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Analysis of SPME flavour chemistry for MSA grass and grain fed beef samples

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Table of contents

The data in this report was supplied by and analysed in collaboration with

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1 Summary

1.1 Quality assurance

A proportion of the 472 GC-MS analyses for 26 volatile compounds were quality assured. The identification of the compounds was checked against the mass spectra and linear retention indices for authentic compounds. The peak areas were also checked. Forty three GC-MS runs appear to have suffered from an instrumental fault and were omitted from statistical analysis of the data and some peak areas were corrected. The remaining data was found to be excellent.

1.2 Training

The data produced by these analyses is complex and requires some experience to analyse and quality assure effectively. Training has been provided both during a visit to AFBI by Dr Jerrad Legako and through email correspondence.

1.3 FlavourBlue storage

The final quality assured excel database has been stored on the AFBI server, which is backed up regularly.

1.4 Data analysis and findings

It is important to note that the data discussed in this section were acquired by the staff and students of Texas Tech University and that they have academic ownership of these data. While AFBI have been commissioned by MLA to conduct a preliminary analysis of this data, the final analysis must be conducted in collaboration with TTU.

I. SPME analysis of volatile compounds from the grilled steak used for MSA consumer panels shows some significant differences due to ageing, cut and diet which shed some light on the nature of the effects of these factors on flavour.
II. Surprisingly, there were few consistent effects of USGrade, marbling, ribfat and other carcase measurements on the flavour volatiles.
III. The data confirms previous findings that volatiles from similar pathways generally tend to follow the same trends. This offers the possibility that certain compounds may act as markers for important flavour compounds which are difficult to analyse routinely.
IV. There are many findings from this data set that will allow much greater analysis than has been possible in this report and should result in several refereed scientific publications.

1.5 Implications for MSA

The data under discussion in this report is extensive and complex and this report can only address the scientific findings in part. These will be addressed more completely in scientific papers planned by Jerrad Legako and co-workers. Nevertheless, some conclusions can be drawn about the relevance of the findings for MSA.

- While all the relationships between meat production and flavour are not yet understood, a rational explanation can be proposed for how meat production and processing can influence flavour formation and release and thereby flavour as perceived by the consumer.
- The findings presented in this report demonstrate that both flavour volatiles and consumer scores are influenced by factors such as muscle/cut, days aged and diet.
- IMF appears to be an important driver for flavour perception, and it is probable that a certain level of IMF is needed to achieve the most desirable flavour release.

Knowledge of the optimum level of IMF for flavour release and the impact of different levels on flavour liking will assist prediction of flavour liking.

- Differences in consumer liking between different muscles is likely to be influenced by flavour differences as well as tenderness. Only some of this difference is explained through variations in IMF.
- Days aged is likely to show a curvilinear impact on flavour liking with an optimum level for most consumers. Further information may be available from existing MSA data.
- Diet has significant effects on flavour, only some of which may be explained by IMF. A controlled experiment with consumers from different countries eating beef from both diets is required.
- Section 7.2 proposes how MSA might be developed to include the prediction of flavour and Section 7.3 summarises these findings and how they may be applied to the development of MSA.
- Recommendations for further work and a flavour workshop are included.

2 Background

2.1 The Importance of Beef Flavour

The Meat Standards Australia (MSA) system has been tested in Northern Ireland, Ireland, Korea, Japan, France and USA. In all cases it has been found to predict eating quality effectively with only slight variations due to the cultural differences between countries (Polkinghorne, personal communication). Nevertheless, some areas have been identified where MSA predicts a little less well and where questions remain.

MSA mainly predicts tenderness. This is not surprising as the science of tenderness is much better understood than that for flavour. However, recent studies have shown that consumers in USA, NI and Australia find that flavour liking is as important as tenderness for the overall eating quality. In many cases these attributes are correlated in consumers' minds and prediction of one also predicts the other. However, in some cases it is believed that discrepancies between the predicted and actual MQ4 scores are due to flavour differences.

One of the suspected causes of differences between predicted and actual MQ4 scores and between consumers from different cultures is from grassfed versus grain/concentrate-fed beef. Liking for these products tends to be closely associated with previous experience of the consumer. Can volatile analysis be used to identify those differences between concentrate and grassfed beef that are responsible for the flavour differences and the impact on consumers from different cultures?

2.2 Use of Marker Compounds for Flavour

The compounds contributing to beef flavour have been the subject of much study (Mottram 1991, Elmore and Mottram 2009), and include water soluble taste compounds and volatile, fat soluble aroma compounds. Most of the key odour compounds identified are either formed by the Maillard reaction, thermal oxidation of lipids or other breakdown reactions, or are terpene-based odour compounds derived from the plant material consumed by the animals (Mottram 1991).

Many key odour compounds in beef have low odour thresholds and are present at extremely low concentrations. Techniques have been developed for their quantification (Hofmann and Schieberle 1998) but they are difficult to detect by routine GC-MS procedures. However, there are many interrelationships between the pathways of the Maillard reaction and the thermal oxidation of lipid. Recent research at AFBI has shown that some volatile compounds, although not key odour compounds themselves, can show a relationship both with flavour and with parameters affecting eating quality (Farmer et al. 2012) .

This work demonstrates that readily monitored volatile compounds are associated with other compounds from the same flavour formation pathway. These compounds are also associated with concentrations of precursors and processing parameters. Certain compounds are also associated with flavour acceptability and may act as marker compounds for desirable beef flavour.

2.3 Previous Flavour Contracts conducted for MLA

Modern flavour analysis methods are increasingly simple in operation and are becoming more adaptable to routine analysis. Nevertheless, some experience is required to know which volatile compounds to look for. Two short projects have been funded by Meat and Livestock Australia (MLA) at the Agri-Food and Biosciences Institute (AFBI) and Texas Tech University (TTU) to evaluate the potential for monitoring flavour volatiles alongside Meat Standards Australia (MSA) consumer testing. Work at AFBI for MLA has comprised:

• Training to a student at Texas Tech University to enable them to conduct analyses following the protocol and to help them analyse the data.

• Development and validation of a flavour analysis and data collection protocol.

• Development of an automatic data analysis process to collate the data.

• Advice on the analysis of data.

The work conducted to date shows some interesting relationships between production and meat grading factors and the volatile analyses.

The following reports by AFBI to MLA describe this work in more detail:

• PROJECT NUMBER V.EQT.1006. Feasibility Study to Evaluate the use of SPME Volatile Collection in Beef for Linkage to Consumer Flavour Evaluation. Final Report: May 2010

• PROJECT NUMBER V.EQT.1006 & V.EQT.1103. Protocol for the analysis and quantification of flavour volatiles from beef prepared according to MSA cooking protocols. July 2010.

• PROJECT NUMBER V.EQT.1103. Development of automated data analysis method, data evaluation and protocol development for use of SPME volatile collection in beef for linkage to consumer flavour evaluation. Interim Report: July 2010. Final Report: November 2010.

The last of these projects outlined how flavour analysis could assist MSA to deliver eating quality predictions which encompassed flavour characteristics as well as tenderness. It was proposed that the MSA-predicted score could either be modified for the target consumers based on their perceptions of flavour, or be qualified by descriptors that will enable consumers or purchasers to make their own decisions.

The aim of this project was to extend the analyses conducted previously to establish the relationships between flavour compounds, consumer scores and treatments.

3 Objectives

The projects' purpose is to evaluate the results of SPME analysis relating to MSA grass and grain fed beef samples that have both objective and consumer eating quality results tested at Texas Tech University (TTU). Following quality assurance of data from TTU, AFBI was asked to supervise identification of odour compounds by TTU, consolidate final data in the AFBI FlavourBlue database and conduct analyses on the flavour volatiles relative to eating quality parameters.

The objectives of this project, as set out in the Agreement between Meat and Livestock Australia and the Agri-Food and Biosciences Institute, were as follows:

1. To conduct quality assurance on the raw data.
2. To supervise the identification of compounds and to add results to a unified data base.
3. To analyse the grass fed samples with reference to relationships with muscle, ageing, fat content, other potential grading inputs and consumer scores.
4. To contrast these results with those reported on the earlier grain fed data.
5. To review the results and report on any, or the lack of any, difference or trend between the grass and grain fed data.
6. To combine suitable data and further analyse and report on any relationships between flavour chemistry, muscle, days aged, intramuscular fat and other potential grading inputs, diet and to consumer sensory scores.
7. On applicable samples to examine differences and consistency of consumer scoring to grass and grain fed samples by country (Australia, USA, France and South Africa) and potential relationships to flavour parameters.
8. To attend a flavour workshop in Australia including presentation of the project findings and participation in discussion regarding current flavour knowledge and priority future work.

The following section, Section 4, of this report describes the methods applied to these objectives. Section 5 describes the outcome of the quality assurance investigations outlined in Objectives 1 and 2. In Section 6, the specific issues outlined in Objectives 3 to 7 are addressed under the headings 6.2 to 6.5, respectively. Section 7 evaluates the implications of this work for further developments, including recommendations for a flavour workshop.

4 Methods

4.1 Training and Supervision

During 2010 and 2011, Dr Legako acquired 472 flavour analyses on 26 volatile compounds from grilled beef samples by GC-MS, using the protocol previously developed between TTU and AFBI[1]. He had confirmed identities with authentic compounds and used these to determine the quantities of volatile compounds collected. Nevertheless, he lacked the experience and guidance to fully evaluate the very complex data and some wrong determinations were present in his first data set. To overcome this, Dr Legako spent two weeks at AFBI during September 2012 and this was used to fine-tune an automated quantification procedure (see Section 4.2) which mainly overcame the problems. Dr Legako took this method back to TTU and used it to further analyse his data. Further advice was provided by email as required.

4.2 Quantification and collation of GC-MS data

Due to the quantity of data produced by GC-MS analysis of flavour, it is impractical to quantify all volatile compounds manually. Therefore, it is necessary to put in place an automated quantification method, based on the known mass spectrum and retention time of the compounds.

Using the Agilent Chemstation Data Analysis software a Quantitation Database identifying all the compounds of interest was created. For each compound a target ion was chosen from its mass spectra. This target ion was selected from a typical mass spectra alongside one or two qualifier ions. For each compound the ratio of the peak areas of the target ion and the qualifier(s) should be constant (+/- 5%) for all the data runs. The accuracy of this ratio for any given sample run is indicated by the “Q” value (0-100). The higher the “Q” the greater the certainty of fit that the compound has been correctly identified. Another factor which can be selected to contribute to the”Q” value is the retention time. For each compound a specific integration file or events file was also created. This ensures that the integrated peak area for each compound is calculated using exactly the same integration parameters throughout all the data runs. When the quantitation database has been developed for all the compounds of interest it was then saved as a specific method file. All the data files were then processed using that specific method file. Finally all the processed data files were collated together using a “macro” designed by the AFBI Biometrics department to produce the final detailed spreadsheet.

4.3 Quality Assurance of GC-MS data

The data received from Dr Legako included GC-MS analyses conducted during several months in two different years. These were divided into batches on the basis of the month collected. The number of data runs within each month varied substantially.

[1] Farmer, L.J. & Hagan, T.D.J. Protocol for the analysis and quantification of flavour volatiles from beef prepared according to MSA cooking protocols. July 2010

Confirmation of identity of the compounds was conducted by comparing the Mass Spectra Library Match on the NIST05a Library in the Agilent MS Chemstation Data Analysis software and also checking the reported Linear Retention Indices (LRI) of the compounds on equivalent gas chromatographic columns against scientific literature and AFBI analyses of the authentic compounds conducted previously. LRI information was received from TTU very recently so these checks are still ongoing.

The proposed quality assurance protocol was to select a minimum of three runs from each of the five sets of data and check the peak areas for all 26 compounds. For the set TTU 0811 there were over 200 runs, so instead, three runs were quality assured every 100 data files. The runs quality assured are listed in the Table 4.3a below.

Table 4.3a. List of data files checked for all compounds

Batch	Data Files Checked
TTU0410	039, 046, 053, 066, 073, 086, 091, 106, 112, 123, 134, 146, 152, 158, 168, 178.
TTU0510	003. 010, 019, 028, 032, 036, 044.
TTU0611	003, 015, 023, 031, 038, 046, 054, 081.
TTU0711	003, 008, 043, 053, 060, 066, 071.
TTU0811	002, 016, 027, 038, 057, 096, 109, 117, 128, 138, 163,179, 199, 213, 229, 245, 253

Further checks were conducted to check any discrepancies in peak areas or retention times. This involved producing graphical presentations of all compounds for all data files displaying the compound peak areas (e.g. Figure 4.3a) or compound retention times (e.g. Figure 4.3b). These were then examined and checked for major anomalies. Any such anomalies were checked.

Figure 4.3a: Example of peak area check for four compounds from Batch TTU0811 200- 264

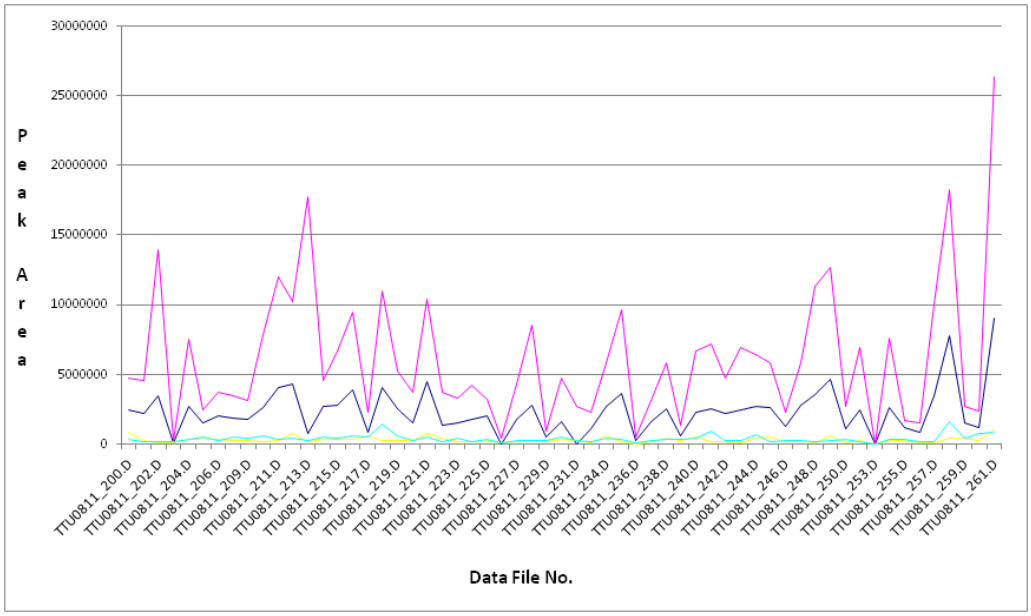
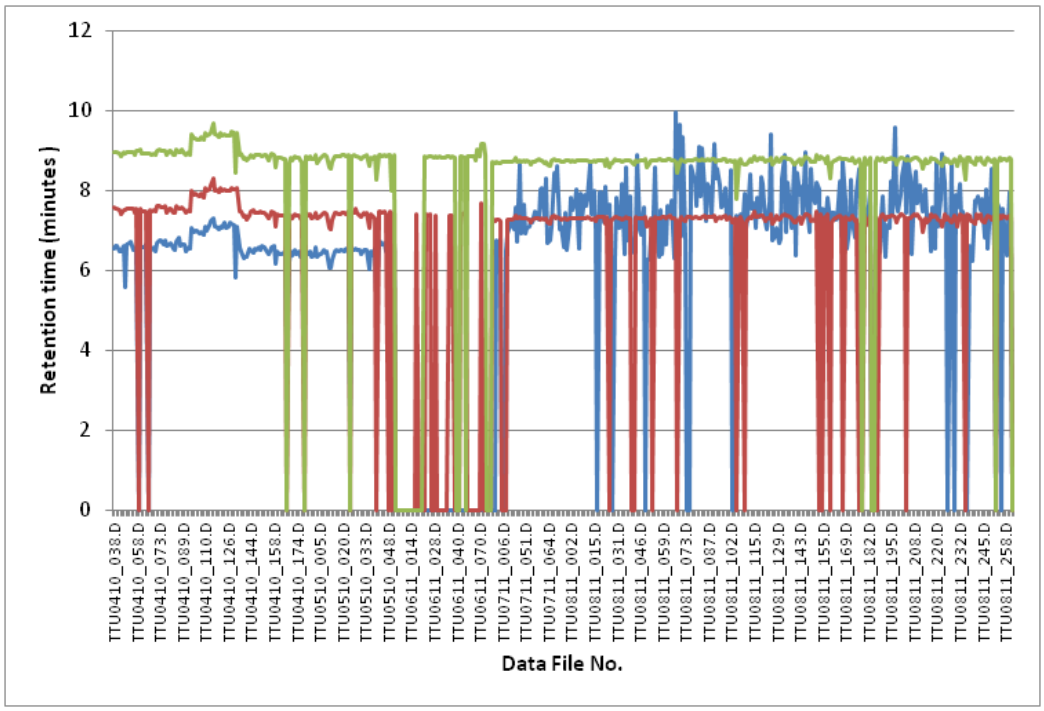


Figure 4.3b. Example retention time check for three compounds for all runs.



4.4 Statistical Analysis

The analysis of the effect of various factors, fitted as fixed effects, on the quantities collected of volatile compounds was determined using REML variance components analysis, which utilizes the linear mixed model methodology. In all cases Animal Number was fitted as a random effect. REML was conducted both for all the grassfed, feedlot and combined data

(excluding analyses conducted during 06/112) and for selected balanced groups within the total data set. The significance of each of the various factors was assessed by comparing a Wald statistic against either the appropriate F- or Chi-squared distribution.

In addition, principal components analysis, using the correlation method, was conducted on the volatile compounds and other continuous variables were then correlated back on to the principal components thus derived.

All analyses were conducted using GenStat version 15.1.

Many statistical analyses were conducted. Those presented in this report are those which gave the most effective means of determining answers to the queries identified in the objectives.

Other evaluations conducted but not reported herein include:

- Various options were considered to use preference mapping techniques with the groups of 10 consumers used in MSA testing. External preference mapping (against instrumental data) was dismissed as invalid as the method requires all samples of a set of treatments to have been consumed by the same consumers, and this was not done. Internal preference mapping generally requires sensory profiling to have been conducted and these data were not available.

- The possibility of analysing groups of compounds by considering them as repeated measures within compound groups was evaluated. While this showed similarities and differences between compounds within groups, it did not add to the arguments presented and was not included here.

4.5 Secure storage of FlavourBlue database

The FlavourBlue database (stored in Excel format) containing the quality assured results of the 472 analyses conducted to date, and to which may be added future analyses, is stored on the AFBI server. This server is backed up on a nightly basis to prevent any accidental loss or damage to the data source.

