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UNDERSTANDING CONSUMER LIKING OF BEEF WITH PARTICULAR REFERENCE TO FLAVOUR

THESIS PRESENTED BY
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PhD dissertation submitted in fulfilment of the requirements for the PhD in Food Science and Technology.
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Contents

Figures **1**
Tables **3**
Declaration **6**
Acknowledgement **7**
Abstract **9**
Publications and Presentations **11**
Chapter 1 Scientific Literature Review **14**
1.1 Introduction **14**
1.2 Factors influencing beef eating quality **14**
1.2.1 Pre-slaughter factors 14
1.2.2 Post- slaughter factors 17
1.3 Linking beef grading systems with eating quality **21**
1.4 Linking consumer liking with the sensory attributes of a product **23**
1.5 Differences between consumers from different countries or regions **25**
1.6 Aroma and flavour of beef **27**
1.6.1 Physiology of aroma and flavour 27
1.6.2 Understanding the beef aroma and flavour 28
1.6.3 Factors influencing volatile compounds of beef 33
1.7 Measurement of volatile compounds **35**
1.7.1 Headspace method for collection of volatile compounds 36
1.7.2 Solid phase microextraction technique 37
1.8 Aims of the study **41**
1.9 References **43**
Chapter 2 Consumer Perceptions of Beef- A comparison of consumers from Northern Ireland (NI), Republic of Ireland (ROI) and Great Britain (GB) **59**
2.1 Introduction and Objective **59**
2.2 Materials and Methods **60**
2.2.1 Experimental design 60
2.2.2 Quantitative Descriptive Analysis (QDA) 63
2.2.2.2 Sensory profiling panel cooking protocol 64
2.2.3 Sensory Consumer Panel Evaluation (Belfast, Cork, Reading) 65
2.2.4 Warner Bratzler Shear Force (WBSF) 71
2.2.5 Statistical design and analysis 72
2.3 Results **74**

2.3.1 Instrumental analysis 74
2.3.2 Quantitative Descriptive Analysis 76
2.3.3 Consumer Panels 81
2.3.4 Consumers' socioeconomic survey in the three regions 87
2.3.5 Cluster analysis of the consumer panel 103
2.4 Discussion 106
2.4.1 Factors affecting the eating quality of beef 106
2.4.2 Understanding the differences between regions 111
2.4.3 Multivariate analysis of consumer preference with corresponding sensory characteristics and instrumental measurements 123
2.5 Conclusion 134
2.5.1 Future Direction 135
2.6 References 135
Chapter 3 Investigation of the Effect of Value-added Processes and Muscles on Beef Quality 142
3.1 Introduction and Objectives 142
3.2 Material and Methods 144
3.2.1 Source of Samples 144
3.2.2 Treatment information 144
3.2.3 Consumer study design by UNE 146
3.2.4 Collection of flavour samples 146
3.2.5 Flavour volatile analysis 146
3.2.6 Sugar analysis 148
3.2.7 Dry matter analysis 149
3.2.8 pH measurement for value-added samples 150
3.2.9 Statistical analysis 151
3.3 Results 152
3.3.1 Dry matter analysis 152
3.3.2 pH analysis 153
3.3.3 Weight analysis 154
3.3.4 Consumer liking of beef 155
3.3.5 Flavour volatile analysis 157
3.3.6 Sugar analysis 163
3.3.7 Stepwise regression analysis 166
3.4 Discussion 166

3.4.1 Effect of value-added processes, animal sex and muscles on moisture content and pH ... 167
3.4.2 Volatile profile of grilled beef ... 168
3.4.3 Understanding the effects of value-added process and muscles on sugars and sugar phosphates ... 174
3.4.4 Linking the instrumental results to consumer analyses ... 176
3.5 Conclusion ... 184
3.5.1 Future Direction ... 185
3.6 References ... 186
Chapter 4 Development of an Accessible and Robust Headspace Solid Phase Microextraction Technique for the Analysis of Cooked Beef Samples ... 193
4.1 Introduction and Objective ... 193
4.2 Materials and Methods ... 194
4.2.1 Experimental design ... 194
4.2.2 Product procurement and preparation ... 196
4.2.3 Volatile analysis ... 196
4.2.4 Statistical analysis ... 200
4.2.5 Method selection ... 200
4.3 Results and Discussion ... 201
4.3.1 General criteria ... 201
4.3.2 Specific criteria ... 204
4.3.3 The advantages and disadvantages of the different extraction methods ... 242
4.4 Conclusions ... 244
4.4.1 Future Direction ... 244
4.5 References ... 245
Chapter 5 Investigation of the effects of packaging and ageing period on the eating quality and flavour of two beef muscles ... 250
5.1 Introduction ... 250
5.2 Material and Methods ... 251
5.2.1 Product procurement and preparation ... 251
5.2.2 Consumer sensory panels ... 253
5.2.3 Sample selection for chemical and instrumental analysis ... 253
5.2.4 Volatile analysis ... 254
5.2.5 Dry Matter analysis ... 255
5.2.6 Statistical analysis ... 256
5.3 Results ... 256

5.3.1 Dry matter analysis 256
5.3.2 Consumer liking of beef 258
5.3.3 Volatile analysis 259
5.4 Discussion 268
5.4.1 Volatile profile for beef 268
5.4.2 Interaction effects of muscles, packaging and ageing on volatile compounds 269
5.4.3 Effects of muscle, packaging and ageing on volatile compounds 272
5.4.4 Linking the instrumental results (dry matter and volatile) to consumer panels analysis 279
5.5 Conclusion 284
5.5.1 Future Direction 284
5.6 References 285
Chapter 6 General Discussion and Future Directions for Research 290
6.1 Significance of the results 290
6.2 Implications for the beef industry 294
6.3 Recommendations for future work 295
6.4 References 298
Annex 300

Figures

Figure 1.1 Carcass suspension method, reproduced from (Emma, 2014).....18

Figure 1.2 Schematic diagram illustrating the formation of volatile compounds derived from Maillard reaction, lipid thermal degradation and thiamine degradation, reproduced from Dashdorj et al. (2015). 29

Figure 1.3 Schematic diagram of a SPME syringe equipped with SPME fibre, reproduced from Zhang et al. (1994).....38

