	0.09	0.24	0.09	0.90	0.002	0.25	0.46		
22.43	2,4-heptadienal	0.13	0.13	0.06	0.19	0.008	0.13	0.13		0.086
30.17	2,4-decadienal	0.08	0.15	0.09	0.17	0.05	0.12	0.13		
	Pyrazines									
18.77	methylpyrazine	1.26	1.03	1.31	1.45		0.99	1.55	0.015	0.03
20.12	2,6-dimethylpyrazine	1.81	1.91	2.18	2.29		1.84	2.28		0.014
20.24	2,5-dimethylpyrazine	0.67	0.42	0.83	0.63		0.51	0.76		
20.41	ethylpyrazine	0.22	0.14	0.20	0.24		0.15	0.25	0.043	
20.71	2-3-DMP	0.34	0.25	0.28	0.31		0.23	0.36	0.023	
21.55	2_ethyl_5_methyl pyrazine	0.79	0.62	0.68	0.90		0.64	0.87		
21.713	2_ethyl-3-methylpyrazine	1.18	1.03	1.10	1.32		0.97	1.36		
21.98	trimethylpyrazine	1.03	0.91	0.81	1.04		0.81	1.10		
23.2	3-ethyl-2,5-dimethylpyrazine	0.27	0.21	0.21	0.23		0.19	0.28	0.041	
23.848	2,3-diethyl-5-methylpyrazine	0.17	0.19	0.15	0.15		0.16	0.17		
24.82	3,5-dimethyl-2-isobutylpyrazine	0.03	0.04	0.03	0.05		0.03	0.04		
27.1	2-(3-methylbutyl)-3,5-dimethylpyrazine	0.98	1.11	0.93	1.30		1.00	1.19		
	Sulphur									
13.69	dimethyl disulphide	0.30	0.28	0.43	0.73	0.03	0.28	0.61	0.008	
14	2-methylthiophene	0.91	2.33	0.44	0.99		1.53	0.83		

21.67	dimethyl trisulphide	0.17	0.22	0.13	0.25	0.22	0.17	
23.27	methional	0.07	0.04	0.05	0.07	0.04	0.08	0.047
29.45	2-acetyl-2-thiazoline	0.02	0.01	0.02	0.02	0.02	0.02	