[2] *Analyses conducted on this date had unusually low peak areas and were not comparable with other analyses. Presumably this was due to an instrument problem.*

5 Results and Discussion – Quality Assurance of Data

5.1 QA of compound identification

For compounds eluting after octane, Linear Retention Indices were calculated from daily n-alkane analyses. The LRI values calculated from Dr Legako's runs are in general agreement with those reported in scientific literature and/or previously obtained in our laboratories using equivalent columns.

5.2 QA of ion peak areas

For the initial quality assurance screening of over 50 runs for all the compounds a total over 1500 peak areas were checked. The ions used were the same as used by Dr Lekago of TTU. No correction was made if the peak areas obtained was within a tolerance of +/- 15%. The number of peak area edited was between 1 and 2 % of those checked. Quality assurance of peak areas and retention times using the charts described previously resulted in the checking of between 6-8 data files for each of the 26 compounds.

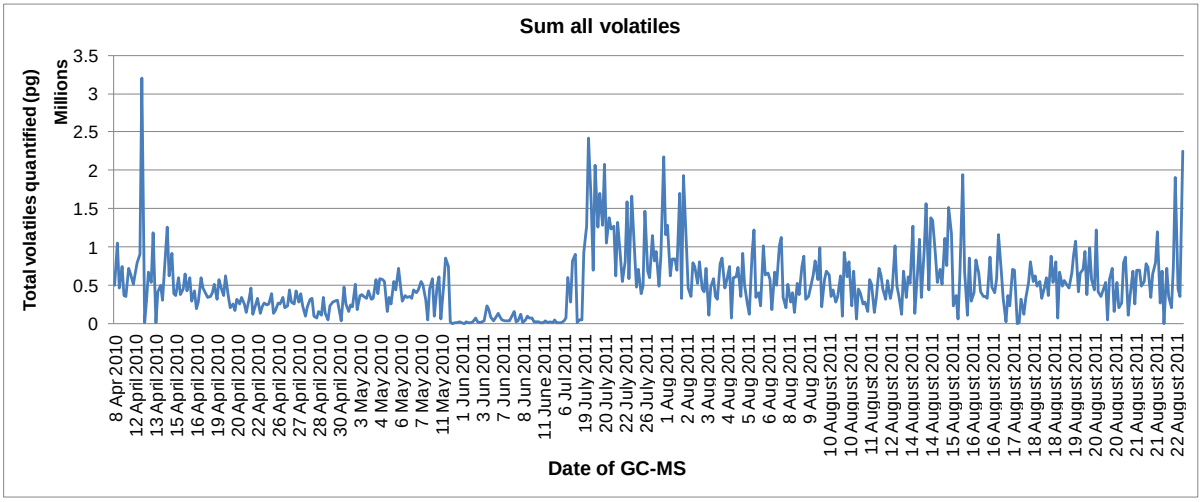
Most of the GC-MS data was accurate with only 30-40 amendments required (less than 2.5%).

5.3 Effect of date of analysis

Statistical analysis of the data indicated that the quantities of most/all volatile compounds was significantly ($P<0.001$ in all cases) affected by the date of analysis. This effect was mainly due to the analytical analyses conducted during June 2011 being unusually low in volatiles. This is illustrated for the total measured volatiles in Figure 5.3a. The GC-MS runs recorded during June 2011 appear to suffer from an instrumental fault and have been omitted from subsequent statistical analysis.

An internal standard, 4-octanol, was used throughout but gave inconsistent results. This is not uncommon for flavour volatile analyses, due to the difficulties of adding to a solid matrix. While alkane standards were run throughout the analyses, the concentrations used were not consistent, limiting their application as an external standard. In future, a quantitative external alkane standard including alkanes and a polar compound such as bromobenzene should be run daily to check column performance and allow for adjustments to the data to take account of changes in instrument sensitivity.

Figure 5.3a. Total quantity of volatiles measured (pg) over period of analysis



5.4 Effect of fibre

A number of different SPME fibres were used during the analyses and it would be expected that these may differ systematically in the quantities of volatiles they collect. This need not be a problem provided the use of fibres is balanced across treatments.

A comparison of the number of times that the fibres were used in each month of analyses showed that, as is normal, different fibres were selected for use at different stages (Figure 5.4a). There is no evidence of any consistent differences between fibres in the total volatiles collected (Figure 5.4b). The effect of date of use (as discussed in Section 5.3) appears to be greater than any differences between individual fibres.

Figure 5.4a. Frequency of usage of each fibre throughout experimental period

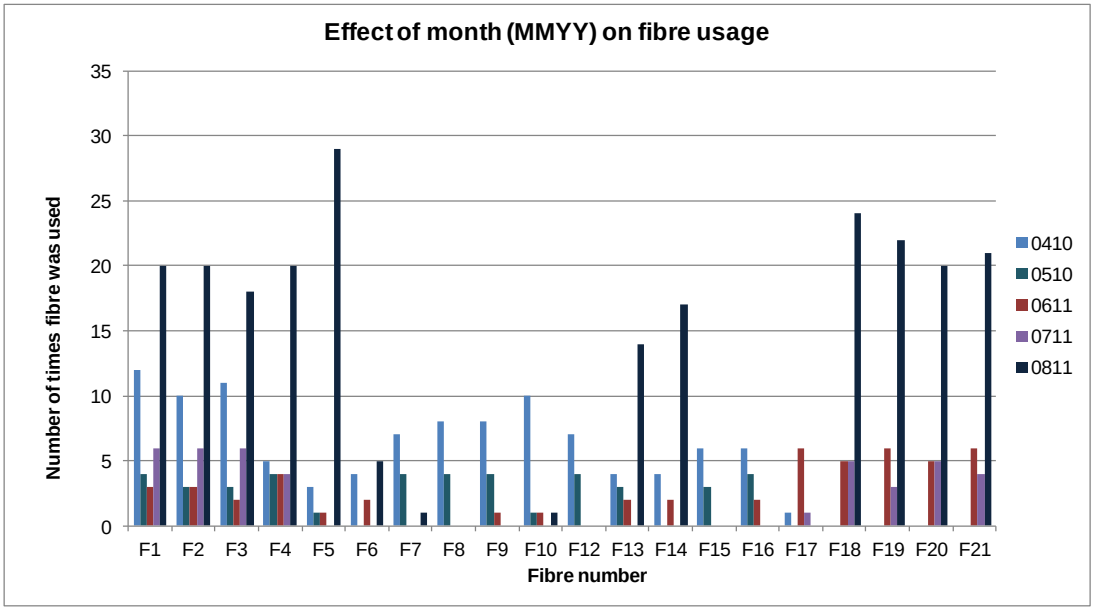
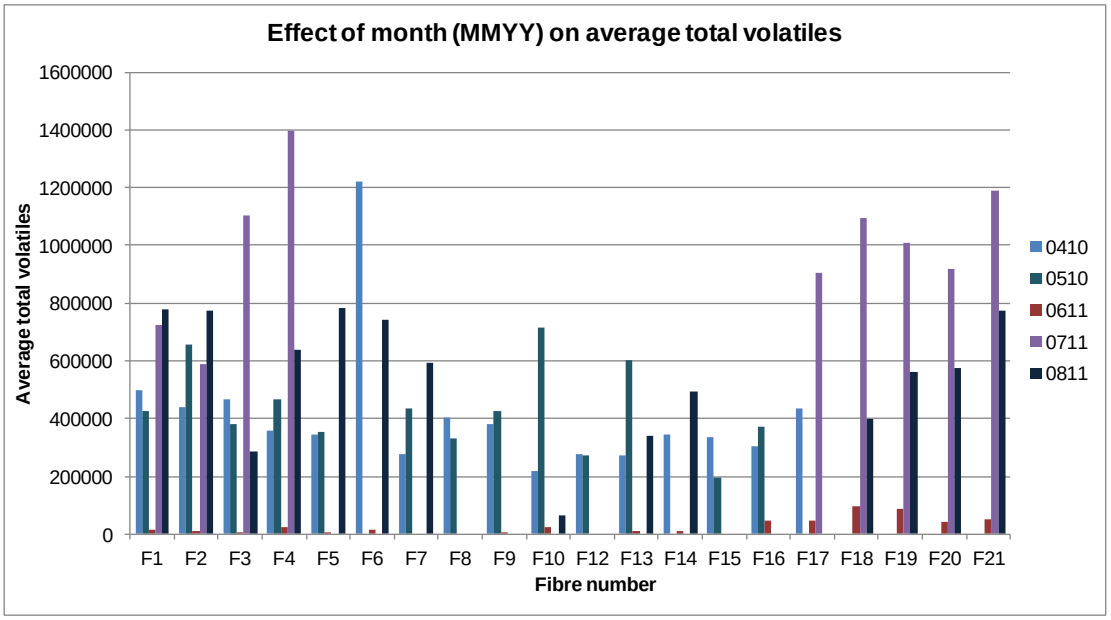


Figure 5.4b. Average total volatiles collected for each fibre for each month of analysis



5.5 Effectiveness of quality assurance

The level of accuracy achieved both for identification and quantification of volatile compounds is evidence that the training on validation and quantification techniques introduced during Dr Legako's visit to AFBI in 2012 was successful.

The protocol for analysis and quantification of volatiles should be revised to emphasise the need for the analyst to (a) run a consistent external standard on all days of analysis, (b) balance the use of fibres across treatments and days of analysis and (c) include specific validation and quantification procedures.

6 Results and Discussion – Analysis of Data

6.1 Overview

It is important to note that the data discussed in this section were acquired by the staff and students of Texas Tech University and that they have academic ownership of these data. While AFBI have been commissioned by MLA to conduct a preliminary analysis of this data, the final analysis will be conducted in collaboration with TTU.

The data were obtained from several separate experiments conducted during 2010 and 2011. The analyses for volatile compounds were conducted on selected samples from larger experiments. Table 6.1a shows the numbers of samples for the various treatments within these experiments.

Statistical analysis on the whole data set will inevitably be affected by the different treatments in each experiment. This would result in confounding effects where the source of differences would be difficult to clearly identify. For this reason, statistical analysis has also been conducted on selected data sets from within this design, chosen to allow the effects of specific factors to be identified in a reasonably balanced design.

6.2 Grassfed samples: Impact of muscle, ageing, fat content, other potential grading inputs and consumer scores on flavour volatiles

The grassfed animals included 321 analyses of volatiles from grilled beef from ca 20 animals slaughtered in Australia; samples were analysed from 8 muscles, of which two were aged for 5 days, two for 5 and 21 days and four for 5, 21 and 70 days (Table 6.1a). This final group was selected for statistical analysis as this had 13-18 analyses per treatment.

6.2.1 Effect on volatile compounds

Analysis of four cuts aged for three different ageing periods were conducted by REML variance components analysis (Table 6.2a).

Table 6.2a. Numbers of observations for each treatment

CUT	OUT005	STR045	TDR062	TOP073
D_AGED				
5	15	16	14	13
21	18	14	17	16
70	17	13	16	16

Table 6.1a. Numbers of samples analysed for flavour volatiles from different treatments

FEED[a]	HGP YES/NO	Abattoir	City	Country	USgrade	HANG	CUT	D.AGED	Total[b]
0	0	0	0	0	0	0	0	0	2
F	YES	ACC	Brisbane	Aust	HighStd	SS	OUT005	7	2
								48	2
							RMP131	7	1
								48	1
							STR045	7	2
								48	2
								70	2
							TOP073	7	2
								48	2
						TX	OUT005	7	1
								48	1
							RMP131	7	2
								48	2
							STR045	7	2
								48	2
								70	1
							TOP073	7	1
								48	1
					LowChoice	TX	OUT005	7	1
								48	1
							STR045	70	1
							TOP073	7	1
								48	1
					Select	TX	OUT005	7	1
								48	1
							RMP131	7	1
								48	1
							STR045	7	1
								48	1
								70	1
							TOP073	7	1
								48	1

FEED[a]	HGP YES/NO	Abattoir	City	Country	USgrade	HANG	CUT	D.AGED	Total[b]
		Friona	Friona TX	USA	High Choice	AT	RMP131	21	4
							STR045	21	2
								22	6
							TDR062	21	2
							TOP073	21	4
					Low Choice	AT	RMP131	21	4
							STR045	21	3
								22	6
							TDR062	21	4
							TOP073	21	4
					Prime	AT	RMP131	21	4
							STR045	21	3
								22	6
							TDR062	21	4
							TOP073	21	4
					Select	AT	RMP131	21	4
							STR045	22	9
							TDR062	21	4
								35	2
							TOP073	21	4
					Standard	AT	RMP131	21	5
							STR045	21	2
								22	7
							TDR062	21	4
							TOP073	21	4
G	NO	Casino	Pretoria	RSA[c]	0	AT	CHK078	5	1
			Sydney	Aust	0	AT	BLD096	5	15
							CHK078	5	16
							OUT005	5	16
								21	16
								70	16
							OYS036	5	16
								21	16

FEED[a]	HGP YES/NO	Abattoir	City	Country	USgrade	HANG	CUT	D.AGED	Total[b]
							RMP131	5	16
								21	16
							STR045	5	16
								21	16
								70	15
							TDR062	5	15
								21	16
								70	16
							TOP073	5	16
								21	16
								70	16
			TWB	Aust	0	AT	BLD096	5	2
							CHK078	5	2
							OUT005	5	2
								21	2
								70	2
							OYS036		4
							RMP131	5	2
								21	2
							STR045	5	2
								21	2
								70	2
							TDR062	5	2
								21	2
								70	2
							TOP073	5	2
								21	2
								70	2
Grand Total									472

a. *a FEED = G or F: grassfed or feedlot*
b. *b Total = total number of flavour analysis GC-MS runs*
c. *c One sample from South Africa was excluded from analyses*

Table 6.2b shows that the main effects on most volatile compounds are due to cut and days aged. There were significant effects on a very few compounds of UMb (1), pHu (1), LTemp (1), Cut.Days aged (2), Cut.UOss (1), Cut.pHu (2), Cut.LTemp (2), D.Aged.UMb (3), D.Aged.UOss (2), D.Aged.Ribfat (6), D.Aged.pHu (6). Only the consistent effects of cut, days aged and the days aged.pHu interaction will be discussed here.

The effect of days aged and cut on volatile compounds are considered for each compound group in turn:

n-aldehydes***:*** All the n-aldehydes show a significant effect of days aged, while some show an effect of cut also. Figure 6.2a illustrates these effects for pentanal and nonanal. The aldehydes between these examples show a similar pattern, with quantities tending to decrease on ageing, especially in striploin.

Table 6.2b. Significant effects on volatile compounds from grassfed beef

	D.Aged	Cut	UMb	UOss	Ribfat	pHu	LTemp	Cut.D.Aged	Cut.UMb	Cut.UOss	Cut.Ribfat	Cut.pHu	Cut.LTemp	D.Aged.UMb	D.Aged.UOss.	D.Aged.Ribfat.	D.Aged.pHu	D.Aged.LTemp	3 way?
n-aldehydes																			
Pentanal	***	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
Hexanal	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
Heptanal	***	**	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
Octanal	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*oss
Nonanal	***	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
Decanal	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**oss *LT
furans																			
2-Pentylfuran	ns	ns	ns	ns	ns	ns	*	ns/*	ns	***	ns	ns	***	ns	*	ns	ns	**	***oss *RF **phu ***LT

	D.Aged	Cut	UMb	UOss	Ribfat	pHu	LTemp	Cut.D.Aged	Cut.UMb	Cut.UOss	Cut.Ribfat	Cut.pHu	Cut.LTemp	D.Aged.UMb	D.Aged.UOss.	D.Aged.Ribfat.	D.Aged.pHu	D.Aged.LTemp	3 way ?
ketones																			
2-Propanone	***	***	P=0.054	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	** mb
2-Butanone	ns	***	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*mb
2,3-Butanedione	**	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*oss
3-Hydroxy-2-Butanone	**	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	ns	ns	ns	*	ns	
Strecker aldehydes																			
Acetaldehyde	***	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
3-Methylbutanal	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	P=0.050	ns	*	ns	*	ns	ns	
2-Methylbutanal	***	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	P=0.054	ns	ns	ns	*	ns	ns	
Methional	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	*	ns	ns	
Phenylacetaldehyde	***	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	

	D.Aged	Cut	UMb	UOss	Ribfat	pHu	LTemp	Cut.D.Aged	Cut.UMb	Cut.UOss	Cut.Ribfat	Cut.pHu	Cut.LTemp	D.Aged.UMb	D.Aged.UOss.	D.Aged.Ribfat.	D.Aged.pHu	D.Aged.LTemp	3 way ?
S-containing																			
Methanethiol	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	*	ns	ns	
Dimethyl sulfide	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*oss
Dimethyl disulfide	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
pyrazines																			
Methyl pyrazine	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	
2,5/6-Dimethyl pyrazine	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*mb
Trimethylpyrazine	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
2-Ethyl-3,5/6-dimehtyl pyrazine	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*phu
Other																			
Heptane	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	*oss
Octane	ns	***	ns	ns	ns	ns	ns	ns	*	ns	ns	*	ns	ns	ns	**	ns	ns	
Benzaldehyde	ns	**	ns	ns	ns	P= 0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	