Figure 1.4 SPME process; (A) Extraction of volatile compounds using SPME fibre and (B) Thermal desorption on GC inlet, reproduced from Kataoka et al. (2000). 38

Figure 2.1 Demonstration of beef striploin cut up and collection of sample for different tests. 63

Figure 2.2 Posting sheet example. 68

Figure 2.3 Distribution of consumers' individual MQ4 score for unsatisfactory (fail), satisfactory everyday quality (3*), better than everyday quality (4*) and premium quality (5*). 85

Figure 2.4 Regional distribution of members of cluster groups (CG) in percentage. 103

Figure 2.5 Second order effect of breed by sex interaction on pHu and cook loss. 107

Figure 2.6 Effect of breed x sex interaction on sensory trained panel attributes, (i) *succulence mouthfeel*, (ii) *juicy external appearance*, (iii)*bloody aroma*, (iv) *bloody external appearance*, (v) *greasy external appearance*, (vi) *red juice external appearance* and (vii) *tight internal appearance*.....109

Figure 2.7 Significant (P<0.001) effect of breed by sex interaction on consumer *tenderness* (TE), *juiciness* (JU), *flavour liking* (FL), *overall liking* (OL) and MQ4 scores of consumer panel. For significance, see Table 2.9. 110

Figure 2.8 Significant (P<0.001) effect of breed by sex interaction on consumer tenderness (TE), juiciness (JU), flavour liking (FL), overall liking (OL) and MQ4 scores of consumer panel. For significance, see Table 2.9. 112

Figure 2.9 Comparison of proportion relative to satisfactory everyday quality (P-WTP) in Belfast, Cork and Reading. 113

Figure 2.10 Consumers' preferred cooking endpoint in three regions.....115

Figure 2.11 Spider diagram showing the consumer consumption frequency of different products.....117

Figure 2.12 Factors that affect consumer motivation for beef choice.....120

Figure 2.13 Effect of cluster group x hanging method x animal sex on consumer MQ4 score. 125

Figure 2.14 Mean consumer MQ4 score of (a) hanging method, (b) animal sex and (c) sample position among four overall cluster groups.....125

Figure 2.15 External preference map for texture on cutting and mouthfeel. 127

Figure 2.16 External preference mapping for overall liking.....130

Figure 2.17 Internal preference maps for overall liking. 132

Figure 3.1 Typical example of chromatograms for control sample and sample treated with ficin, actinidin (kiwi) or phosphate solutions. 163

Figure 3.2 Effect of value-added processes on the quantities of n-aldehydes in relation of control treatment (T1) for (a)Trial 1 and (b) Trial 2. 169

Figure 3.3 Effect of value-added processes on the quantities of Maillard compounds in relation to control treatment for (a)Trial 1 and (b) Trial 2. 170

Figure 3.4 Effect of treatment and muscle on weight added (%). 172

Figure 3.5 Illustration of needling point (addition of extract solution) and sampling point on a meat sample. 173

Figure 3.6 Muscle and sex interaction on (a) tenderness, (b) juiciness and (c) MQ4 score. 178

Figure 3.7 Effect of value-added processes on the consumers' palatability traits (P<0.001). 179

Figure 3.8 Principal component analysis, (a) PC1 vs PC2 and (b) PC1 vs PC3. 181

Figure 4.1 Illustration of methods selected for the trials. 195

Figure 4.2 Changes in the quantities of Strecker aldehydes in relation to the quantity of volatile compound from cooked beef sample aged for 4 days. 235

Figure 4.3 Changes in the quantities of n-aldehydes in relation to the quantity of volatile compound from cooked beef sample aged for 4 days. 237

Figure 4.4 Changes in the quantities of alkenals in relation to the quantity of volatile compound from cooked beef sample aged for 4 days. 238

Figure 4.5 Changes in the quantities of pyrazine compounds in relation to the quantity of volatile compound from cooked beef sample aged for 4 days. 239

Figure 4.6 Changes in the quantities of acid compounds in relation to the quantity of volatile compound from cooked beef sample aged for 4 days. 241

Figure 5.1 Flow diagram for sample preparation (Polkinghorne et al., 2018). 253

Figure 5.2 Average coefficient of variation for volatile compounds. 269

Figure 5.3 Third order interaction of muscle by packaging by ageing period on the quantities (ng/ headspace of 2g of beef sample) of (a) 3-methylbutanal and (b) 2-ethyl-1-hexanol... 270

Figure 5.4 Effects of muscle by ageing on the quantities (ng/ headspace of 2g of beef sample) of (a) 3-hydroxy-2-butanone and (b)2,4-pentadione. 271

Figure 5.5 The effects of muscle type on volatile compounds. 274

Figure 5.6 Effects of packaging on the volatile compounds. 276

Figure 5.7 Effects of post-mortem ageing on volatile compounds. 278

Figure 5.8 Effect of muscle by ageing interaction on (a) tenderness, (b) overall liking, (c) MQ4 and (d) satisfaction. 280

Figure 5.9 Principal component analyses for PC1 versus PC2. 281

Figure 5.10 Principal component analyses for PC1 versus PC3. 282

Tables

Table 1.1 MSA prediction models in different countries. 27
Table 1.2 Common volatile compounds for cooked beef and their flavour characteristics. 31
Table 1.3 SPME fibre coating and the compounds to be analysed. 39
Table 2.1 Details of sample treatments. 61
Table 2.2 Panellist paired up for profiling panel. 64
Table 2.3 Samples used for consumer panels in Belfast, Cork and Reading. 66
Table 2.4 Consumer experimental design for Cork panellists 1 to panellists 60. 67
Table 2.5 Instrumental analysis results. 75
Table 2.6 REML analysis of the effect of ageing period on WBSF and cooking loss. 76
Table 2.7 Abbreviation and definition for sensory attributes. 76
Table 2.8 Mean and average standard deviation of the intensity scores for the sensory attributes from sensory trained panel. 79
Table 2.9 The effect of hanging, breed, sex, sample position and region on the mean of consumer palatability traits of beef. 82
Table 2.10 Number of sample (n), mean, standard deviation, minimum and maximum of consumers' individual MQ4 scores for different quality grades. 84
Table 2.11 Consumer willingness to pay for products at different grades. 86
Table 2.12 Distribution of consumers in various socioeconomic groups. 88
Table 2.13 Effects of socioeconomic status on consumer sensory scores and WTP. 89
Table 2.14 Distribution of consumers in various behavioural factor groups. 91
Table 2.15 Effects of consumer behaviour and preferences on sensory socres and WTP. 92
Table 2.16 Distribution of consumer consumption frequency of each product. 94
Table 2.17 Effects of consumer consumption frequency on sensory scores and WTP. 95
Table 2.18 Importance level for consumer motivation of beef choice 99
Table 2.19 Effects of consumer motivation on beef choice on sensory scores and WTP 100
Table 2.20 Mean differences in consumer overall liking across various cluster groups and significant interaction associated with overall cluster groups. 104
Table 2.21 Coefficients for MQ4* and MQ3* models. 121
Table 2.22 Final MQ4 model for different regions and countries. 122
Table 2.23 MSA boundaries between fail, 3*, 4* and 5*. 123
Table 2.24 Pearson's correlation coefficients (r-value) between consumer panel and instrumental analysis. 127
Table 2.25 Percentage variation of principal components for external and internal preference mapping of overall liking. 129
Table 3.1 Value-added treatments. 145
Table 3.2 Samples analysed for volatile compounds. 148
Table 3.3 Samples analysed for sugar content. 149
Table 3.4 Samples analysed for dry matter content. 150

Table 3.5 Samples analysed for pH. 151
Table 3.6 Dry matter analysis results. 153
Table 3.7 Effect of treatment and muscle on ultimate pH (pHU) and enhanced pH (pHE). 154
Table 3.8 Effects of treatment and muscle on raw weight, processed weight and weight added. 155
Table 3.9 Effect of treatment, muscles and animal sex on consumer sensory scores. 156
Table 3.10 Volatile compounds included in analysis. 158
Table 3.11 Effect of treatment, muscle and sex on the quantities of volatile compounds (ng/ headspace of 2g of beef sample) for Trial 1. 161
Table 3.12 Effect of treatment, muscle and sex on the quantities of volatile compounds (ng/ headspace of 2g of beef sample) for Trial 2. 162
Table 3.13 Effect of value-added treatment, muscle and sex on the concentration (uM/wet gram) of sugar and sugar phosphates. 165
Table 3.14 Regression analysis for consumer sensory score. 166
Table 3.15 Coefficient of variation for each volatile compound on value-added treatment. 173
Table 4.1 Number of samples used for each method in Trial A and Trial B. 195
Table 4.2 Effect of the length of time on response peak area for sample exposed under room temperature. 199
Table 4.3 Justification for scores for the ease of use. 201
Table 4.4 Justification for scores for amount of sample required. 203
Table 4.5 Justification for scores for flexibility of the method. 203
Table 4.6 Volatile compounds identified in Trial A. 205
Table 4.7 Volatile compounds identified in Trial B. 208
Table 4.8 Number of compounds detected in each compound category by method. 213
Table 4.9 Score allocation for the range of volatile compounds detected. 214
Table 4.10 Mean, standard deviation and coefficient of variation of volatile compounds quantities (ng/ headspace of 2g of beef sample) based on four methods in Trial A. 215
Table 4.11 Trial B: Mean standard deviation and correlation variation of volatile compounds quantities (ng/ headspace of 2g of beef sample) for samples with different ageing period (A). 218
Table 4.12 Total quantities (ng/ headspace of 2g of beef sample) of volatile compounds in each compound category. 230
Table 4.13 Score allocation for the quantity of volatile compounds detected. 231
Table 4.14 Number of compounds in each reproducibility classes in Trial A and Trial B. . 232
Table 4.15 Scores allocation for method reproducibility. 233
Table 4.16 Scores allocation for the ability to differentiate beef samples aged to different periods by each volatile compound category. 242
Table 4.17 General and specific criteria for selection of extraction method. 243
Table 5.1 Number of samples in each treatment. 254
Table 5.2 Dry matter and moisture content result. 257

Table 5.3 Mean consumer sensory scores of muscle type, packaging method, ageing period and interaction effects. 258

Table 5.4 Volatile compounds identified in beef samples. 260

Table 5.5 Effect of muscle (M), packaging (P) and ageing period (A) on the quantities (ng/ headspace of 2g of beef sample) of volatile compounds. 265

Declaration

I, Fui Shien Chong, hereby declare that this dissertation is the result of my own independent work and has not been previously submitted for any diploma, degree, fellowship or other identical recognition. I clarify that, to my best knowledge, all the information and material obtained from other sources, have been duly acknowledged in this dissertation.

Student: Fui Shien Chong

Signature: Irene Chong
Date: 30th April 2020

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Abstract

This thesis reports the consumer perception for beef eating quality with particular reference to flavour. A study was conducted to investigate if there are regional differences in consumer perception of beef between consumers from different regions. Consumers were recruited from Cork, Belfast and Reading to represent consumers from the Republic of Ireland (ROI), Northern Ireland (NI) and Great Britain (GB). Consumers from Reading scored significantly higher in palatability traits (aroma liking, tenderness, juiciness, flavour liking, overall liking) compared to the other regions although all consumers received portions of same samples. However, consumers from these three regions showed similar preferences towards beef, which indicated that consumer studies conducted in ROI and NI are representative of those in GB. Consumers from Reading were less concerned about the origin of beef and healthiness of beef product. Higher consumption frequency of low-quality cuts was reported by consumers in Reading and thus they gave higher scores for palatability traits when they consumed striploin steak. Four cluster groups were observed using hierarchical cluster analysis and these cluster groups were described as “fastidious”, “tender beef liker”, “bull beef liker” and “easily pleased” consumers. These cluster groups exhibited different scoring patterns and/or preference for beef.

Research was commissioned to evaluate the effects of enhancement (kiwi, fig and phosphate solutions) and tenderisation on meat quality with instrumental and chemical analyses. Sugar concentrations were significantly increased and sugar phosphate concentrations were significantly decreased for enhanced beef samples compared to untreated beef samples. A clear muscle effect was observed on volatile composition but the enhancement effects were generally small, probably due to the variation of the extraction method.

Automatic solid phase microextraction (SPME) methods were developed to improve the labour intensive manual SPME method. Three general criteria were considered, including flexibility of the method, amount of sample required and ease of use. Specific criteria such as detection range of volatile compounds, quantity of compounds detected, reproducibility and the ability of the method to differentiate beef samples processed under different conditions. Two methods were selected, automatic SPME-

cored beef (CAR/PDMS fibre) and automatic SPME- liquid nitrogen homogenised beef (DVB/CAR/PDMS fibre), each had their own advantages and disadvantages.