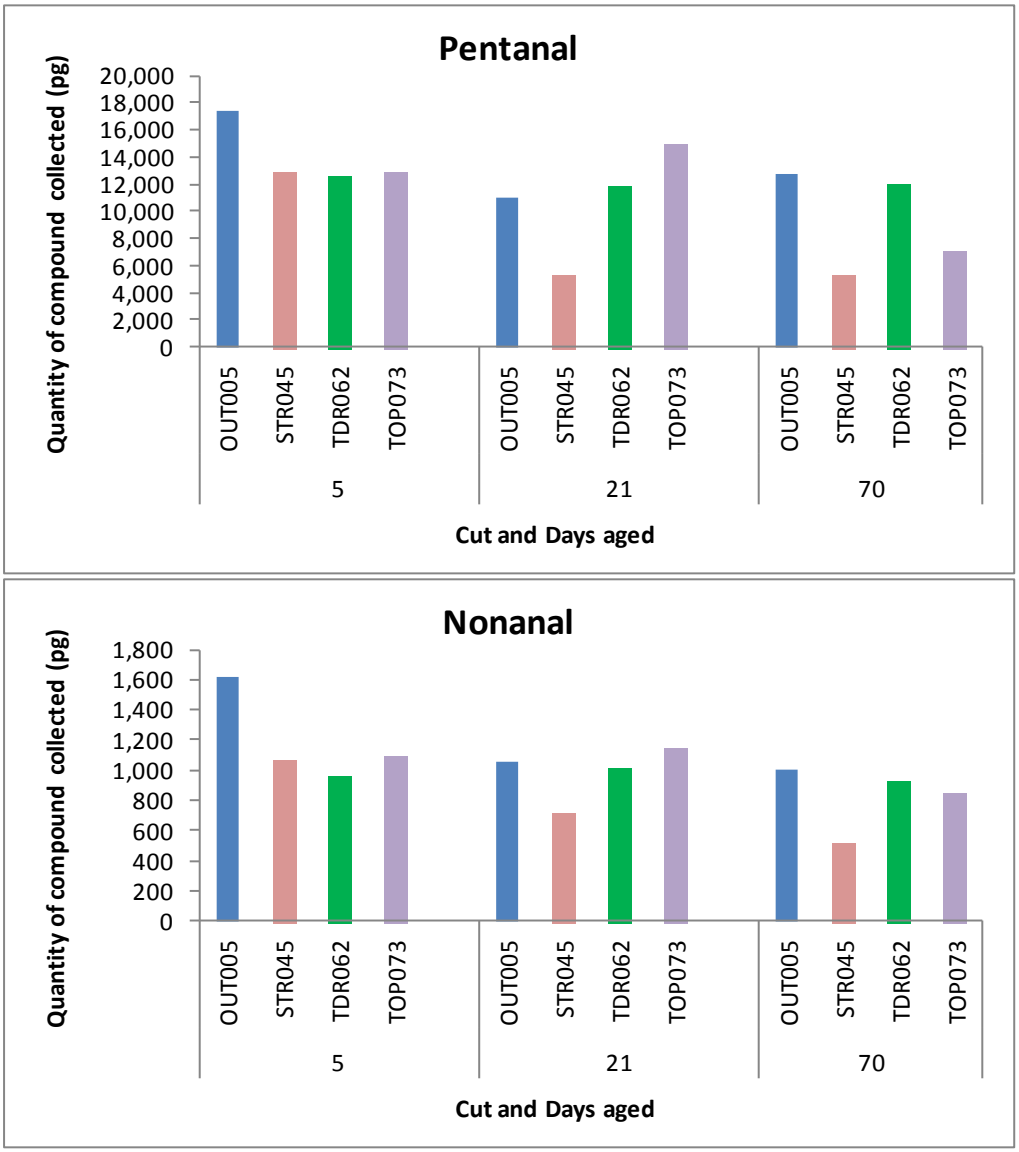
	D.Aged	Cut	UMb	UOss	Ribfat	pHu	LTemp	Cut.D.Aged	Cut.UMb	Cut.UOss	Cut.Ribfat	Cut.pHu	Cut.LTemp	D.Aged.UMb	D.Aged.UOss.	D.Aged.Ribfat.	D.Aged.pHu	D.Aged.LTemp	3 way ?
						1													

*Analyses were conducted for Cut * D.aged * UMb or UOss or Ribfat or pHu or Ltemp separately. Where the significane for Cut, D.Aged or Cut*D.Aged differed, the significance quoted is that found in three of these analyses. Differences were small.*

P is quoted only for probabilities approaching P=0.05 (P<0.06).

x No analysis possible for Trimethylpyrazine as none was detected in the headspace from these samples

Figure 6.2a. Effect of days aged and cut on quantities of n-aldehydes



The longer chain decanal and 2-pentylfuran follow different patterns, with increases during ageing and higher levels detected in striploin. These compounds are formed by the oxidation of lipids. Although the n-aldehydes are not generally major odour compounds themselves, many key odour compounds are derived from lipid thermal oxidation and recent research has demonstrated that compounds formed by the same pathway are often correlated (Farmer et al. 2012). **These results indicate that the formation of odour compounds by lipid oxidation is affected by cut/muscle and days aged.**

The Strecker aldehydes are mainly affected by ageing and little by cut. This is illustrated in Figure 6.2b. Although there appear to be differences in effect of ageing between cuts, these interactions are not significant. This increase in Strecker aldehydes, derived from the Maillard reaction between amino acids and sugars, is consistent with recent findings on the relationships between flavour precursors and volatiles, and is likely to be caused by the

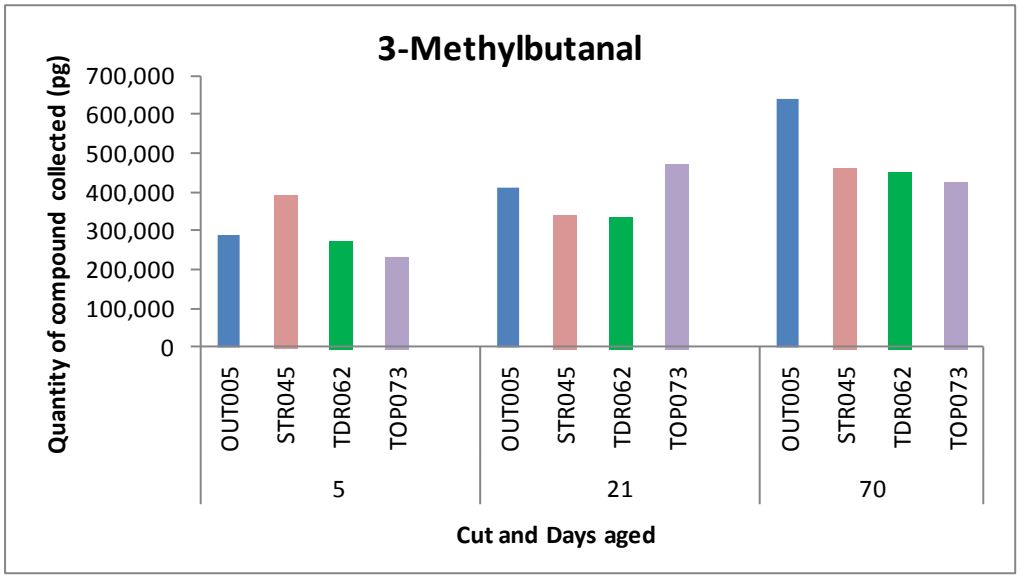
formation of free amino acids and sugars through proteolysis and glycolysis (Farmer et al. 2012).

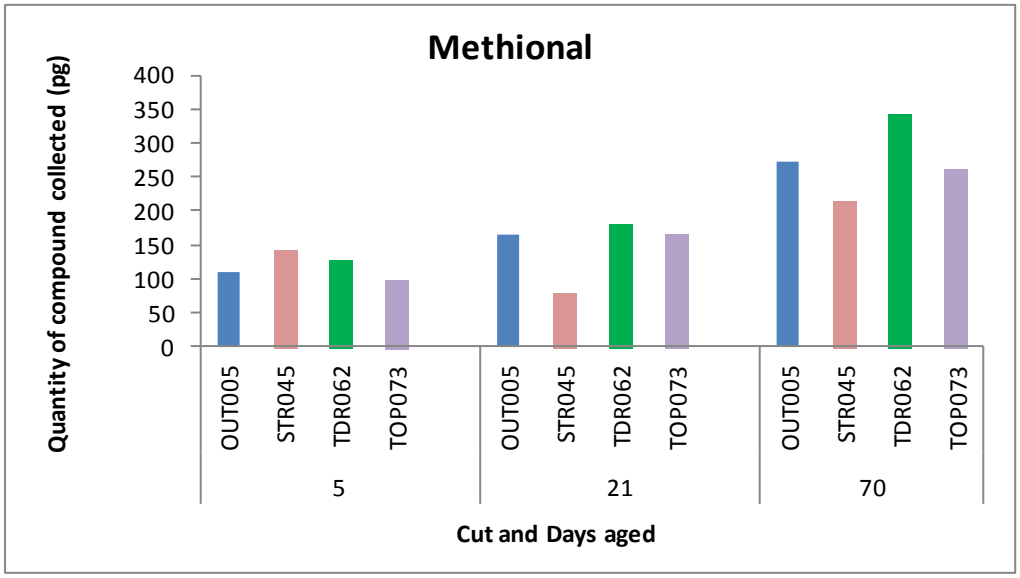
Sulphur-containing compounds are highly odorous and, as well as being related to other important odour compounds, make an important contribution to odour in their own right. Of the compounds quantified, methanethiol shows a very highly significant effect of days aged, increasing with ageing to 21d but decreasing again by 70d (Figure 6.2c). In contrast, dimethyl sulphide is consistently higher in grilled beef from OUT005 and TOP073. It is very likely that this will influence the overall flavour of these cuts.

2,3-butanedione and 3-hydroxy-2-butanone are reactive compounds expected to participate in further reaction with Maillard intermediates to give Strecker aldehydes, pyrazines and other odour and flavour compounds. Both compounds show the pattern illustrated in Figure 6.2d, with significant effects of both ageing and cut. Like the sulphur-containing compounds, the highest levels are found in the 21d aged meat, with OUT005 and TOP073 giving higher quantities than STR045 and TDR062.

Thus, all the Maillard–derived volatile compounds are heavily influenced by days aged while only some are affected by cut/muscle. This probably relates to the quantities of precursor compounds in the raw meat.

Figure 6.2b. Effect of days aged and cut on quantities of Strecker aldehydes




Methional
Quantity of compound collected (pg)
400
350
300
250
200
150
100
50
0
OUT005
STR045
TDR062
TOP073
5
21
70
Cut and Days aged

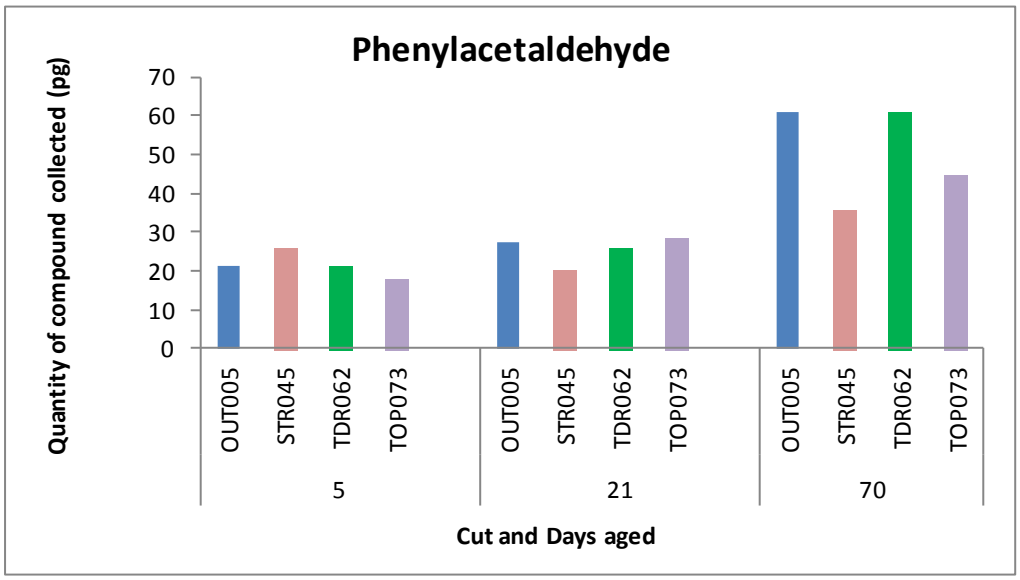
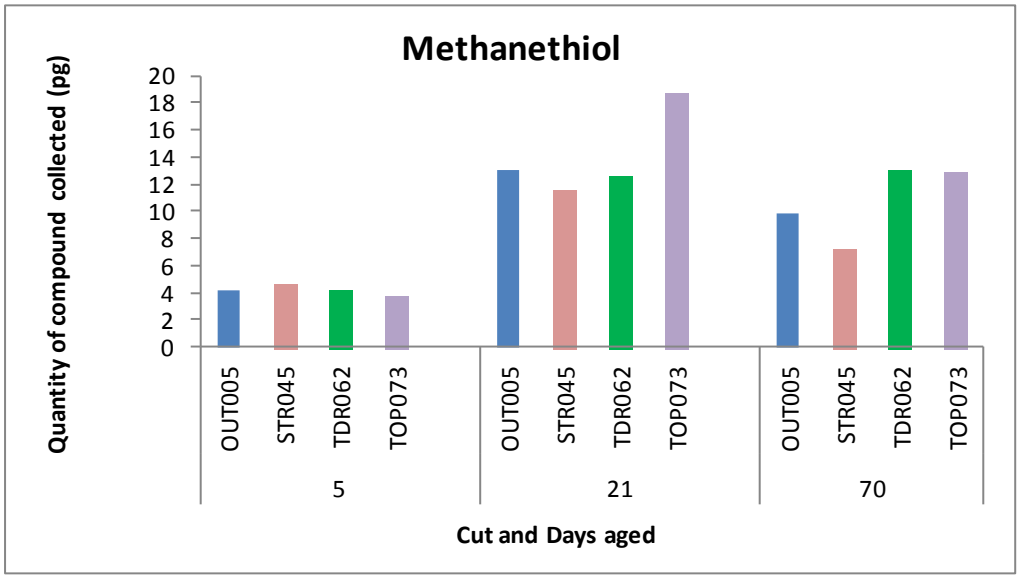

Phenylacetaldehyde
Quantity of compound collected (pg)
70
60
50
40
30
20
10
0
OUT005
STR045
TDR062
TOP073
5
21
70
Cut and Days aged

Figure 6.2c. Effect of days aged and cut on quantities of sulphur compounds



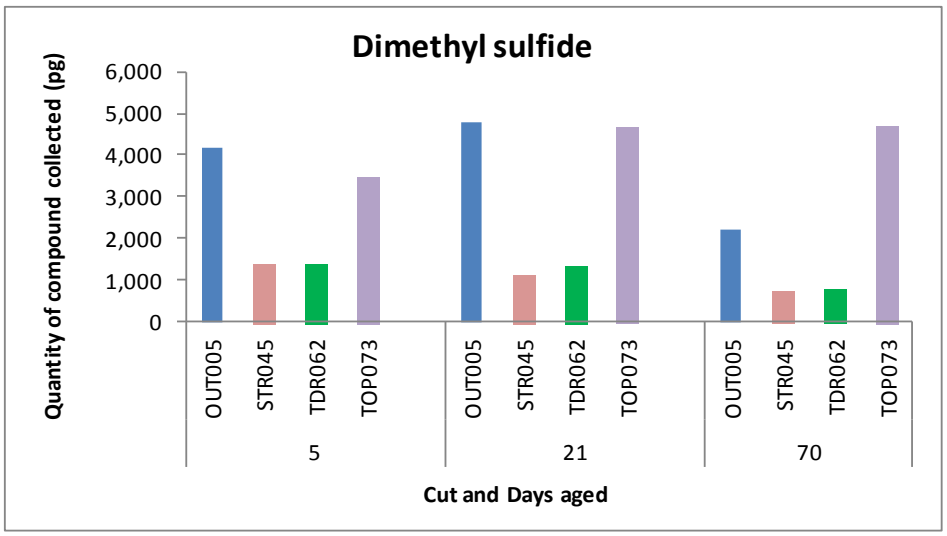
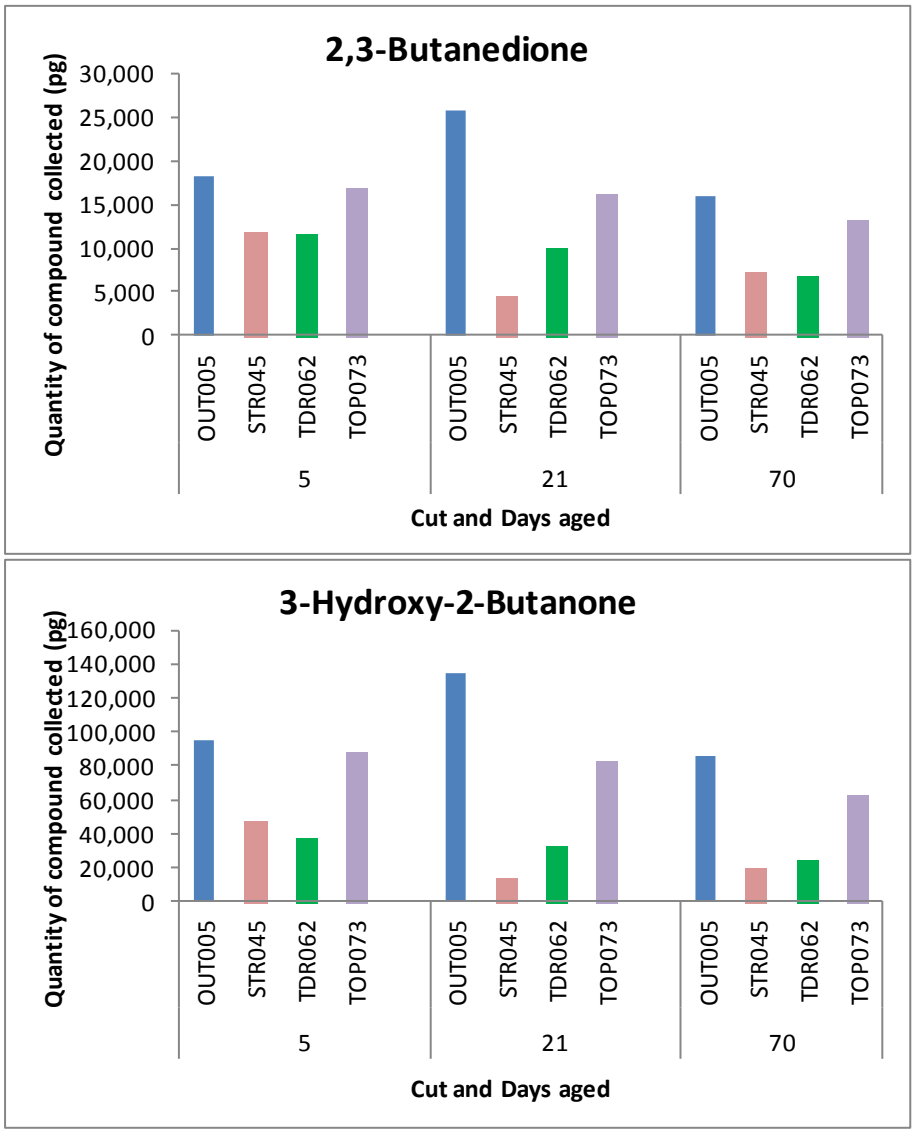


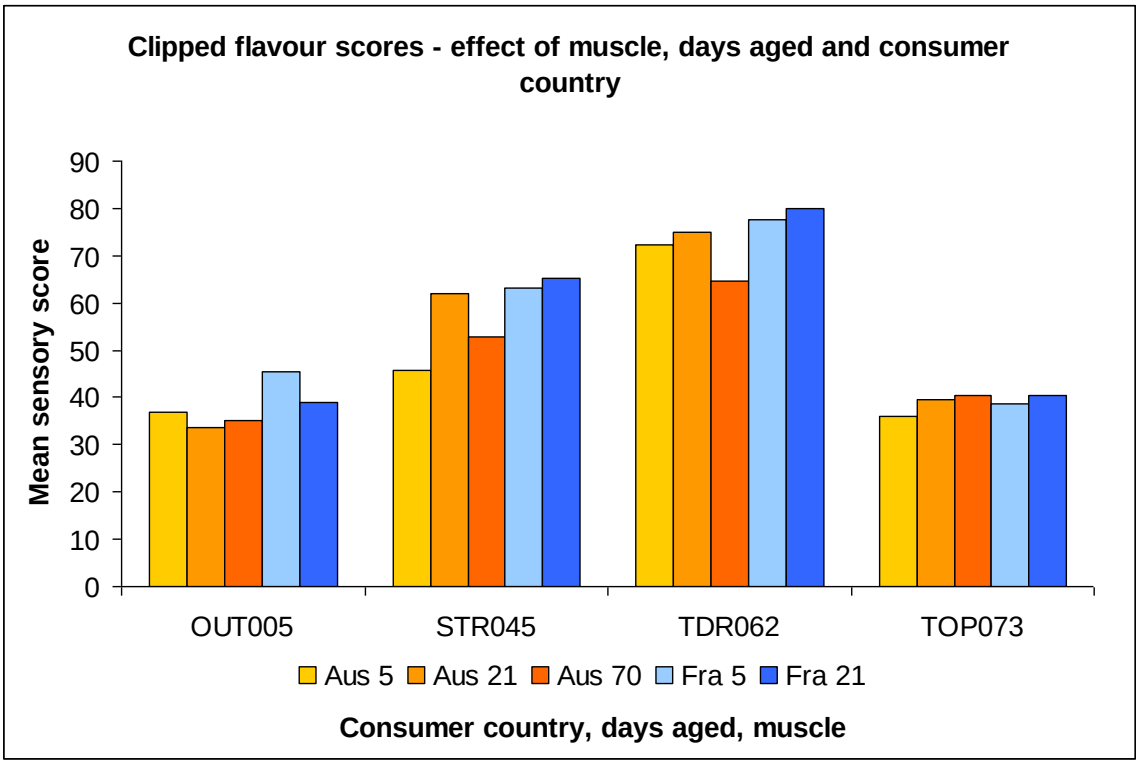
Figure 6.2d. Effect of days aged and cut on quantities of 2,3-butanedione and 3-hydroxy-2-butanone



6.2.2 Effect of cut and days aged on consumer scores

Analysis of the same samples for consumer scores showed a clear effect of muscle/cut ($P<0.001$), as expected, but no significant effect of ageing for either Australian or French consumers (Figure 6.2e). Neither was there any significant interaction between cut and ageing. It may be observed that the scores followed a similar pattern for Australian and French consumers, though the absolute scores differed slightly.