A study conducted in this thesis showed that post-mortem ageing (14, 21 or 49 days), muscles (rump, striploin) and packaging methods (vacuum skin packaging, overwrapped, modified atmosphere packaging) had significant impact on the quantities of volatile compounds. Beef aged for longer period had higher quantities of Strecker aldehydes, n-ketones and pyrazines. Differences in lipid content may explain the differences in the quantity of volatile compounds, which was clearly indicated using principal component analysis. These data indicated that modified atmosphere packaging induced generation of lipid degradation compounds may have reduced the consumer liking for these beef samples.

Publications and Presentations

Research Papers (Published)

Chong FS, O'Sullivan MG, Kerry JP, Moloney AP, Methven L, Gordon AW, et al. Understanding consumer liking of beef using hierarchical cluster analysis and external preference mapping. Journal of the science of food and agriculture. 2019.

Chong FS, Farmer LJ, Hagan TDJ, Speers JS, Sanderson DW, Devlin DJ, et al. Regional, socioeconomic and behavioural- impacts on consumer acceptability of beef in Northern Ireland, Republic of Ireland and Great Britain. Meat science. 2019;154:86-95.

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Chong FS, Hagan TDJ, Legako JF, Kerry JP, O'Sullivan MG, Farmer LJ. Optimisation of solid-phase microextraction based techniques for the analysis of volatile compounds of cooked beef.

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Chong FS, Farmer LJ, Hagan TDJ, Moloney AP, Kerry JP, O'Sullivan MG. Consumer acceptability of beef in regions of the British Isles? 64th International Congress of Meat Science and Technology; 2018; Melbourne, Australia.

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Chapter 1 Scientific Literature Review

1.1 Introduction

Meat, particularly beef, remains an essential source of protein for most of the population in the United Kingdom and the Republic of Ireland. The sensory quality of beef can be highly variable, and this greatly influences consumer dissatisfaction (Farmer et al., 2016). The eating quality of beef is very important to ensure consumer satisfaction and guarantee future repurchase intent. Consumers are the most vital component of the food production chain. Consumers demand beef products to be produced using sustainable farming practices, nutritious, safe, and most importantly, of good eating quality (Realini et al., 2009). Therefore, the beef industry needs to understand the consumer perception of beef products as it greatly influences profitability. If the beef industry can deliver better value products by improving the consistency, quality and/or price, revenues are expected to increase. This chapter will review the eating quality of beef and consumer perception of beef with particular focus on how beef flavour influences eating quality.

1.2 Factors influencing beef eating quality

A wide range of factors affect consumer liking and acceptability of beef. These include, but not limited to, the production or breeding method at the farm and other factors outside the farm gates (Henchion et al., 2017). This section will focus on the eating quality of beef and how consumers perceive beef products.

1.2.1 Pre-slaughter factors

It is suggested that many factors influencing meat quality are directly linked with the animal and its growing environment (Dannenberger et al., 2006). These factors including breed, growth rate, animal age, fatness of animal, animal sex, slaughter practice and animal stress management. Those that are related to the thesis are animal breed, sex and age, and these will be discuss in this section.

1.2.1.1 Breed

Beef originates from a large pool of pure bred and crossbred animals. Researchers in Poland suggested that pure black and white beef had greater eating quality compared to dairy x Belgian Blue bulls and Aberdeen Angus and Charolais crosses (Groth et al., 1999). The eating quality of beef from steers across range of breeds was assessed by 504 consumers in a Meat and Livestock Commission trial in 1986. The breeds selected including dairy breed (Holstein/ Friesian), continental x suckler and Hereford x Friesian. Interestingly, the results showed that there was no significant breed difference in eating quality (Matthews, 2011). A study focused on pure breed steers showed that Aberdeen Angus had reported higher scores for overall acceptability, flavour and juiciness compared to Holstein and Charolais (Sinclair et al., 2001). Several studies compared the differences in flavour liking of beef among different breeds with similar age and feeding regimes and concluded that the difference in flavour liking can be attributed to the difference in intramascular fat, such as Belgian Blue x Holstein versus Angus x Holstein crossbred steers (Keady et al., 2017), Simmental versus Hereford bulls (Mandell et al., 1997) and Angus versus Hanwoo cattle (Van Ba et al., 2013). A trial investigating the heritability of beef flavour and intensity of flavour from 1066 carcasses representing 12 beef breeds established heritabilities of (weak to moderate) 0.00-0.18 and 0.06-0.22, respectively (Pratt et al., 2013). Some breeds also have unique flavour characteristics. For example, double-muscled bulls from Spain are reported to give meat with a higher intensity of acidic flavour while rustic breeds had higher intensity of liver flavour for certain ageing periods compared to beef from other genotypes (Campo et al., 1999). Overall, the breed effects are small if the post-slaughter handling is strictly controlled (Matthews, 2011).

1.2.1.2 Gender

Pre-slaughter issues such as animal gender have been at the forefront of beef industry interest and debate. The major issue for consideration related to cattle sex is the treatment of bulls. Intensive studies were conducted to compare the eating quality of bulls and steers using different production systems. Most of the reviews indicated that the eating quality of steers is better compared to bulls, particularly in the tenderness

of meat (Moran et al., 2017, Venkata Reddy et al., 2015). Flavour of the meat is highly associated with the fat content in the beef, which can be manipulated with sex, which is very much caused by the degree of fatness as steers rated better in eating quality compared to bulls due to high intramuscular fat (Therkildsen et al., 2017). Less characteristic beef flavour with higher intensities of bloody and livery flavour are reported in bulls compared to heifers (Gorraiz et al., 2002). Fisher et al. (2001) suggested that the meat is generally tougher for bulls compared to steers and no significant effect was discovered for age on tenderness. Studies from Beef Blueprint suggested ways for young bulls to be included in a quality specification if they meet some other special requirements, including young bulls not more than 15 months of age at slaughter and the minimum ageing period of 14 days utilised from slaughter to retail sale (Matthews, 2011). The benefit of ageing bulls for a minimum of 14 days is supported by Fisher *et al*. (2001) and Johnson et al. (1988).

1.2.1.3 Age

The literature is consistent that generally an increase in age of animal affects eating quality by decreasing the tenderness (Bouton et al., 1978, Harper, 1999, Reagan et al., 1976). This is probably due to changes in collagen; older animals have a higher proportion of collagen with heat stable cross-links (Robins et al., 1973). In addition, ossification is directly proportional to the maturity of the animal. A study reported that carcasses with lower ossification scores normally have a lower incidence of dark cutting (McGilchrist et al., 2012). Flavour intensity was found to be highest from older group of cattle; including 8 to 9 years old cows and 2 years old bulls (Dransfield et al., 2003, Zembayashi, 1994). The intensity of roasted residual beef flavour after swallowing is found to be higher in older (22 months) bulls compared to younger bulls (Nian et al., 2017). However, no differences are found in meat flavour in the beef from 14, 19 and 24 months steers (Warren et al., 2008). Breed may be a factor that interacts with age on some unique flavour characteristics. Higher intensity of fishy flavour was found in beef from 14 months old Holstein steers compared to beef from Angus, but this effect was not observed for 19 or 24 months old steers. Beef from 24 months Holstein steers had a lower intensity of acidic flavour and higher intensity of rancid flavour (Warren et al., 2008).

1.2.2 Post- slaughter factors

1.2.2.1 Suspension method

Carcass suspension method has a major influence in enhancing the eating quality of beef. The traditional hanging method for beef is straight hung in which the carcass is hung by the Achilles tendon. During the process of rigor mortis, the rear leg muscles contract because the spine is curved and there is less tension on them. This causes the muscle fibres to overlap and results in tough meat (Sørheim and Hildrum, 2002). For aitch hung or tenderstretch (TS), the carcass is hung or suspended by the pelvic bone using an S-shaped hook. This creates a 90° angle when the carcass's leg drops down which causes the *longissimus dorsi* muscles to stretch and they cannot contract during the rigor mortis process, thus resulting in tender meat (Figure 1.1). Commercially, the beef industry applies the TS hanging method by suspending the carcasses through the pelvic ligament or obturator foramen (aitch bone). Both methods increase the tension on the beef loin and leg but some minor muscles have the risk for shortening (Hwang et al., 2002). TS can decrease Warner-Bratzler Shear Force and increase the sarcomere length of *gluteus medius, semimembranous* and *longissimus* of beef but has limited effects in *psoas major* and *semitendinosus* muscles (Bouton et al., 1973). Although TS was scientifically proven to be more effective in improving eating quality, it have not been widely adopted in some countries due to higher costs and perceived inconvenience. To overcome this issue, the Meat Standards Australia grading scheme promotes the TS hanging method by offering the potential to increase returns and obtaining superior grade results (MSA, 2007).

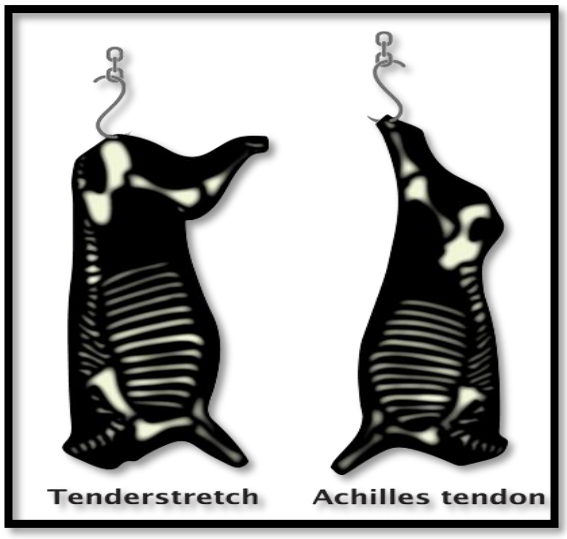


Figure 1.1 Carcass suspension method, reproduced from (Emma, 2014).

1.2.2.2 Post-mortem ageing

Meat ageing is a process of resting the meat for a period after slaughter under low temperature for a defined period of time (Lawrie, 1998). Wet ageing is widely used in Ireland and England, where meat is aged in vacuum pack for a certain period of time, usually 7 days, 14 days or 21 days. The ageing process enhances eating quality by breaking down the muscle fibres through the activation of proteolytic enzymes such as caspases, cathepsins and calpains (Longo et al., 2015). Calpains are sensitive to the temperature and pH of the meat. This enzyme is responsible for the degradative changes during post-rigor conditioning (Lawrie, 1998). Ageing has been associated with reducing variation in tenderness, which can be caused by breed or sex of animal (Monsón et al., 2005). A study investigated the effect of ageing in several beef muscles, the authors reported that tenderness of *infraspinatus* improved steadily until 29 days, *semitendinosus* achieved maximum tenderness with 7 days aging, while there was no effect on *semimembranosus* and a limited effect on *longissimus* after 15 days ageing (Janz and Aalhus, 2004). The ageing period has different effects in different beef muscles probably because of different levels of connective tissue in the muscles (Lawrie, 1998). To date, most of the research focused on the effects of short-term ageing (up to 35 days). Lee et al. (2008) reported that ageing up to 35 days had no significant impact on bloom development on *longissimus thoracis*. In addition, top sirloin butt steaks aged for 7 days and 14 days were significantly ($P<0.05$) more vivid,

yellower, redder and had higher oxymyoglobin percentages compared to those aged for 28 days and 35 days. Other research reported that *psoas major*, *gluteus medius* and *supraspinatus* beef that was wet aged for 21 days and 35 days had higher rancid or metallic flavour according to trained panellists (Yancey et al., 2005). Samples were aged to an extended period of 63 days and the study reported that the longer ageing period reduced retail colour stability yet increased the tenderness of *longissimus lumborum* and *gluteus medius* steaks (Colle et al., 2015). However, limited research has explored the effect of extended ageing (over 40 days) on consumer acceptability and meat quality. The eating quality of meat aged for 14, 28 and 42 days was investigated and the results showed that flavour rating and overall impression were higher for *gluteus medius* and *longissimus thoracis* beef aged for 14 days compared to 28 and 42 days while no change in beef flavour for *longissimus dorsi* and *infraspinatus* was observed (Adcock et al., 2015). On the other hand, a study showed that all the consumers' palatability traits increased from 2 weeks to 12 weeks of ageing (Hughes et al., 2014).