Figure 6.2e: Effect of cut and ageing (5, 21, 70d) on mean consumer scores for Australian and French consumers (nb. only Australian consumers sampled 70d beef)



6.3 Effect of cut at 5 days aged on volatiles and consumer scores from different countries

The greatest number of muscles were evaluated by both volatile analysis and consumer panels in different countries at 5 days ageing with 17 or 18 samples of eight muscles analysed for volatiles. Ten of the compounds showed significant differences between cuts at 5 days (Table 6.3a). Figures 6.3 (a-d) compares the quantities of the n-aldehydes, Strecker aldehydes, ketones and sulphur compounds (expressed relative to striploin, for ease of comparison).

Only two of the n-aldehydes show significant differences ($P<0.05$; Table 6.3a). RMP131 and OUT005 have significantly greater quantities of octanal than OYS036 and CHK078 ($P<0.05$, by least significant difference), while for nonanal, RMP131 and OUT005 have significantly greater quantities than CHK078, STR045, OYS036 and TDR062. The remaining aldehydes, although not significant, show similarities in pattern, with RMP131 and OUT005 having relatively high levels of all the aldehydes and OYS036 and STR045 tending to have lower levels. OYS036 also has the lowest levels of two of the ketones, most of the Strecker aldehydes and dimethylsulphide. RMP131, OUT005 and in some cases TOP073 tend to have higher levels of these compounds. While chemical fat analyses were not available for these samples, the analyses of USA cuts suggested that generally STR045 and TDR062 had more intramuscular fat than TOP073 and RMP131. **Therefore, lower quantities of volatiles detected from the former muscles may be due to slower flavour release. No**

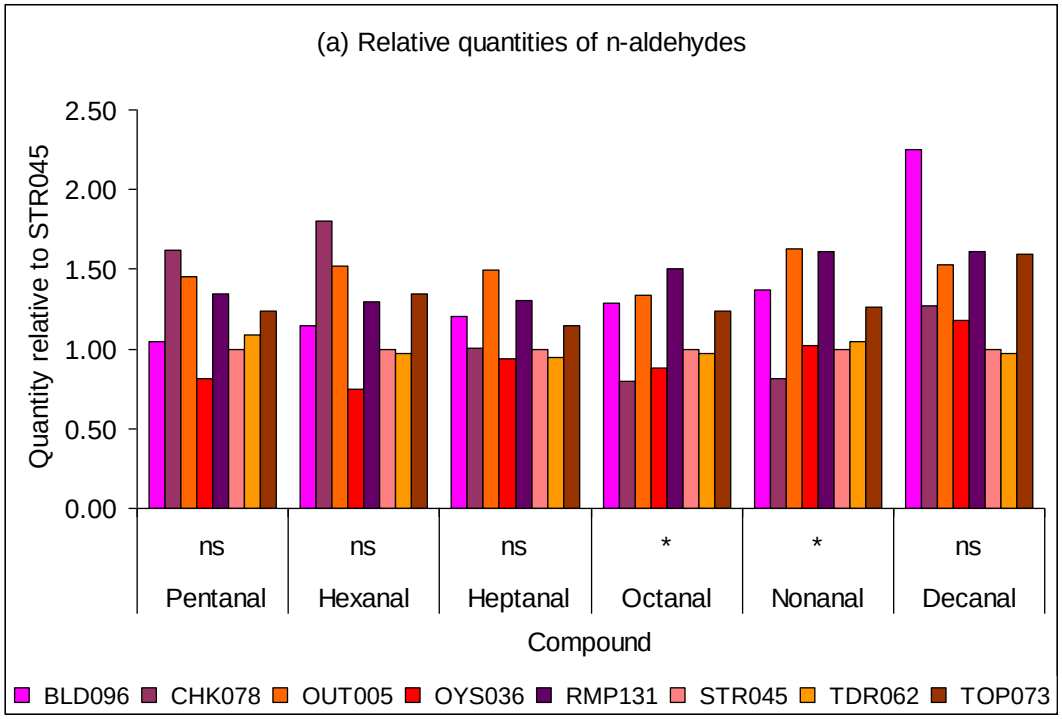
flavour precursor analyses were conducted. The extent to which these differences in volatiles from the various muscles is due to differences in intramuscular fat levels and/or in precursor compounds will require further study.

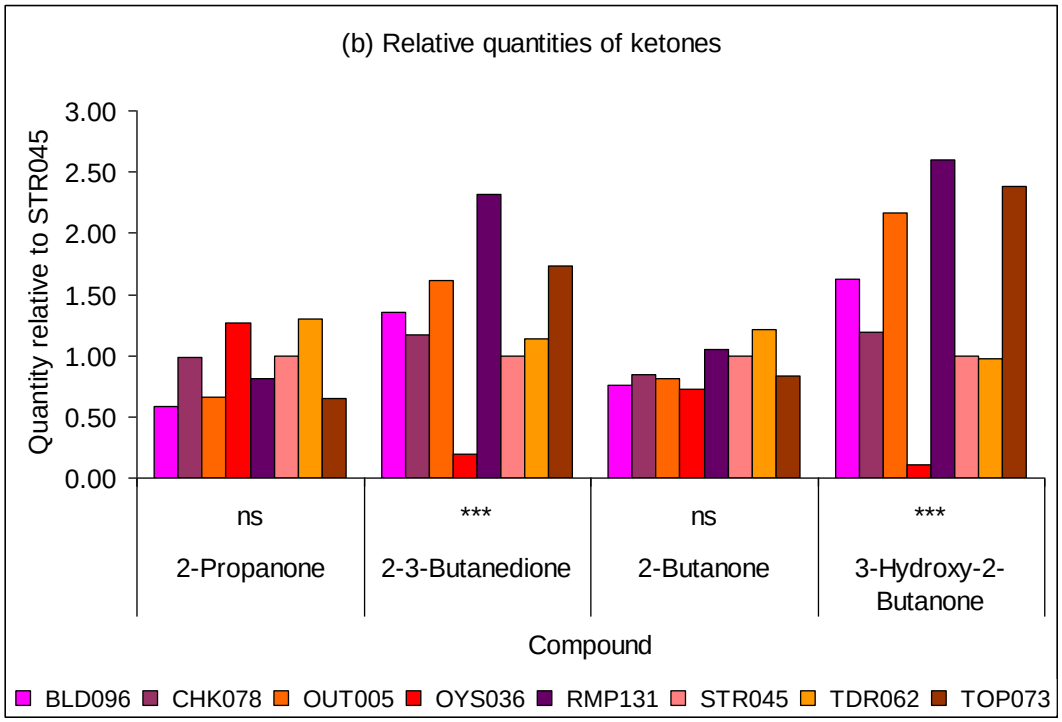
While these differences are consistent and interesting, comparison with the data presented in Section 6.2 suggests that **differences between muscles are least marked at 5 days ageing and may increase at later stages of ageing**.

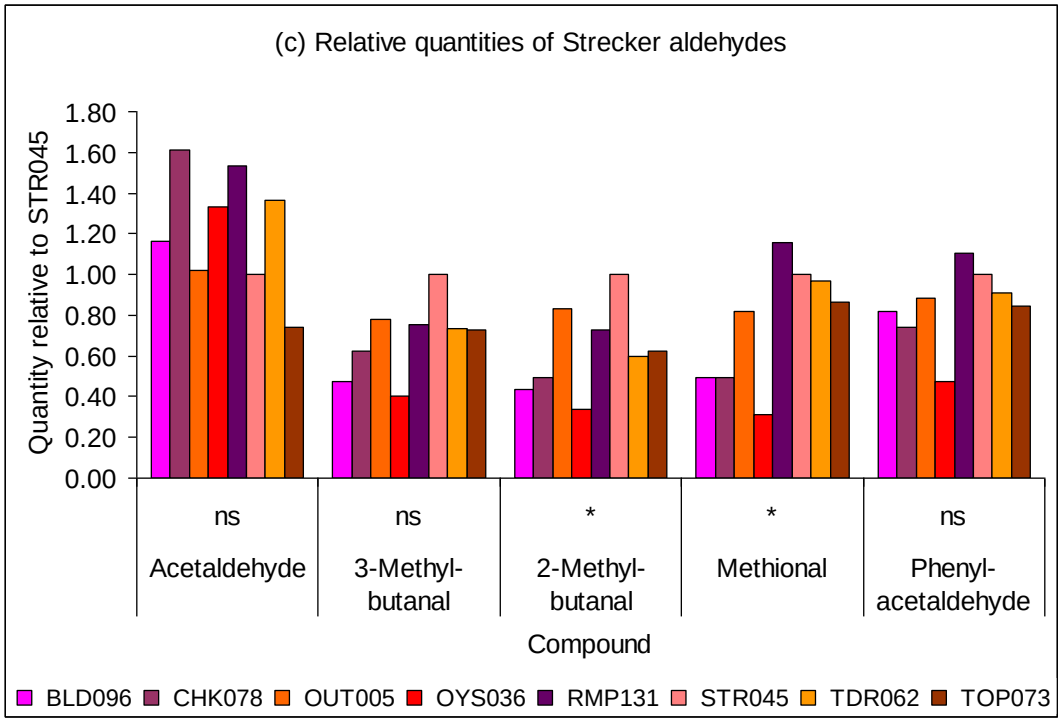
Table 6.3a. Effect of cut at 5 days aged on quantities of volatile compounds

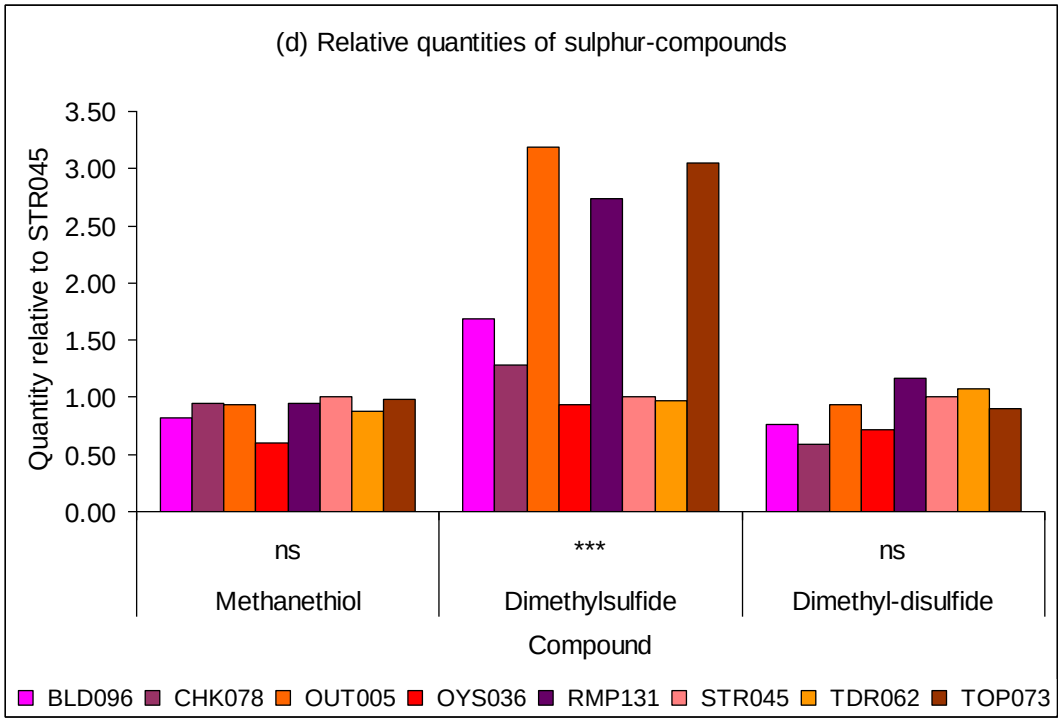
Compound	Sig (Cut)	Compound	Sig (Cut)
n-aldehydes		2-Methylbutanal	*
Pentanal	ns	Methional	*
Hexanal	ns	Phenylacetaldehyde	ns
Heptanal	ns	***S-containing***	
Octanal	*	Methanethiol	ns
Nonanal	*	Dimethyl sulfide	***
Decanal	ns	Dimethyl disulfide	ns
Furans		***pyrazines***	
2-Pentylfuran		Methyl pyrazine	ns
Ketones		2,5/6-Dimethyl pyrazine	ns
2-Propanone	ns	Trimethylpyrazine	not detected
2-Butanone	ns	2-Ethyl-3,5/6-dimethyl pyrazine	ns
2,3-Butanedione	***	***Other***	
3-Hydroxy-2-Butanone	***	Heptane	**
Strecker aldehydes		Octane	*
Acetaldehyde	ns	Benzaldehyde	***
3-Methylbutanal	ns		

Figure 6.3 (a-d). Effect of muscle on the quantities of compound classes







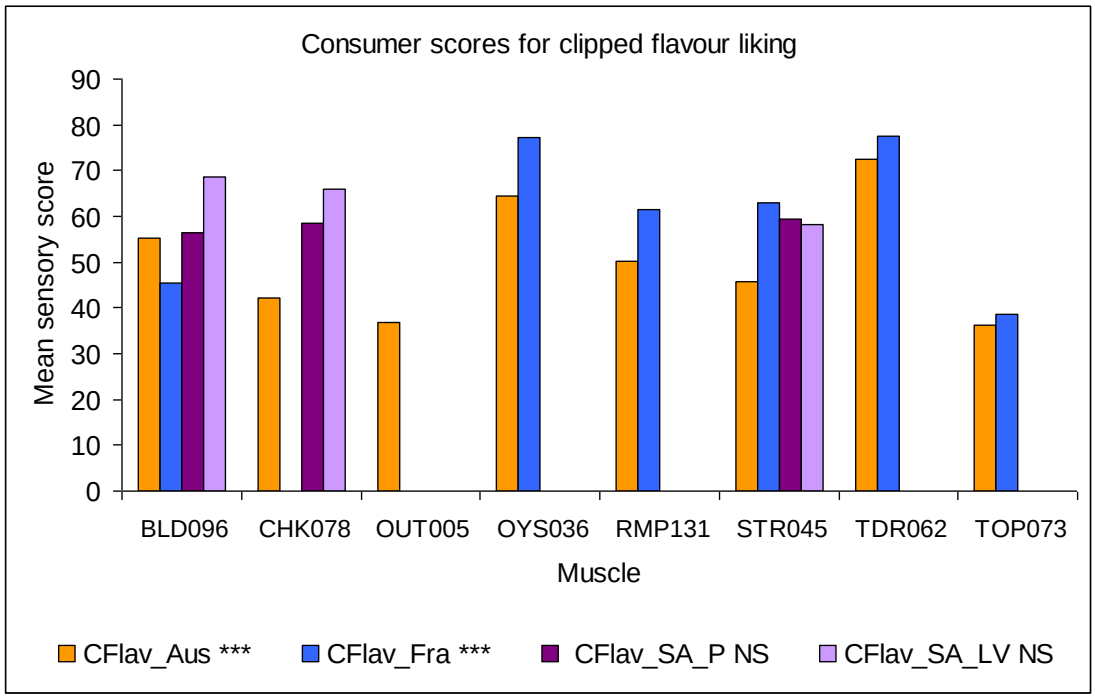


The same eight muscles from these animals were assessed by consumers from Australia, France and South Africa (Pretoria (P) or rural districts (LV)), though only Australian consumers assessed all eight muscles (Figure 6.3e). Although these data are for flavour liking, they follow the same pattern as those for tenderness or MQ4 due to the so-called "halo effect" which means that these scores tend to correlate quite closely. Statistically, these data have been analysed (using Wald statistics) for significant interactions between country and muscle, for six muscles (Australia and France) and for three muscles (Australia

and South Africa, both regions). The data show the expected differences between muscles/cuts (P<0.001) for the French/Australian data, though the three muscles assessed by the Australian and South African assessors did not differ. Neither analysis shows a significant Cut.Country interaction but both showed a significant effect of country, with French and South African consumers scoring at least 9 points higher than Australian consumers for these samples (P<0.001; P<0.01, respectively).

Thus, while consumers from different countries have used the sensory scale differently, there is no evidence that they have different preferences for the flavour of different muscles at 5 days ageing. They like TDR062 and OYS036 the best and TOP073 and OUT005 the least.

Figure 6.3e. Consumer scores from different countries for eight muscles aged for five days



6.4 Grainfed samples: Impact of cut, ageing, fat content, other potential grading inputs and consumer scores on flavour volatiles

The grainfed animals included 33 animals slaughtered in Australia with four muscles aged for 7 and 48 days, and 15 animals slaughtered in USA, aged for 21 or 22 days (Table 6.1). Both groups of carcases were US graded. These data were not analysed together due to the difference in ageing. An analysis of the latter group is presented.

The US grading system grades beef according to its marbling and maturity on the following scale:

- Prime
- Choice

- Select
- Standard

Some of these grades can be further subdivided using “High”, “Low” etc. The grade is allocated to carcases not individual muscles.

6.4.1 Analysis of USA feedlot beef for effect of USGrade and cut on volatiles

Table 6.4a shows the design for the comparison of USGrade and muscle for American feedlot beef aged to 21 or 22 days.