1.2.2.3 Packaging method

The appearance of the product is one of the main factors that affects consumers' beef purchase decision (Grunert et al., 2004). Therefore, packaging or packaging-related properties have a significant impact on consumer perception of beef quality. Packaging methods range from air permeable packaging methods (e.g. overwrap) to methods employing barrier materials in bulk such as gas flushing, vacuum packaging and modified atmosphere packaging. Packaging systems that are commonly used in the beef industry are modified atmosphere packaging (MAP), conventional overwrapped packaging (OWP) and vacuum skin packaging (VSP). OWP is a rather simple packaging system, whereby the beef product is sealed using film, allowing oxygen to diffuse into the packaging (Millar et al., 1994). This type of packaging has been reported to give a lower shelf-life compared to MAP and VSP (Lorenzo and Gómez, 2012). Development of packaging systems has been supported by the beef industry, as they believe that new packaging systems contribute to beef product safety. In addition, new packaging systems can also improve the availability of ready-to-eat-meals. Thus, there are different drivers for the beef industry and consumers to select

preferred packaging systems. Packaging systems influence consumer perceptions of beef quality as packaging can affect beef colour or meat colour stability (Grobbel et al., 2008). High oxygen MAP (commonly 80% oxygen and 20% carbon dioxide) improves the colour stability of beef products (Yam, 2009). Interestingly, some studies reported that this gas mixture has a detrimental effect on beef tenderness, caused by the protein and lipid oxidation (Clausen et al., 2009, Estévez, 2011, Sørheim et al., 2004, Zakrys et al., 2008).The VSP packaging system is a relatively new technology, where the beef is placed on a tray and the packaging film is heated then tightly wrapped or shrunk around the beef and packaging tray once the vacuum is drawn. This packaging system decreases the rate of microbial growth and promotes longer shelf-life (Vázquez et al., 2004). However, the beef colour will turn purple which affects consumer acceptability. In a European study, 54.7% of consumers accepted MAP beef while 73% of the consumers accepted vacuum packaged beef (Van Wezemael et al., 2011). Other advances in packaging include active packaging using bioactive or chemoactive components. Active packaging methods are categorised into antioxidant, oxygen scavengers, carbon dioxide emitters, antimicrobial packaging and moisture absorbers (Realini and Marcos, 2014). The beef industry is looking for future packaging methods with high flexible material, higher barrier properties and less materials (McMillin, 2017).

1.2.2.4 Enhancement

Enhancement technologies, extensively applied in the pork and poultry industries, have been adopted by the beef industry in recent years to produce more tender, juicy and consistent products. With the increased popularity of value-added beef products, muscles which are traditionally marketed as lower quality cuts can be improved using enhancement treatment. Sodium lactate, sodium chloride and sodium tripolyphosphate solutions have been proved to improve beef tenderness and meet consumer expectations (McGee et al., 2003, Molina et al., 2005, Robbins et al., 2002, Vote et al., 2000). Another approach to improve meat tenderness is to decrease the connective tissue of the beef by adding exogenous enzymes to control extensive proteolysis. Ficin, papain, *Bacillus subtilis* protease, *Aspergillus oryzae* protease and bromelin are commonly used as meat tenderisers which are approved by United States federal

agencies (CFR, 2009). A previous study proved that these enzyme treatments improve tenderness through collagenous protein degradation (Sullivan and Calkins, 2010). Recent research has also shown that the proteolytic kiwifruit extract, actinidin, achieved a higher controlled tenderising effect on the myofibrillar structure (Aminlari et al., 2009, Christensen et al., 2009). However, the effect of actinidin is less pronounced on collagen, limiting its effectiveness on beef cuts with high connective tissue content.

In order to classify carcasses based on their quality, it is important to have a classification or grading system to place or define values of cattle or carcasses for pricing purposes. Generally, such grading systems involve ranking cattle, carcass or even individual muscles in a hierarchy for the traits of interest.

1.3 Linking beef grading systems with eating quality

Within the beef industry, there are many grading systems introduced to increase the consistency of eating quality and transparency in the supply chain. Many of these grading systems rank carcasses into hierarchy grades using yield, carcass fat, carcass shape, conformation scores or texture scores (Polkinghorne and Thompson, 2010).

Mandatory EC Beef Carcass Classification Regulations were introduced into the United Kingdom in 1992. Under this regulation, all abattoirs in Europe slaughtering more than 75 cattle per week need to classify carcasses based on EUROP grid. The EUROP grading scheme involves estimation of carcass conformation and degree of fatness (Polkinghorne and Thompson, 2010). Under the EUROP grid, conformations of carcasses are classified into five classes: E, U, R, O and P; with U, O, and P being subdivided into lower (-) and upper (+) bands. The degree of fatness is divided into five main classes, from 1 (not fat) to 5 (very fat); classes 4 and 5 are divided into fatter (H) and leaner (L) bands. Video image analysis has been extensively employed to automate visual assessment of fat and conformation classes on the EUROP grid (Craigie et al., 2012). Video image analysis has been extensively employed to automate visual assessment of fat and conformation classes on the EUROP grid (Craigie et al., 2012).

In addition to the EUROP carcass grading system, the Meat and Livestock Commission (MLC) Blueprint was introduced in the UK to deliver better eating quality for beef; the pilot MLC grading scheme was launch in 1972 (AHDB, 2008). The MLC Blueprint is a pass/fail system in the UK which aims to ensure the eating quality of beef meets consumers' expectations. A number of rules are set to enable carcass classification according to the MLC Blueprint. For example, carcass conformation should be O+ or higher and carcass fat class should be 3 or higher. Furthermore, age of the animal should be less than 30 months. All carcasses need to be hip hung and aged for at least 7 days. However, tighter restrictions apply to bulls; these include carcasses to be less than 15 months old and ageing period is set to be at least 14 days.

Eating quality may not be consistent within the same carcass (Hunt et al., 2014, Lorenzen et al., 2003, Neely et al., 1998). Eating quality is believed to be affected by many factors (Hocquette et al., 2014). It's almost impossible to predict the eating quality with one or two factors. For example, USDA failed to ensure consistent eating quality because of low emphasis on beef tenderness, disregard for cooking method or cut and high reliance on degree of marbling and maturity (Hocquette et al., 2014). In contrast to other grading systems which classify whole carcasses, Meat Standards Australia is a cut-based grading scheme that uses a modelling approach to assure consistency in beef eating quality at a cooked portion level.

In 1996, Meat Standards Australia (MSA) was established by predicting consumer satisfaction or eating quality of beef products with a modelling approach. Unlike other grading schemes, MSA is a cut-based grading scheme (Strong, 2001). The MSA grading system sought to focus on consumer assessment rather than trained panel assessment or objective measurement. A protocol outlining the experimental design and data handling process was produced by Watson et al. (2008) to ensure effectiveness and robust consumer assessment. This protocol addressed all stages of consumer assessment from sample collection, consumer panel preparation, consumer recruitment, cooking, serving, questionnaire design and scoring. Satisfaction score, also known as MQ4 score, is measured by combining *tenderness*, *juiciness*, *flavour liking* and *overall liking*. The muscle portions are assigned into four different grades (ungraded/ 2*=unsatisfactory, 3*= satisfactory everyday quality, 4*= better than everyday quality, 5*= premium quality) based on the eating quality rated by

consumers (MSA, 2007). Palatability Analysis at Critical Control Points (PACCP) approach is established to predict the final eating quality of beef (Henchion et al., 2014). A multiple regression approach was used to develop the MSA prediction model which estimates the consumers' MQ4 score (Thompson, 2002). A series of factors were considered in the model, such as breed, dentition, marbling, muscle, days of ageing, cooking method, fat, *Bos indicus* content, pH-temperature decline rate (shortening), hanging method (tenderstretch/ achilles tendon), cattle handling method, use of Hormone Growth Promotant (HGP) and effect of dark-cutting meat (Polkinghorne, 2006). Extensive consumer testing was carried out and the results were utilized to calculate the MQ4 score. The best equation to predict the MQ4 score in Australia is 0.3 tenderness+ 0.1 juiciness+ 0.3 flavour liking + 0.3 overall palatability (Polkinghorne et al., 2008a). Some prediction traits such as use of hormonal growth promoters and % *Bos indicus* breed did not apply in United Kingdom, Republic of Ireland or other European countries. In addition, the MSA grading system did not include dairy breed, bulls or beef cooked to "well-done" in the equation (Farmer et al., 2010a). Therefore, it is important to understand the consumers from different regions to generate a suitable prediction model.

1.4 Linking consumer liking with the sensory attributes of a product

Meat, a unique sensory product, is one of the least homogenous food products in terms of its characteristics and composition, leading to high variability in sensory attributes. Today, consumers are changing and evolving rapidly in terms of cultural, socio-economic and ethical values. Consumer perception of beef involves consumers' expectation and experience of beef quality (Corcoran et al., 2001). Depending on the mismatch or match between the expectation and experience of consumers, this leads to consumer dissatisfaction or satisfaction which in turn affects future purchase intent.

Extrinsic cues, such as brand, origin of product and price have been found to influence consumer choice of beef products (Barrena and Sánchez, 2009, Bower et al., 2003, McEachern and Schröder, 2004). A body of research focusing on intrinsic cues, included the influence of animal welfare, production systems, traceability, food safety on beef eating quality (Bernués et al., 2003, Font and Guerrero, 2014, Loureiro and

Umberger, 2007, McEachern and Schröder, 2004, Smith et al., 2008). These extrinsic and intrinsic cues influence consumers' preference towards the products, intention to purchase and willingness to pay for the product (Font and Guerrero, 2014, Killinger et al., 2004, Tonsor et al., 2005).

Consumer panels can be applied in different situations such as shelf life testing, new product development, product ingredient changes, product improvement or product maintenance (Civille and Oftedal, 2012). Participants should be selected to represent the population of product end-user. Recruitment of panellists can be carried out in various ways such as established databases, random telephone solicitation, selection from community organisations, advertisements, posters or intercepts at shopping malls. Consumer panels can be conducted in a variety of sites such as in the home, sensory laboratory or mobile lab in a shopping mall, bus or van. The aim of the project can be helpful in determining where the test should take place. Factors such as social pressures, effort, consumption time and convenience should be considered to select an appropriate location (Jellinek, 1985).While consumer sensory tests mainly focus on measuring preference or liking, the tests can be taken one step further for market research by combining them with mathematical modelling to predict features such as product consumption and purchase intent (Munoz, 2002). A range of scale types are available such as frequency of consumption, price scale questionnaire, just about right, importance, line scale, or nine point hedonic. Some consumer panels require screening due to experimental design to meet demographic requirements.

Quantitative descriptive analysis (QDA) is a sensory analysis method using trained panellists (instead of naïve consumers) to give a detailed sensory description or profile of a product including texture, aroma, flavour or aftertaste (Jellinek, 1985). Panellists are recruited based on their consumption frequency, availability and motivation, ability to discriminate basic tastes, sensory acuity and consistency of performance (Civille and Oftedal, 2012). After extensive training has been provided to the panellists, they are able to provide detailed evaluations of product qualities and function like a laboratory instrument. Therefore, it is inappropriate to ask consumers to provide descriptive information or quantify product characteristics because they have not been calibrated and trained for this type of test. QDA has been extensively used to analyse different meats including pork (Gao et al., 2015, Meinert et al., 2007), chicken (Aliani and Farmer, 2005, Peter et al., 2017), lamb (Costa et al., 2018, Murphy

and Zerby, 2004, Oltra et al., 2015) and beef (Drey et al., 2019, Farmer et al., 2012, Luchak et al., 1998).

Consumer data are analysed by employing different methods such as analysis of variance, regression analysis or principal component analysis (Ellekjær et al., 1996, Lea et al., 1997, Tenenhaus et al., 2005). Consumer data can be combined with the data from trained panellists using external preference mapping, which is a technique applied to investigate consumer market segments (Arditti, 1997, Oltra et al., 2009, Oltra et al., 2010, Oltra, 2010). There are two types of preference mapping techniques: external preference mapping (PrefMap) and internal preference mapping (MDPref) (Endrizzi et al., 2014). In PrefMap, priority is given to the perceptual data, which is obtained from the sensory profiling panel. On the other hand, MDPref gives priority to consumer liking scores. The product space accounts for variation in consumers' preference data and the perceptual data is regressed into this product space to explain the difference in preference (Worch, 2013).