Table 6.4a. Numbers of observations for each treatment

CUT USgrade	RMP131	STR045	TDR062	TOP073
Prime	4	9	4	4
HighChoice	4	8	2	4
LowChoice	4	9	4	4
Select	4	9	4	4
Standard	5	9	4	4

Statistical analysis of the effect of Cut, USGrade and interactions between them (Table 6.4b) show that there are few significant effects of USGrade alone, but some significant effects of USGrade.Cut interaction and of Cut.

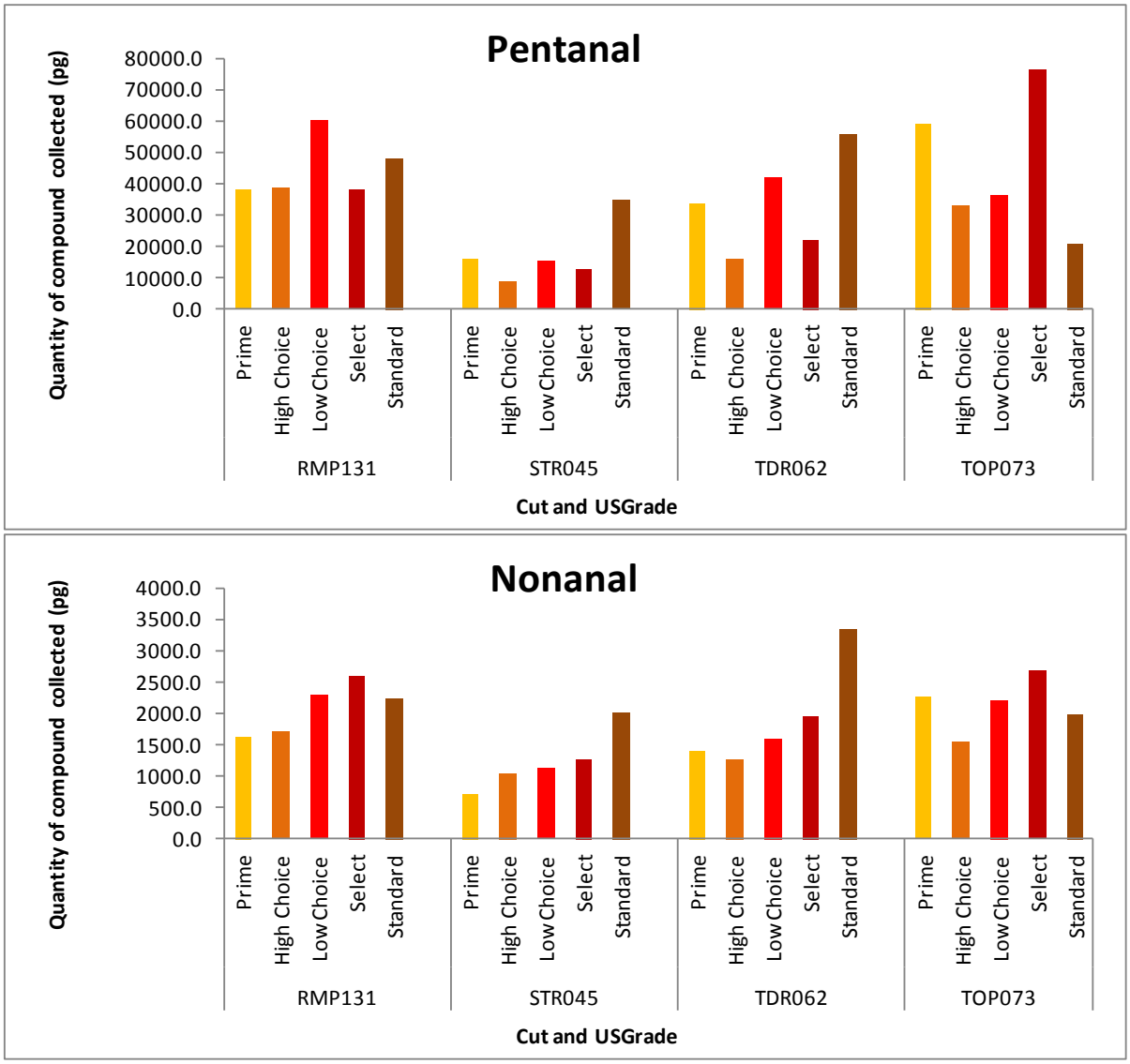
Table 6.4b. Significant effects on volatile compounds

Compound	US-Grade	Cut	Cut. US-Grade
n-aldehydes			
Pentanal	ns	**	ns
Hexanal	ns	**	ns
Heptanal	ns	***	ns
Octanal	ns	***	ns
Nonanal	ns	***	ns
Decanal	ns	ns	*
furans			
2-Pentylfuran	ns	**	ns
ketones			
2-Propanone	ns	***	***
2-Butanone	ns	ns	ns
2,3-Butanedione	ns	*	ns
3-Hydroxy-2-Butanone	ns	***	ns
Strecker aldehydes			
Acetaldehyde	ns	***	***
3-Methylbutanal	ns	ns	ns
2-Methylbutanal	ns	ns	ns
Methional	ns	ns	ns
Phenylacetaldehyde	ns	ns	ns
S-containing			
Methanethiol	ns	ns	ns
Dimethyl sulfide	ns	***	***
Dimethyl disulfide	ns	ns	ns
Pyrazines			
Methyl pyrazine	ns	*	ns
2,5/6-Dimethyl	ns	ns	ns

pyrazine			
Trimethylpyrazine	ns	ns	ns
2-Ethyl-3,5/6-dimethyl pyrazine	*	ns	ns
Other			
Heptane	ns	*	ns
Octane	ns	**	ns
Benzaldehyde	ns	***	***

The n-aldehydes show a significant effect of cut only. All the aldehydes from pentanal to nonanal show a similar pattern and this is illustrated in Figure 6.4a. The striploin gave the lowest quantities of these compounds and this is consistent with the observation for Australian beef in Section 6.2.

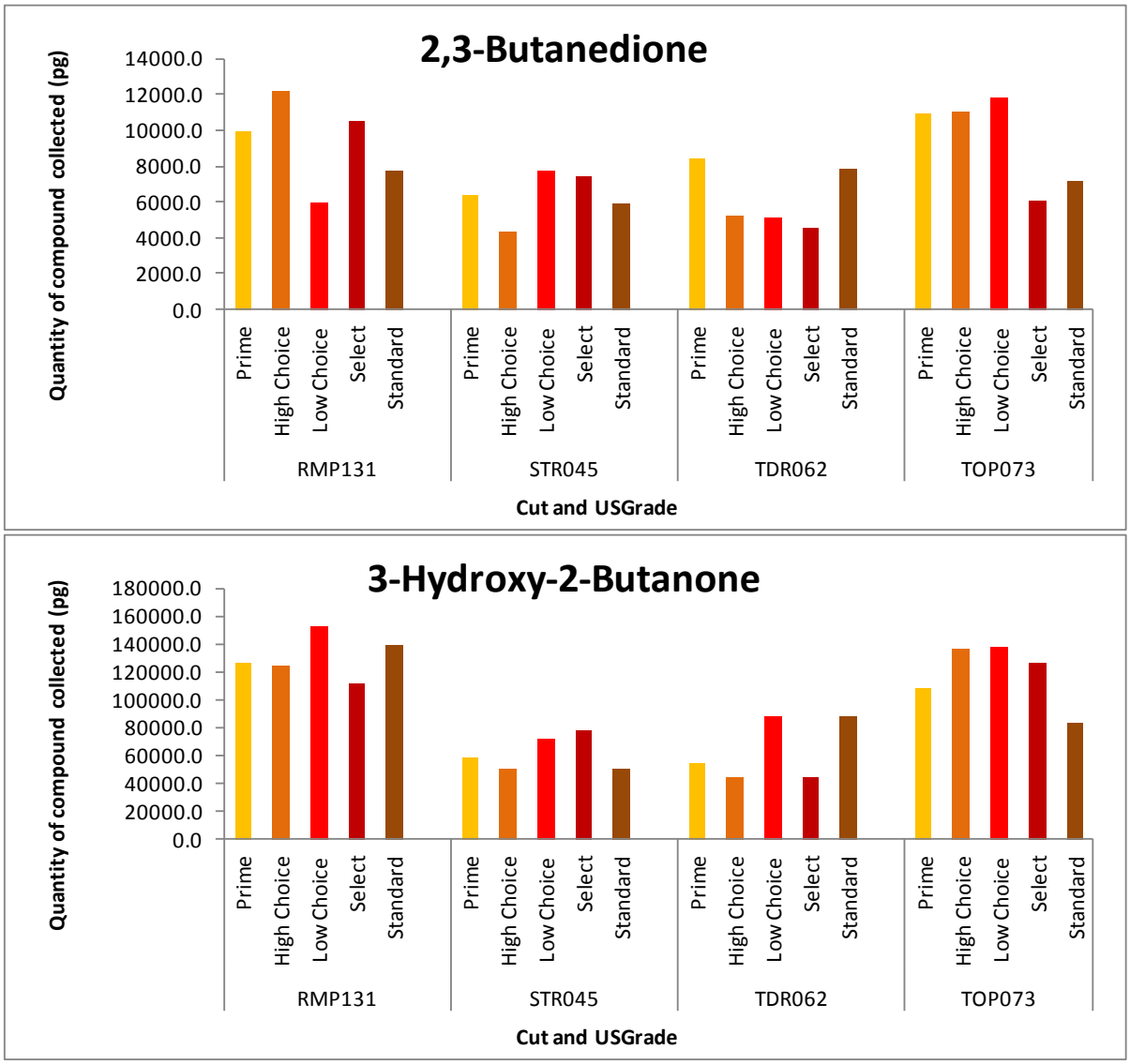
Figure 6.4a. Effect of USGrade and cut on quantities of n-aldehydes



The important precursors, 2,3-butanedione and 3-hydroxy-2-butanone both show significant effects of cut, with less collected from striploin and tenderloin than rump and topside (Figure 6.4b). Again, this effect was also observed for the Australian beef in Section 6.2.

Three compounds showed highly significant interactions, and all gave especially high levels in high choice and select tenderloin (not illustrated). The reason for this is unclear and would require a closer examination of the experimental protocol.

Figure 6.4b. Effect of USGrade and cut on quantities of 2,3-butanedione and 3-hydroxy-2-butanone



Examination of the effect of USGrade x cut x days aged is not advisable due to the small numbers of analyses (1-2) per treatment.

6.4.2 Analysis of USA feedlot beef for effect of USGrade and cut on consumer scores and other parameters

Consumer scores for Flavour Liking are shown in Figure 6.4c. The scores for CMQ4 showed similar trends. These results indicate a significant interaction ($P<0.01$) between USGrade and muscle or cut, i.e. whether USGrade had an effect depended on cut. There was no significant difference of USGrade on flavour liking for TDR062 or TOP073. However, for STR045, Low Choice (but not standard or select) was significantly different to Prime, while for RMP131, Standard grade was significantly different from Select and High Choice (but not Prime). There was no significant main effect of USGrade on flavour liking.

As the samples for analysis were selected to be differentiated on striploin chemical fat, the clear differences in marbling (not shown) and chemical fat (Figure 6.3d) between different USGrades was to be expected. There was a clear ($P<0.001$) difference between muscles in the impact of USGrade on chemical fat as well as significant first order effects of both factors. Increases in fat were accompanied by a reduction of water and protein (not shown).

Thus, cut/muscle had a significant effect on many of the volatile compounds and on consumer scores for flavour liking. However, there were few consistent effects of USGrade on volatile compounds or on flavour liking.

Figure 6.4c. Effect of USGrade and cut on Flavour Liking

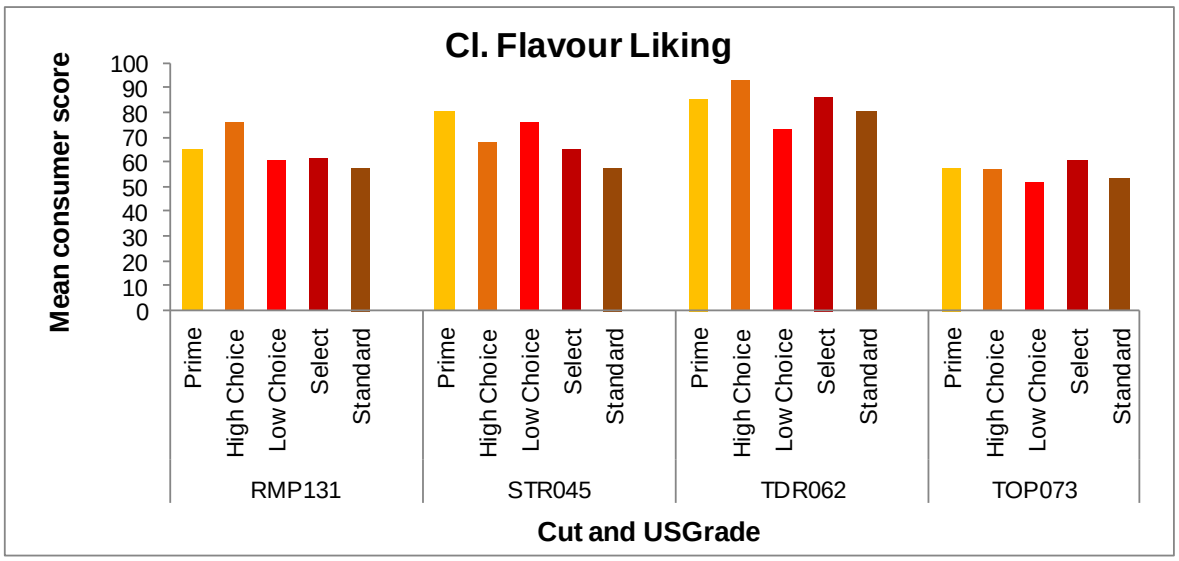
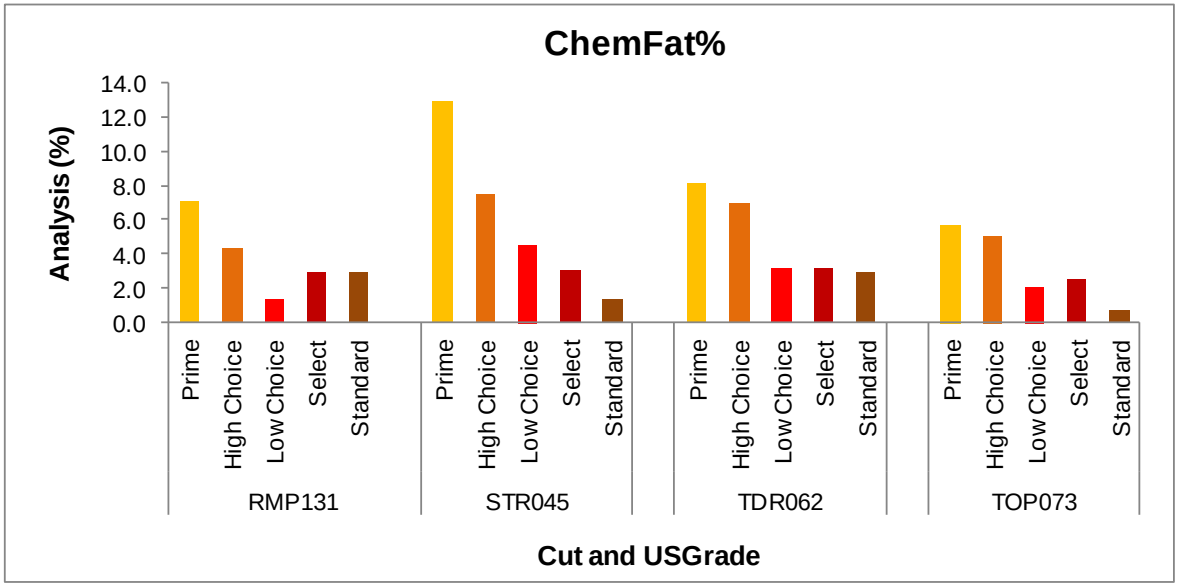


Figure 6.4d. Effect of USGrade and cut on intramuscular fat



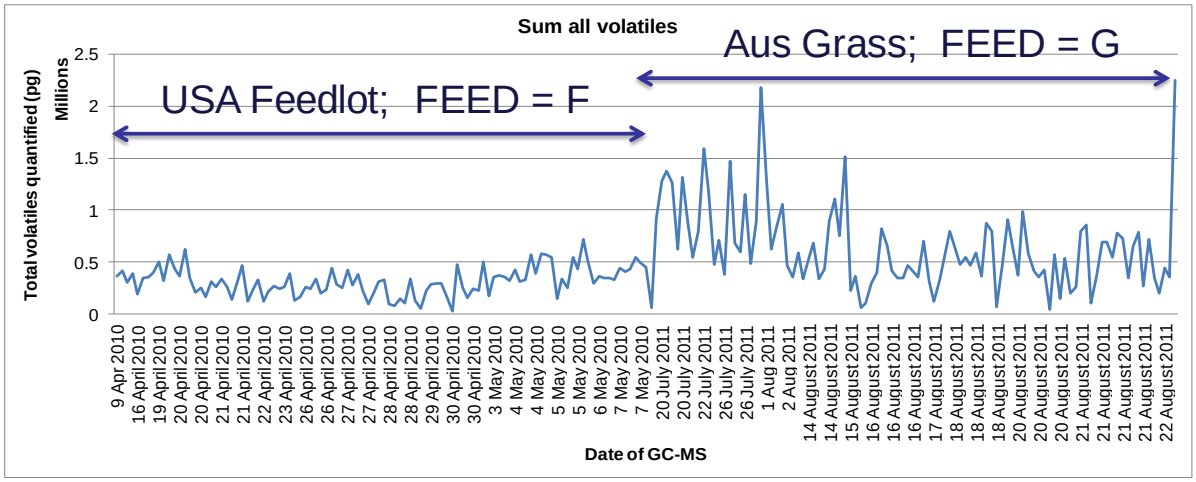
6.5 Impact of feed and cut on flavour volatiles and interactions with other factors

Table 6.5a shows the design for the comparison of feed and muscle using American feedlot and Australian grassfed beef aged to 21 or 22 days. These samples were analysed one year apart, with Australian grassfed beef analysed later, and caution must be exercised in the comparison of these data. Figure 6.5a shows the total volatile compounds collected during the two periods, which shows that the total quantity of volatiles collected in runs from the second period (grass fed beef; feed = G) was greater than for the earlier (feedlot beef; feed = F), but not massively so.

Table 6.5a. Numbers of observations for each treatment

FEED	CUT_AGED	RMP131	STR045	TDR062	TOP073
F	21	21	10	18	20
	22	0	34	0	0
G	21	18	14	17	16
	22	0	0	0	0

Figure 6.5a. Total volatile compounds measured (pg) during collection periods for American feedlot and Australian grassfed beef



Statistical analysis of the effect of Cut, Feed and interactions between them (Table 6.5b) suggest that there are few interactions between Cut and Feed and that the volatile compounds from all cuts are influenced by diet.

Table 6.5b. Significant effects on volatile compounds of feed and cut

	All USGrades included			**Standard and Select only**		
	Feed	**Cut**	**Cut. Feed**	**Feed**	**Cut**	**Cut. Feed**
n-aldehydes						
Pentanal	**	***	ns	*	ns	ns
Hexanal	**	***	ns	*	ns	ns
Heptanal	ns	***	ns	ns	**	ns
Octanal	ns	***	ns	*	**	*
Nonanal	*	***	ns	***	**	ns
Decanal	**	ns	ns	**	ns	ns
Furans						
2-Pentylfuran	***	**	ns	***	ns	ns
Ketones						
2-Propanone	***	***	***	***	***	ns
2-Butanone	ns	ns	ns	ns	*	ns
2,3-Butanedione	***	***	***	**	***	**
3-Hydroxy-2-Butanone	*	***	ns	ns	***	ns
Strecker aldehydes						
Acetaldehyde	ns	***	*	ns	**	*
3-Methylbutanal	***	ns	ns	***	ns	ns
2-Methylbutanal	ns	***	ns	ns	**	ns
Methional	**	ns	ns	ns	ns	ns
Phenylacetaldehyde	***	ns	ns	***	ns	ns
Pyrazines						
Methyl pyrazine	***	*	ns	***	ns	ns
2,5/6-Dimethyl pyrazine	***	ns	ns	***	ns	ns
Trimethylpyrazine	***	ns	ns	***	ns	ns
2-Ethyl-3,5/6-dimethyl pyrazine	***	ns	ns	***	ns	ns

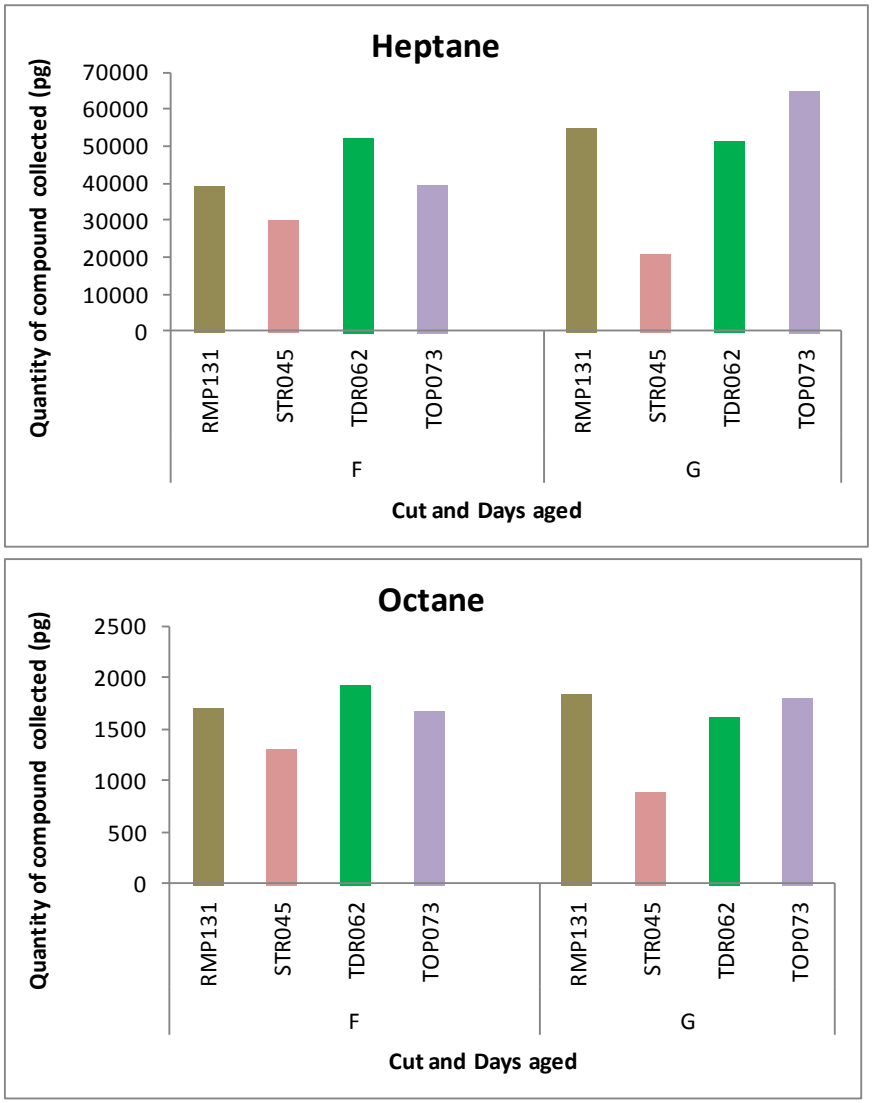
	All USGrades included			Standard and Select only		
	Feed	**Cut**	**Cut. Feed**	**Feed**	**Cut**	**Cut. Feed**
S-containing						
Methanethiol	*	ns	ns	***	ns	ns
Dimethyl sulfide	***	***	***	***	***	***
Dimethyl disulfide	ns	ns	ns	ns	ns	ns
Other volatiles						
Heptane	**	**	*	ns	**	ns
Octane	ns	***	ns	ns	**	*
Benzaldehyde	ns	***	ns	ns	***	ns
Consumer						
Cl_Tender	**	***	**	ns	***	*
Cl_Juicy	***	***	ns	ns	***	ns
Cl_Flavour	***	***	ns	ns	***	*
Cl_Ov Liking	**	***	ns	ns	***	**
CMQ4	**	***	ns	ns	***	ns

Two alkanes have been monitored. These compounds are generally unreactive and might be expected to vary less than many of the more reactive volatile compounds. They have no impact on flavour, but are shown in Figure 6.5b as one indication that there are no large changes due to the two differing experiments conducted on different occasions. In both experiments, striploin had the lowest levels of this compound.

The n-aldehydes showed significant effects of both feed and cut, with high levels in the feedlot beef compared with the grassfed beef (Figure 6.5c). The pattern of differences between the cuts was, nevertheless, the same for both experiments.

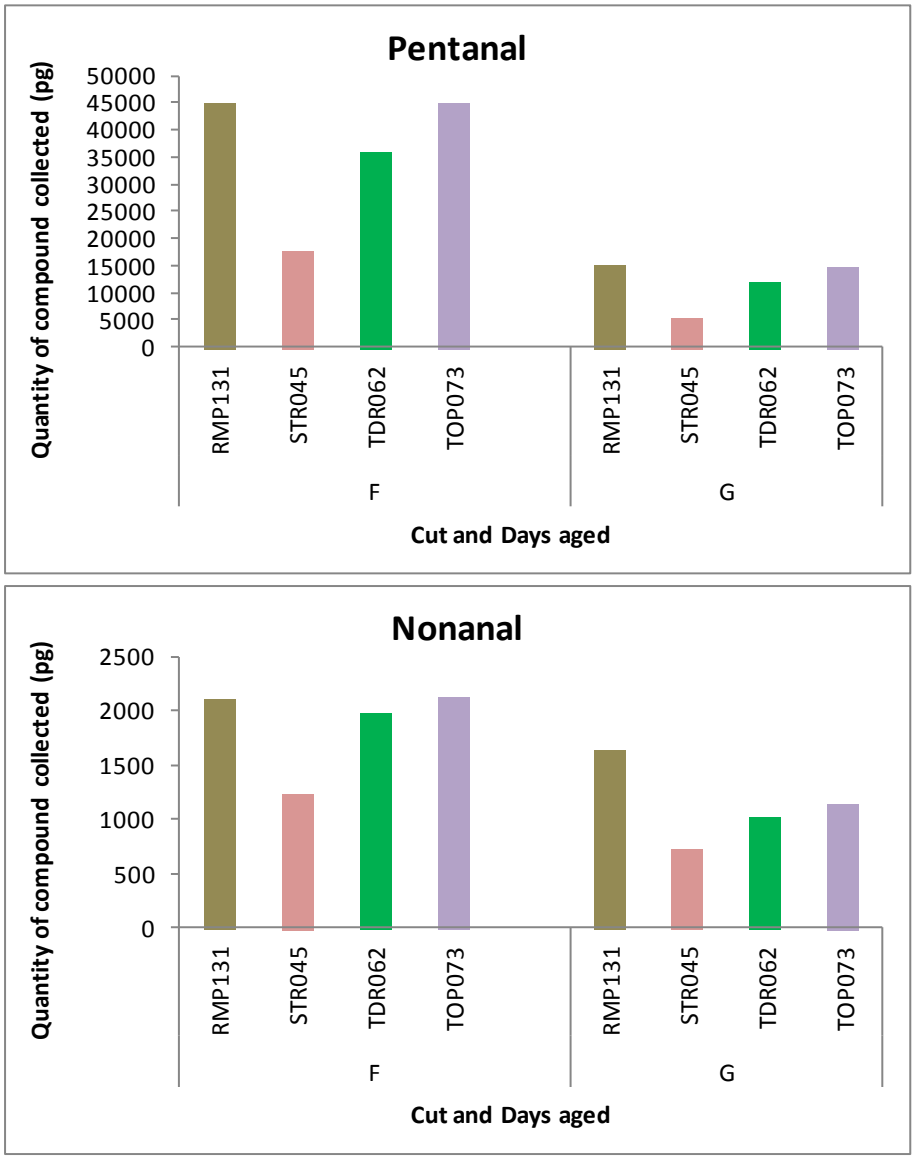
In contrast, some of the compounds formed by the Maillard reaction, e.g. 2,3-butanedione and 3-methylbutanal, showed considerably higher levels in the grassfed beef. Surprisingly, however, some closely related compounds do not show the same effect, Figures 6.5d and 6.5e show 2,3-butanedione and 3-hydroxy-2-butanone, and then 3- and 3-methylbutanal, respectively. **This is a very interesting effect which suggests that some of these reaction pathways are differentially affected by feed.**

Figure 6.5b. Effect of Feed and Cut on quantities of heptane and octane



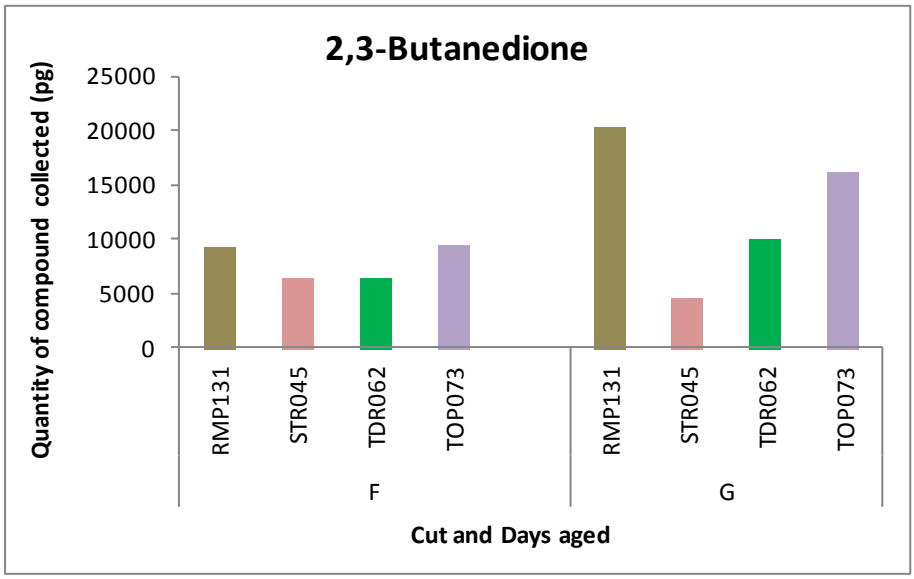
FEED: F = feedlot; G = grass

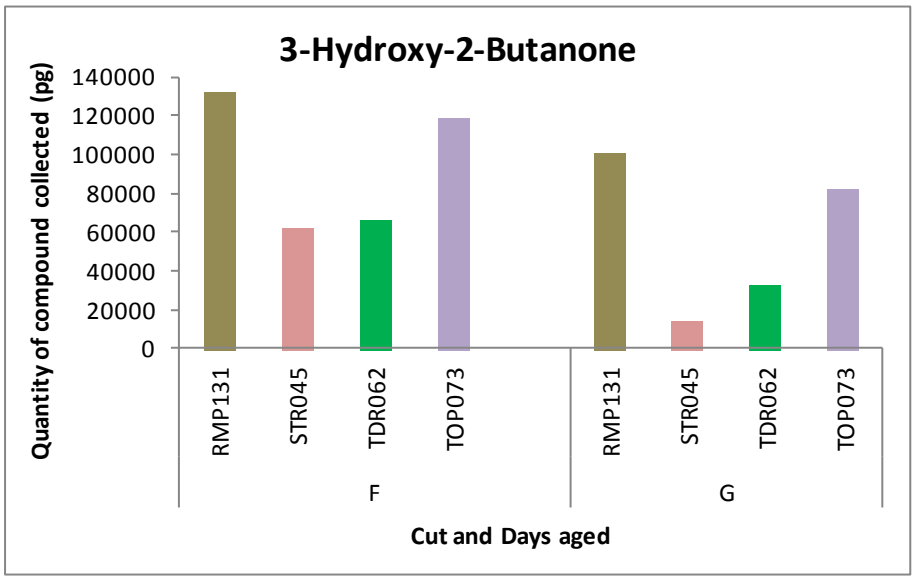
Figure 6.5c. Effect of Feed and Cut on quantities of n-aldehydes



FEED: F = feedlot; G = grass

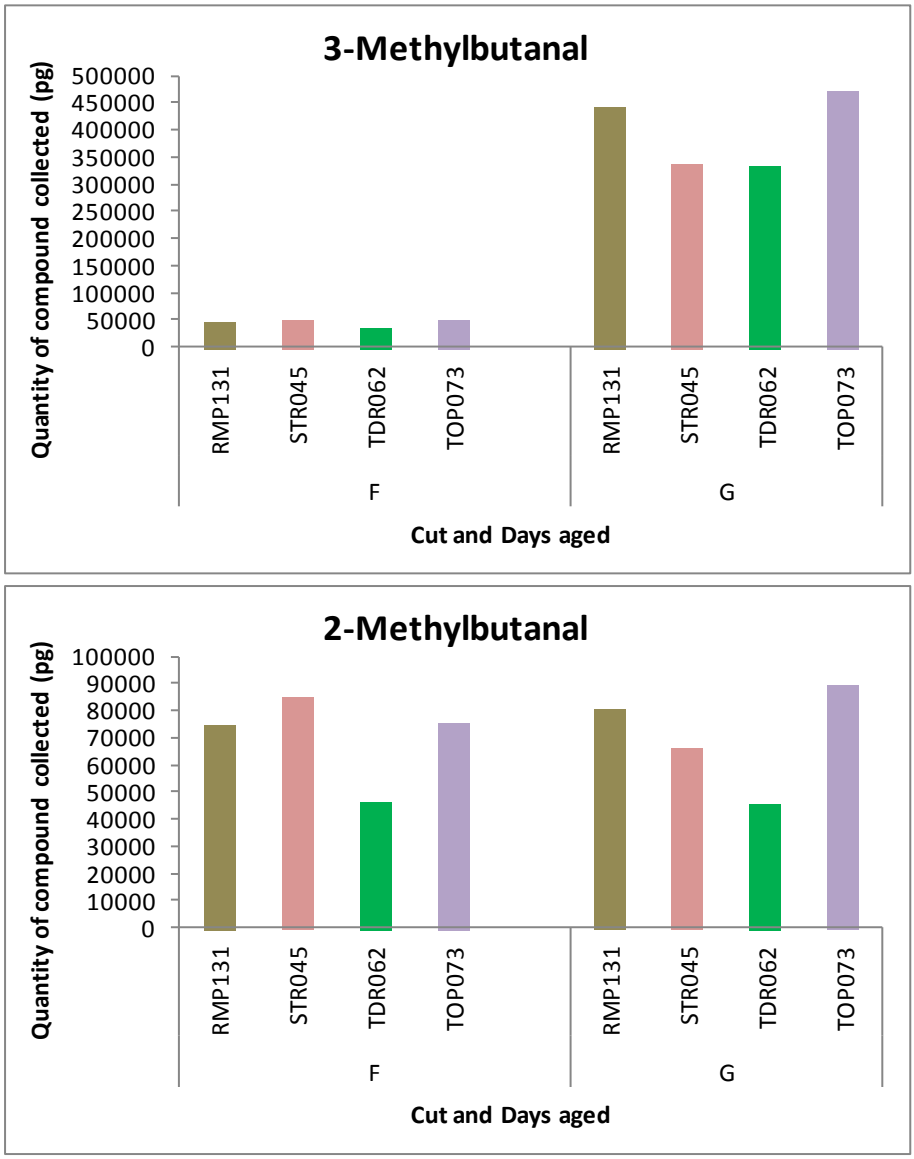
Figure 6.5d. Effect of Feed and Cut on quantities of 2,3-butanedione and 3-hydroxy-2-butanone





FEED: F = feedlot; G = grass

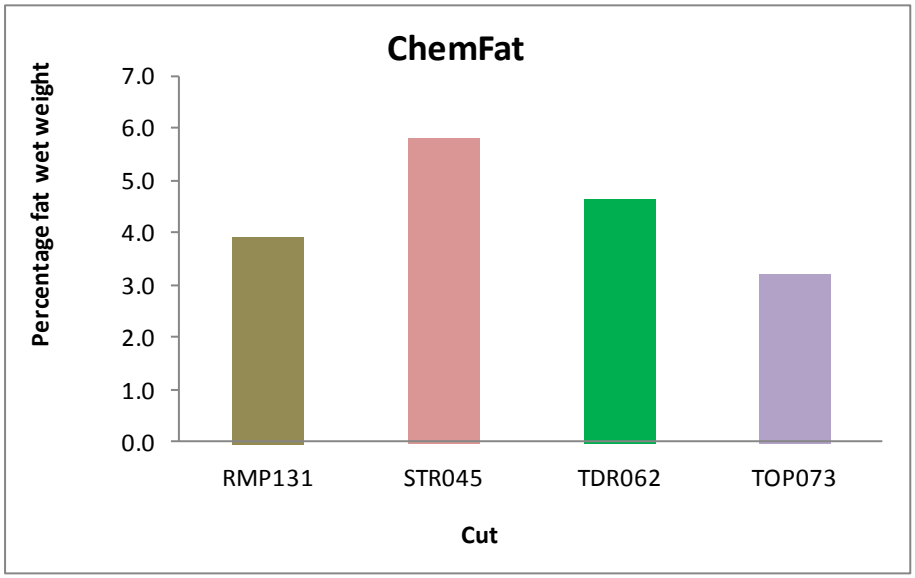
Figure 6.5e. Effect of Feed and Cut on quantities of 3- and 3-methylbutanal



FEED: F = feedlot; G = grass

It is possible that the differences between cuts may be explained, in part, by the fat content of the meat. Intramuscular fat analyses are available for the American feedlot animals only and this is shown in Figure 6.5f. This shows that striploin and tenderloin, on average, have higher fat than the other two muscles. The inhibition of flavour release caused by this fat may be responsible for the lower quantities of odour volatiles observed in these two muscles. Exactly how much fat is required to give optimum flavour release in redmeat is not yet known. It must be borne in mind that lower initial release of volatiles may represent a more balanced and slowly released flavour overall.

Figure 6.5f. Effect of Cut on chemical fat analysis (%)



It is possible that differences between feedlot and grassfed beef may also be explained by fat content. Intramuscular fat analyses are only available for the feedlot beef, but striploin marbling scores are available for all animals. The mean marbling scores (and standard deviations) were, for USA feedlot beef: 489 (238) and for Australian grassfed beef: 302 (53). Thus, it might be expected that more volatiles would be released from the grassfed beef due to the lower fat levels.

The very large standard deviation for the USA beef reflects the wide range of USGrades deliberately selected for this experiment. Selection of only the Standard and Select grades (for USA beef) to compare with the grassfed beef enables the marbling scores to be more closely matched: 264 (77). Table 6.5c shows the numbers of samples falling into these categories

Table 6.5c. Numbers of observations for each treatment

	RMP131	STR045	TDR062	TOP073
F	9	18	8	8
G	18	18	18	18

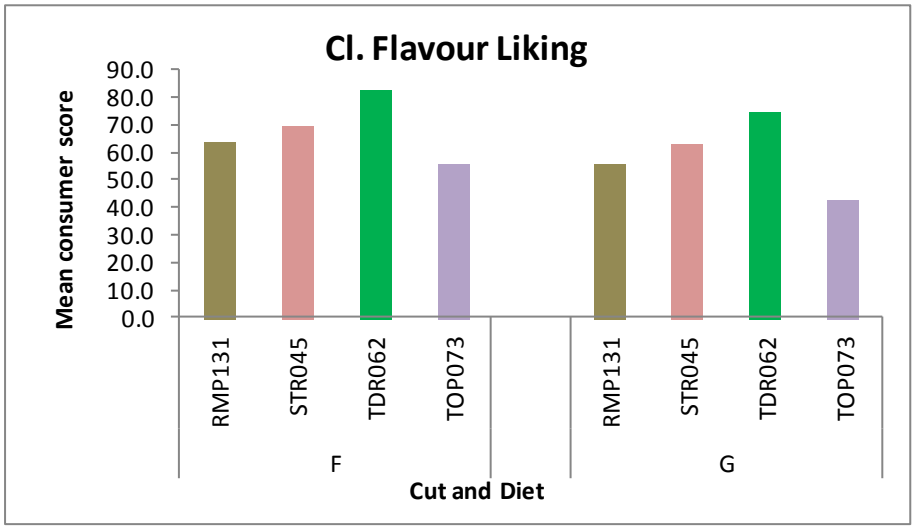
Replotting the graphs in Figures 6.5b to e (not shown), having first matched the feedlot and grassfed treatments for marbling by selecting only standard and select carcases, does not alter the effects illustrated. The total quantities of volatiles measured are increased slightly by omission of the highly marbled USGrades, but only to a small degree. **However, selecting only standard and select carcases does change the significance of the effect of feed, cut and interactions (Table 6.5b). These changes are relatively minor for the effects on quantities of Maillard products (Strecker aldehydes, sulphur compounds and pyrazines) but are substantial for the n-aldehydes fromed by lipid**

oxidation. Thus the effect of feed on the Maillard products is not caused by the differences in marbling but it appears that marbling does have some effect on the quantities of n-aldehydes, but is not the only cause. Some other factor is causing the change in balance of odour compounds. This may be the balance of fatty acids, the presence or absence of antioxidants (which will affect volatile formation from fatty acids) or the levels of other precursors.

Analysis of the consumer scores for the same samples as were assessed for volatiles must be treated with caution as the nationalities of the assessors differed between treatments. As expected, the scores for cuts differed but followed the same pattern (Figure 6.5g). **However, of most interest is the fact that removing the impact of marbling by comparing grass fed beef with select and standard feedlot beef entirely removed the significant difference due to diet** (Table 6.5b).

All the findings in this section should be confirmed by a balanced experiment using the same consumers and conducting the analyses at the same time and/or using an effective external standard.

Figure 6.5g. Effect of Cut and diet on scores for clipped flavour liking



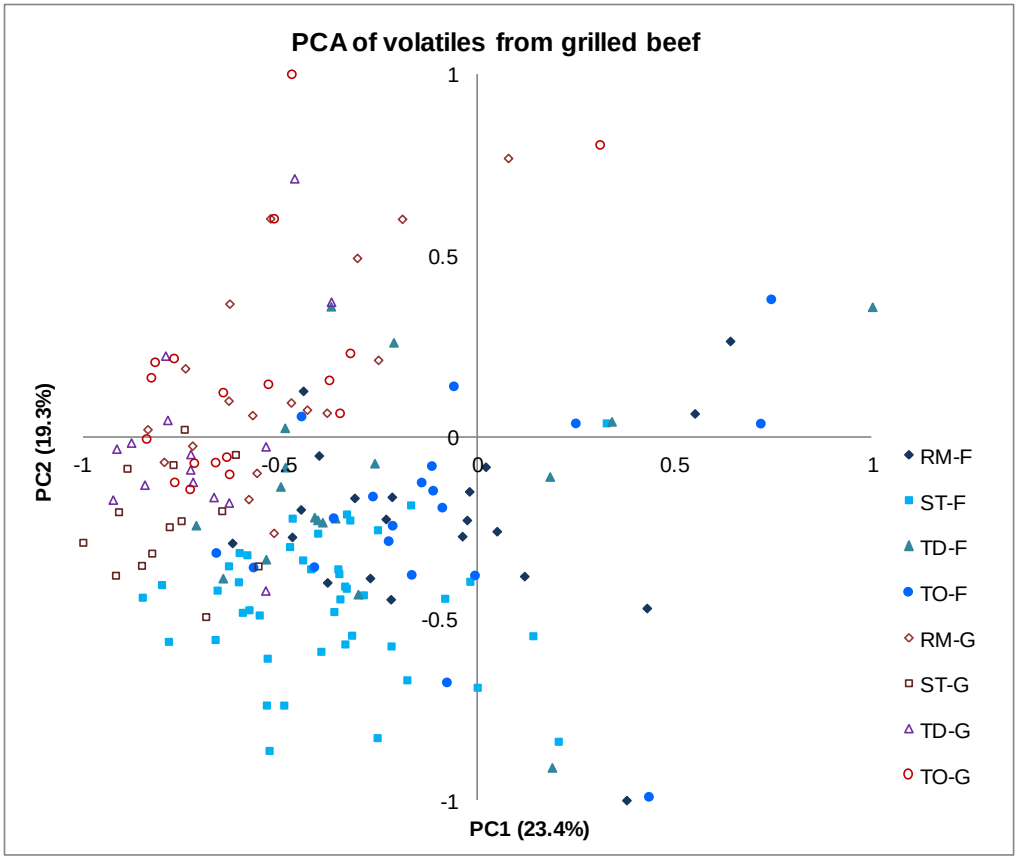
6.6 Principal components analysis showing the relationship between volatile compounds, treatments and other measurements for grassfed and feedlot beef

Principal component analysis (PCA) was conducted on the volatile compounds detected in the samples described in section 6.4a and principal components identified to best discriminate between the individual samples. The resulting plot (Figure 6.6a) shows that the volatile compounds clearly discriminate between grassfed and feedlot beef (again with the caveat that these analyses were conducted on different occasions). The feedlot beef is mainly to the bottom right of the plot, while the grassfed beef is to the top left. In addition there is some separation between muscles with the striploin tending to be located to the

bottom left of the plot. Figure 6.6b shows both the loadings of volatile compounds used to create the principal components, and the correlations with other measurements. Comparison of the two plots indicates that the separation of feed is influenced strongly by the n-aldehydes, while the separation of muscles is related to the overall quantities of many of the volatiles, the highest levels being towards the top right of the plot.

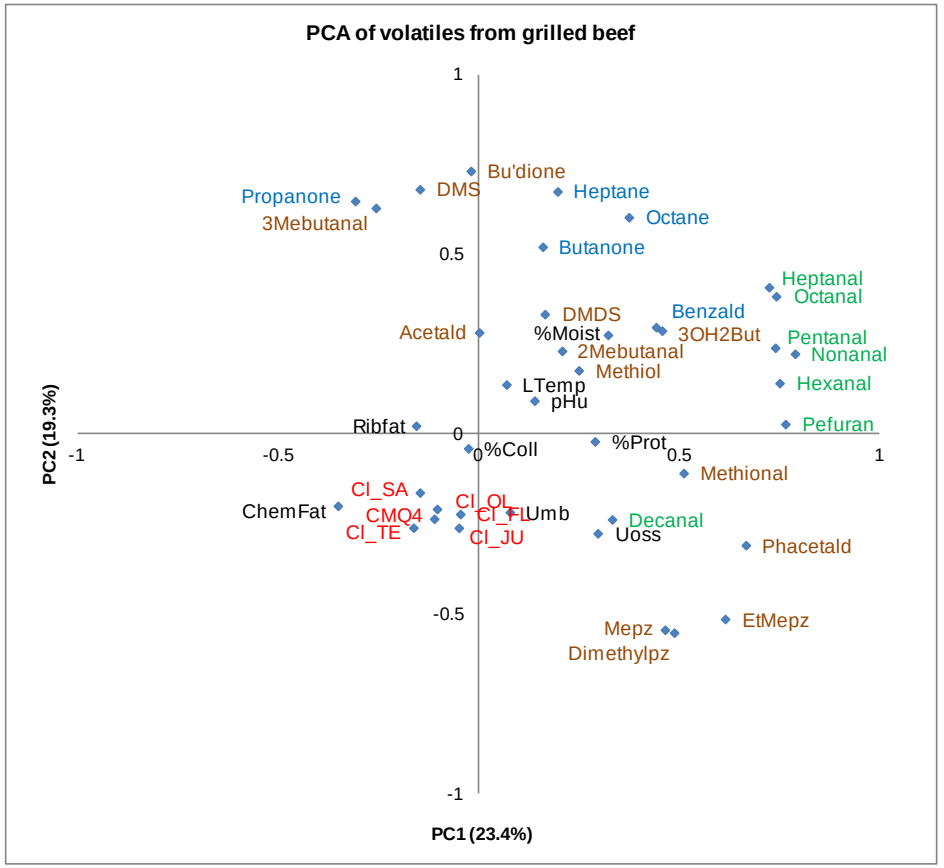
The location of the higher levels of volatiles at a location distant from “chemfat” may illustrate an effect of higher lipid levels in slowing down flavour release by acting as a solvent for the flavour volatiles, but it should be noted that intramuscular fat was only conducted for the USA samples.

Figure 6.6a. Principal Component Analysis of volatiles showing 168 analyses of feedlot and grassfed beef of four cuts



Cuts: RM, ST, TD, TO = RMP131, STR045, TDR062, TOP073; Feed: F = feedlot (blue closed shapes); G = grassfed (purple/red open shapes)

Figure 6.6b. Principal Component Analysis of volatiles showing loadings of volatile compounds and correlations with consumer scores and carcase and meat measurements



Volatile compounds: lipid oxidation products in green, Maillard products in brown and compounds of uncertain source in blue; correlated factors: average consumer scores in red; meat and carcase measurements in black

6.7 Relationships between flavour chemistry, muscle, days aged, intramuscular fat and other potential grading inputs, diet and to consumer sensory scores.

Principal component analysis was conducted on volatile compounds from all samples (421 runs, excluding those recorded during June 2011, as mentioned previously), with the aim to evaluate the relationship between volatiles and consumer scores.

PC1 is heavily weighted towards the left hand side, with only four runs on the right hand side (Figure 6.7a). These four runs do not have extremely high or low quantities of volatiles overall, but they do have especially high levels of pyrazines and/or S-containing compounds. These collections were from two animals but other collections from the same meat were not so extreme. This suggests that the main source of variation is related to the ability of meat from specific animals to produce high levels of certain volatiles combined with perhaps elevated cooking temperature.

Figure 6.7a. PCA of volatile compounds from all runs (PC1 vs PC2)

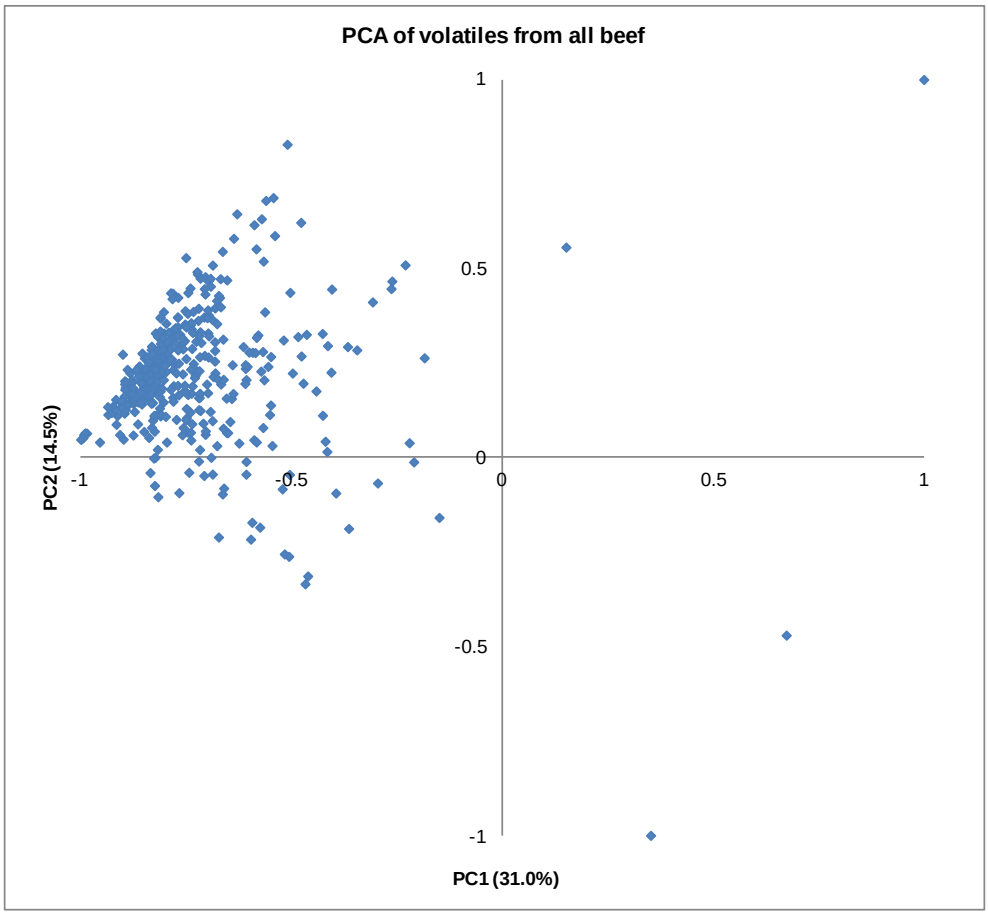


Figure 6.7b. PCA of volatile compounds from all runs (PC2 vs PC3)

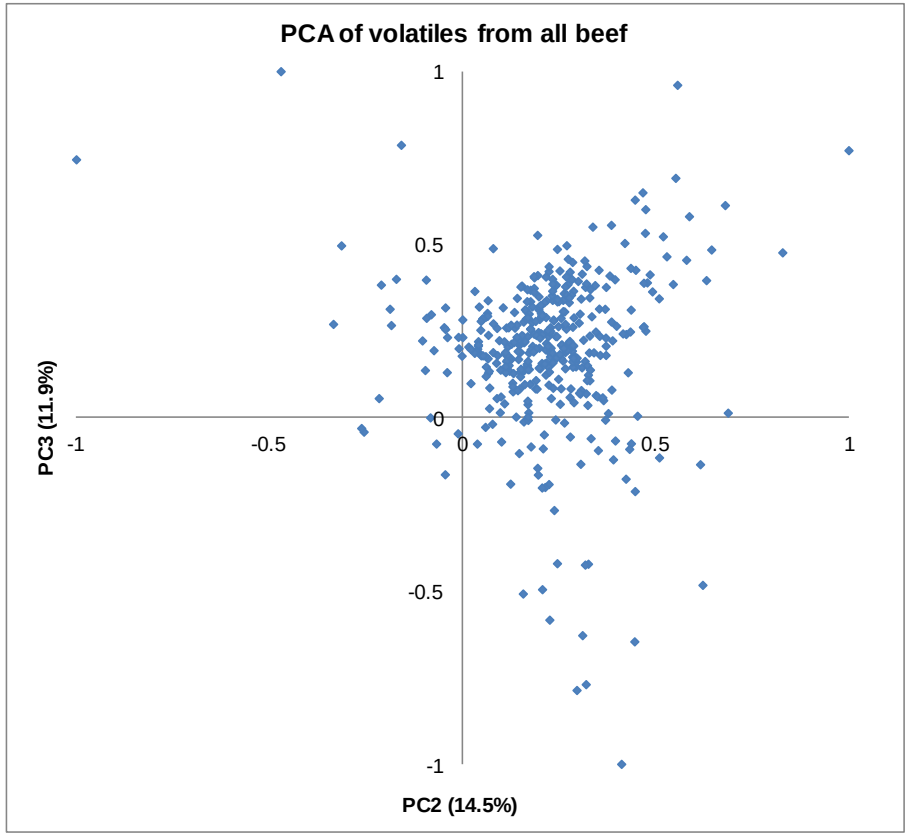
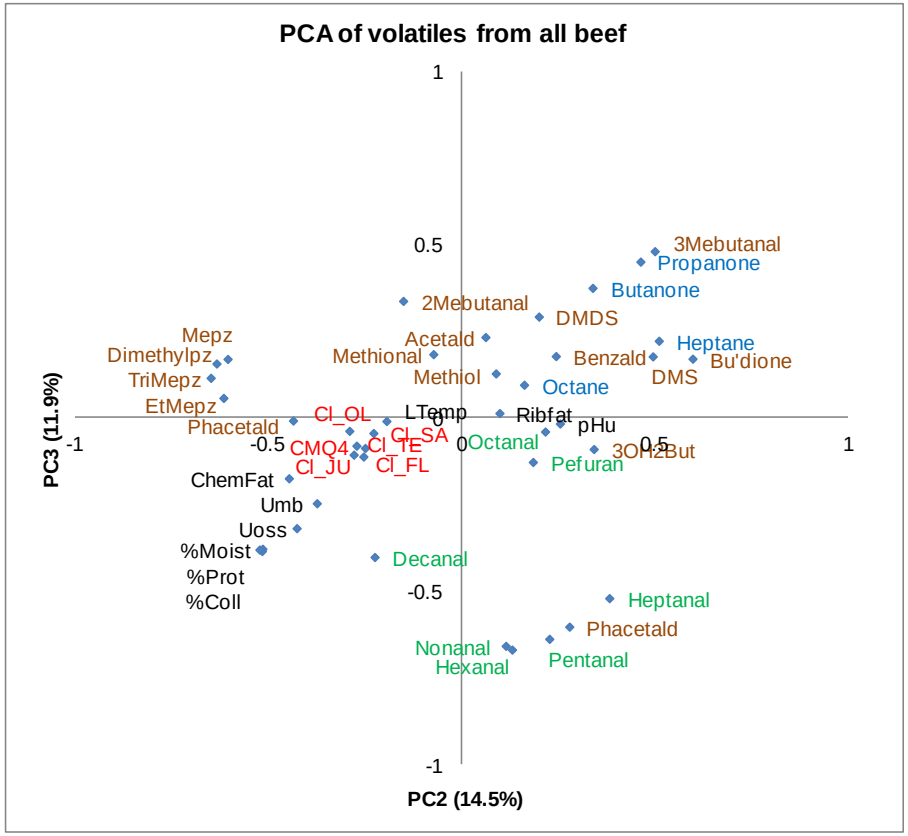


Figure 6.7c. PCA of volatile compounds from all runs (PC2 vs PC3) indicating loadings of volatiles and correlations with other measurements



A plot of PC2 versus PC3 shows a more expected pattern, although it only explains a total of 27% of the variation (Figures 6.7b, c). **Figure 6.7c indicates that PC2 and PC3 are clearly separating the analyses on the basis of compound class, as reported previously (Farmer et al. 2012, 2013).** PC2 separates analyses high in pyrazines from those high in 3-methylbutanal and 2,3-butanedione, while PC3 separates those high in Maillard products (brown) from those high in lipid oxidation products (green).

Correlating other measurements on to these axes indicates that chemical fat and marbling are associated, which is expected, and that that these are located opposite many of the volatile compounds. As mentioned previously, this is expected due to the role of lipid in slowing flavour release. The apparent relationship between higher fat levels and higher pyrazines justifies further investigation. Pyrazines give roasted, toasted flavours and are formed at high cooking temperatures. It may be hypothesised that the higher fat samples drip more fat than water onto the cooking surface, resulting in a higher cooking temperature and more pyrazines.

The consumer scores are all associated together, slightly to the left of the plot, indicating weak association with the samples high in pyrazines. It is unsurprising that only a weak association is seen here as the scores represent only an average liking and for flavour, different subsets of individuals will have their own preferences. **This could indicate another mechanism by which higher fat levels influence acceptability, by modifying the surface cooking temperature. This would require further investigation.**

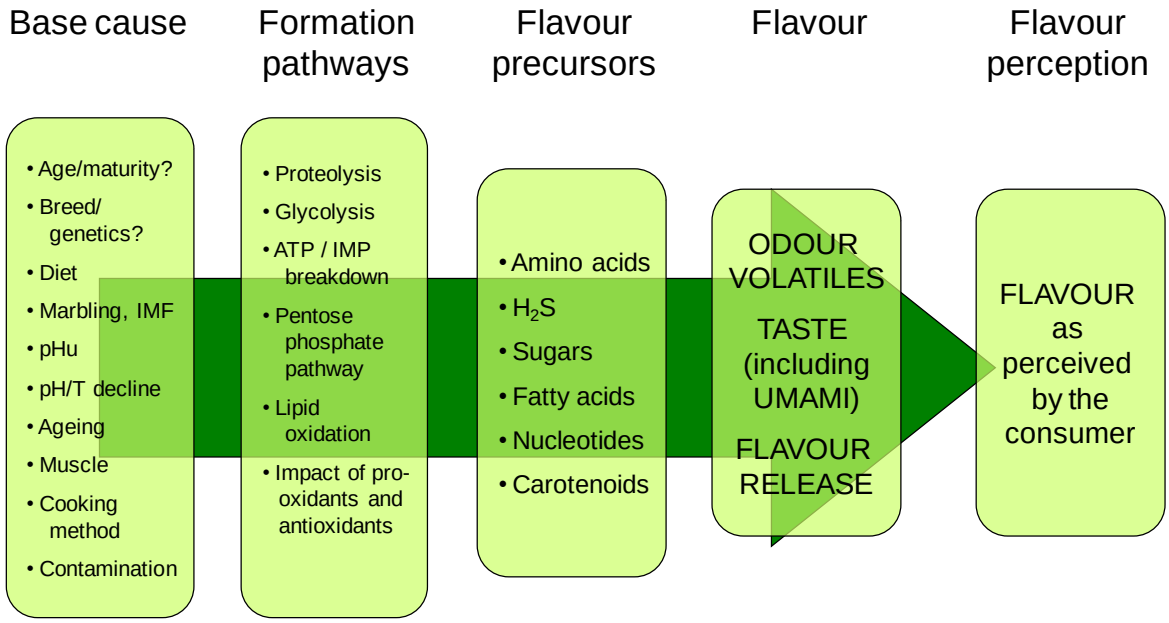
7 Application to MSA

7.1 A rational basis for beef flavour

Changes in consumer liking are often accompanied by changes in the balance of flavour volatiles. In addition, flavour volatiles from related formation pathways tend to respond similarly to treatments, giving the possibility of compounds or groups of compounds acting as "marker compounds" for flavour liking or for specific attributes. While all these relationships are not yet understood, a rational explanation can be proposed for how meat production and processing can influence flavour formation and release and thereby flavour as perceived by the consumer (Figure 7.1).

The findings presented in this report demonstrate that both flavour volatiles and consumer scores are influenced by factors such as muscle/cut, days aged and diet. Section 7.2 proposes how MSA might be developed to include the prediction of flavour and Section 7.3 summarises these findings and how they may be applied to the development of MSA.

Figure 7.1. Relationship between meat production and processing parameters and flavour as perceived by the consumer



7.2 How might flavour be predicted in MSA

The overall aim is to improve the delivery and consistency of good eating quality beef through:

- improved prediction of eating quality, i.e. MSA grade

- improved management of eating quality, ensuring flavour is appropriate for the expected customer

Three options have been considered for using flavour information to enhance the predictive power and usefulness of MSA.

i. Monitor flavour compounds on-line

Modern analytical techniques provide a number of options to monitor volatile compounds in real time. These include versions of mass spectrometry that omit the time consuming separation by chromatography and measure all the volatiles at once by mass spectral techniques such as atmospheric pressure mass spectrometry (APCI), PTR or SIFT-MS. In addition, the relevance of marker compounds makes an electronic nose a possibility for volatile compounds. The use of such techniques for real-time measurement on-line in a meat plant is attractive. However, there are a number of difficulties:

- Quick MS techniques are often insensitive and/or imprecise. Because they are unable to separate the compounds and as many share the same fragment ions, it is impossible to state with certainty the compounds represented by the ions detected by APCI, PTR or SIFT-MS. In addition, they are usually unable to detect to sufficiently low concentrations.
- Conventional GC-MS, whether with SPME or dynamic headspace analysis, with run times of 30-60 minutes, is too slow for on-line analysis.
- The electronic nose may be effective but models are sensor dependent.
- There would be challenges in devising sufficiently robust models of these analytical tools for a meat plant environment.
- **Volatile compound detection needs to be performed on <u>cooked</u> meat**
- Most of these techniques would be too expensive for routine use

For these reasons, it is recommended that analytical tools be used to understand the impact of treatments on flavour rather than as a monitoring tool.

Developments in analytical capability are moving fast and it is possible that cost-effective real time meat plant analysis may become possible in the future.

ii. Modify MSA score for beef flavour

The current MSA system predicts tenderness very well. However, it is less effective at predicting flavour liking. One method for improving the flavour prediction would be to use grading information to predict tenderness, juiciness and flavour liking separately. These predictions could then be combined to give the overall MQ4.

Thus, instead of predicting MQ4 using an equation such as:

*MQ4 = constant + fn AGEING + fn pHu + fn HANG + fn HANG*AGE +*
…… etc etc

Tenderness (TE), juiciness (JU), flavour liking (FL) and overall liking (OL) would be predicted separately using the same type of equation:

*TE/JU/FL/OL = constant + fn AGEING + fn pHu + fn HANG + fn HANG*AGE +*
…… etc etc

These could then be combined using the same equation as already developed:

MQ4 = 0.3 TE + 0.1 JU + 0.3 FL + 0.3 OL

The advantages of this method would be that it uses and builds on the prediction system already used by MSA.

The disadvantage is that this system does not differentiate between different flavour characteristics exhibited by different production systems or muscles and, therefore, does not meet the needs of groups of consumers with specific likes and dislikes.

iii. Qualify MSA score with flavour descriptors

In order to capitalise on the diversity of flavours from beef and the specific likes and dislikes of subsectors of the beef-eating population, a system is required that highlights these different characteristics where they occur. Beef with specific characteristic flavours would be classified according to its MSA grade and its specific flavour note.

The advantages of this approach are that it builds on current MSA grade and would provide increased clarity to consumers: beef with differences in flavour, but similar MSA scores, are not marketed as the same product.

This would require research to identify these flavour characteristics and where they occur. Two methods could be used:

a. Combination of consumers and trained panels

i. to enable the likes and dislikes of consumers to be expressed in terms of the descriptive language used by trained assessors

b. Link flavour compounds to consumer and profiling data

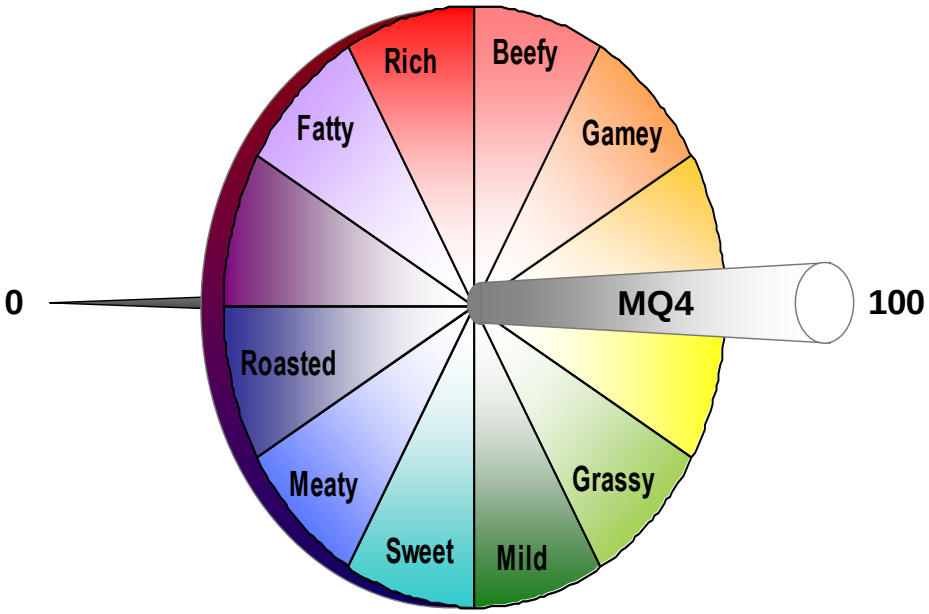
i. To enable the causes of flavour differences to be better understood and allow the prediction of the flavour impact of factors not yet tested

ii. Where possible, other compounds also influencing flavour, such as flavour precursors (from which volatile odour compounds are formed during cooking) and taste compounds should be monitored also.

Some data is already available from the research funded at TTU and work conducted elsewhere. This could be supplemented by research on the specific flavour notes derived from different muscles, ageing and cooking method.

The flavour qualification approach could also be combined with the flavour modification option described in ii.

Figure 7.2. MSA system with flavour characteristics



7.3 Application of findings to MSA

A comprehensive knowledge of beef flavour may be utilised by MSA in four areas:

i. At farm and feedlot level where diet, management and genetics might be manipulated to achieve an enhanced or desired flavour outcome or to avoid a detrimental impact.
ii. In the MSA model algorithm as discussed in 7.2. An encouraging indicator is that aside from diet most identified flavour base causes (see Fig.7.1) are current grading inputs and therefore available to use in modified flavour prediction.
iii. Industry management and recommendation of individual cut ageing to optimise eating quality.
iv. Improved consumer and food service industry recommendations regarding selection and control of cooking techniques and temperatures for alternative muscles.

Table 7.3 lists the main findings detailed in this report and their potential application to the further development of MSA.

Table 7.3 Main findings and their potential application to MSA

	Main finding	Application to MSA
1	**Quality assurance** procedures are now working well. The requirement for extensive training for flavour analysts has been demonstrated. There is a clear effect of date of analysis on quantitative results.	The protocol for analysis and quantification of volatiles should be revised to emphasise the need for the analyst to (a) run a consistent external standard on all days of analysis, (b) balance the use of fibres across treatments and days of analysis and (c) include specific validation and quantification procedures.
2	The data reported here confirms previous findings that **compound groups** from similar reaction pathways tend to "behave" similarly. This indicates that certain compounds may be markers of flavour liking or specific flavour characteristics.	This confirms that an understanding of flavour volatiles may help to understand flavour liking and may assist in the prediction of characteristic flavour notes. This raises the possibility that, in the future, certain compounds may act as markers for detection in cooked meat by e.g. an electronic nose or that precursors may be found to predict flavour.
3	While **intramuscular fat** (IMF) is not available for all samples, there appears to be a clear effect of IMF on release of volatiles. As expected from previous work, more IMF slows down flavour release. There is evidence that reducing differences in IMF (through equalising marbling scores) between grassfed and feedlot beef removes differences in consumer flavour liking scores. This data used different consumers and samples.	IMF appears to be an important driver for flavour perception, and it is probable that a certain level of IMF is needed to achieve the most desirable flavour release. Knowledge of the optimum level of IMF for flavour release and the impact of different levels on flavour liking will be needed for prediction of flavour liking. Information on the exact level of IMF needed for flavour liking may be available from existing MSA data, though a mechanism to disassociate from tenderness is needed. Alternatively, an experiment could be devised to identify the plateau point beyond which IMF has no further effect

	Main finding	Application to MSA
		on flavour volatiles released.
4	**Muscle/cut** has a clear effect on the quantities of volatile compounds. It appears that this can in part be explained by differences in IMF affecting the release of flavour. There is no evidence of differences between consumers from different countries in their liking for flavour from the different cuts.	Differences in consumer liking between different muscles is likely to be influenced by flavour differences as well as tenderness. While some of this difference is explained through variations in IMF, the relative importance of IMF and flavour precursors such as fatty acids, sugars, amino acids etc is unknown.
5	**USGrade** has no consistent effect on flavour volatiles or consumer scores	
6	**Days aged** has significant effects on the formation of odour volatiles, especially those derived from the Maillard reaction. The formation of these compounds is strongly associated with flavour liking. Effects on consumer scores are less clear, probably because the formation of more volatiles does not always lead to more liking.	Days aged is likely to show a curvilinear impact on flavour liking with an optimum level for most consumers. Further information on this may be available from existing MSA data. An experiment with controlled ageing times would enable the links to volatile compounds to be established.
7	**Diet** of the animal appears to affect volatiles with grass fed beef having more of certain Maillard products and feedlot-beef having more n-aldehydes from lipid oxidation. Differences in average consumer liking scores appear to be due to IMF differences. These results were confounded by consumer country and date of analysis.	The analysis of volatiles offers a potential explanation of the qualitative flavour differences reported by consumers from different countries. A controlled experiment with consumers from different countries eating beef from both diets is required.
8	Data on **consumer country** from this experiment was available for some of the treatments analysed for flavour	These data confirm the findings obtained previously for the MQ4 score, that consumers from different countries rank

	Main finding	Application to MSA
	volatiles. The limited evidence available suggests that there were differences in the use of scale, but no significant differences between countries in scores for days aged or muscle/cut.	beef from the main treatments in a consistent manner. However, specific information on liking of different diets needs further controlled experimentation (see 5. above).
9	Impact of **surface cooking temperature**. There is some circumstantial evidence that this may have an effect on volatiles and consumer flavour liking. Further work would be required.	

7.4 Recommendations for further work

The conduct of consumer trials and analyses for compounds important for flavour are expensive and, where possible, use should be made of samples for which consumer panels have already been conducted. There are relevant trials being conducted in Australia, Poland and France and a multinational approach is recommended. In addition, experiments planned as part of research funded by the Department of Agriculture and Rural Development in Northern Ireland may lend themselves to a joint approach. The following studies are suggested:

a. Reanalysis of existing data to determine if there is evidence of an optimum IMF concentration and optimum days ageing for flavour liking on an individual muscle basis. Statistical methods to disassociate the scores for flavour liking from tenderness would be beneficial.

b. A limited student study on the effect of diet and breed on volatiles from beef samples in Poland and France is planned with a PhD student visiting AFBI in Northern Ireland. The potential to add additional chemical and statistical analyses should be considered to allow the determination of precursors, antioxidants, and full statistical analysis of the data.

c. A controlled experiment on the effect of grass versus feedlot diet(s) and muscle on volatiles, consumer scores, IMF, precursors and antioxidants should be conducted.

d. Effect of cooking method and IMF on flavour liking and volatile formation. This could be linked with a study on cooking methods.

7.5 Recommendations for a Flavour Workshop

A number of scientific groups have been evaluating meat flavour for MSA and while bilateral talks have been held between most of these, there has not been a workshop where the progress made by the different groups can be shared and the best potential for future development discussed. It is proposed that such a workshop be held in Australia within the next 6 months.

The format could comprise short presentations (20 minutes each) in the morning and a discussion session in the afternoon. Alternatively, a longer two day meeting would enable participants to describe their non-MSA meat flavour work which may extend the discussion to new areas of relevance to MSA.

Suggested invitees are listed in Table 7.5. This list is not intended to be exclusive.

Table 7.5. Scientists with an interest in MSA and flavour who might be invited to a flavour workshop

Name	**Affiliation**	**Expertise**
Alex Ball	MSA – EQ R&D Manager	MSA, animal, genetics & meat science
Rod Polkinghorne	Consultant	MSA, animal and meat science
John Thompson	UNE/ consultant	MSA, animal and meat science
Ray Watson	U. of Melbourne	MSA, Statistician
Dave Pethick	Murdoch University, Perth	MSA, animal and meat science
Damien Frank	CSIRO - North Ryde	Flavour
Conor Delahunty	CSIRO - North Ryde	Flavour
Robyn Warner	CSIRO - Werribee	Animal and meat science
Peter Watkins	CSIRO - Werribee	Flavour analysis
Linda Farmer	AFBI, Northern Ireland	Meat flavour, meat quality, MSA
Alan Gordon	AFBI, Northern Ireland	Statistician
Terence Hagan	AFBI, Northern Ireland	Meat flavour, sensory and flavour analysis
Jerrad Legako	Utah State University	Flavour analysis, MSA consumer

		panels
Chance Brooks	Texas Tech University	Flavour analysis, MSA consumer panels
Mark Miller	Texas Tech University	MSA consumer panels, meat science

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