1.5 Differences between consumers from different countries or regions

Many papers have discussed whether the consumers from different countries or regions have similar perception of beef. In fact, the term "meat" has different meanings in different countries. For example, the term "meat" refers to edible meat for Ghanaian and Chinese consumers while consumers from Argentina refers mainly to bovine skeletal muscles (Liu et al., 2017, Ohene-Adjei and Bediako, 2017, Pavan et al., 2017). For Italian consumers, the term "meat" also consists of processed meat products while the definition is very diverse among Australian consumers (Dalle Zotte et al., 2017, Warner et al., 2017). Some markets might have well defined traditional practices that apply to the presentation, production or use of beef products which affects the local consumers' attitudes. The outcome of sensory evaluation of meat products can also be heavily influence by consumer age groups, gender, ethnicities and cultures (Bekker et al., 2017).

Differences between consumers from different regions in the United States of America are reported, where consumers rated top sirloin steak the lowest in Philadelphia while consumers in San Francisco rated top loin steaks the lowest (Neely et al., 1998). Minor demographic differences between Korean and Australian consumers were reported by Hwang et al. (2008) and Thompson et al. (2005). On the contrary, previous research stated that all consumers judge beef product similarly provided that they get the beef cooked to their preferred endpoint, whether they are from South Korea, Australia, South Africa or Northern Ireland (Egan et al., 2001). A study comparing Spanish and United States (US) consumers found that all consumers preferred US beef over European beef until the consumers were informed that the former had not originated locally, at which point that the consumers switched their preferences to locally produced beef (Sánchez et al., 2012).

The strategy to apply a common grading system globally depends heavily on whether consumers from different markets and cultures are consistent in their beef quality ranking and sensory ratings. To investigate the feasibility of this strategy, MSA has conducted large consumer study involving 67,900 Australian consumers and 13,140 consumers from France, Northern Ireland, Republic of Ireland, South Korea, South Africa and Japan for approximately 15 years (Bonny et al., 2017, Farmer et al., 2009, Farmer et al., 2010b, Hocquette et al., 2011, Hwang et al., 2008, Legrand et al., 2012, Polkinghorne et al., 2011, Polkinghorne and Thompson, 2010), and the results showed that this grading system is internationally applicable. Interestingly, the prediction models and the satisfaction scores were slightly varied in different countries as showed in Table 1.1. The best prediction model in Northern Ireland is 0.2 *tenderness* + 0.1 *juiciness* + 0.4 *flavour liking* + 0.3 *overall liking*. Therefore, the author proposed to further analyse if the consumer preferences differ between regions, including Northern Ireland (N.I), Republic of Ireland (R.O.I) and Great Britain (G.B).

Table 1.1 MSA prediction models in different countries.

Country	Tenderness (a)	Juiciness (b)	Flavour liking (c)	Overall liking (d)
Australia	0.3	0.1	0.3	0.3
France	0.3	0.1	0.3	0.3
Japan (Grill)	0.3	0.2	0.2	0.3
Japan (Shabu-shabu)	0.2	0.2	0.4	0.2
Northern Ireland	0.2	0.1	0.4	0.3
USA	0.3	0.1	0.3	0.3
Poland	0.2	0.1	0.4	0.3

MQ4 score= (a) tenderness + (b) juiciness + (c) flavour + (d) overall liking (Hocquette et al., 2014).

1.6 Aroma and flavour of beef

1.6.1 Physiology of aroma and flavour

Flavour is detected by humans in a complex sensory system of tissues in the mouth, on the tongue, nasal cavities and sinus. Aromas are detected by the olfactory receptors, basic tastes are identified by the gustatory sensory cells while the somatosensory perception is detected by the trigeminal nerves. Smells play a vital role in flavour perception and gustatory flavours (Mozell et al., 1969, Shepherd, 2005). Distinct cell types on the human tongue express unique taste receptors that detect one of the five basic tastes; umami, sweet, salty, sour and bitter (Chandrashekar et al., 2006). Trigeminal nerves are connected to the sinus cavity and the mucous membrane in the mouth, which in turn integrate with aromas and tastes (Laska et al., 1997). The combination of these three senses determines the overall flavour characteristics (Cerf-Ducastel et al., 2001).

Although meat flavour is a mixture of odour and taste, juiciness and mouthfeel of meat might also influence the consumer perception of beef flavour (Farmer, 1992, Robbins et al., 2003). The overall flavour is detected by three senses, including the somatosensory perception by trigeminal nerves, aroma by the olfactory receptors and basic taste by the gustatory sensory cells.

1.6.2 Understanding the beef aroma and flavour

Back in early days, tenderness of beef was identified as the most important attribute influencing palatability, with beef flavour rated as the second most important attribute (Boleman et al., 1995, Miller et al., 2001). Since then, the Beef Customer Satisfaction Survey reported that tenderness and flavour equally contribute to overall liking of beef (Lorenzen et al., 1999, Neely et al., 1998, Neely et al., 1999, Savell et al., 1999). In 2011, the National Beef Quality Audit (NBQA) in the United States identified that beef flavour was more important compared to tenderness (Igo et al., 2013, Sitz et al., 2005). Common flavour attributes such as sour, bitter, liver-like, warmed-over, gamey and metallic were negatively associated with flavour liking of beef while fat-like, umami, brown, roasted, beefy, sweet and salty were positive flavour characteristics (Adhikari et al., 2011, Maughan et al., 2012).

Raw meat generally has a bloody, metallic and salty taste with sweet aroma resembling serum (Wasserman, 1972). A previous study showed that no meaty aroma formed after fillet steak was heated on a skillet at 104°C for 1min, however, after cooking at 171°C, a meaty aroma was reported (Macleod and Ames, 1986). This indicated the importance of heat in the production of pleasant aroma normally found in cooked beef.

Cooked meat flavour is derived from precursors, such as the constituents of fats and low molecular weight water-soluble compounds and has been reviewed by Legako (2016), MacLeod (1994) and Mottram (1991).

This section will focus on the volatile compounds, sugars and sugars phosphates which contribute to beef flavour and aroma. A variety of low molecular weight volatile compounds such as alcohols, sulphur-containing compounds, nitrogen-containing compounds, esters, hydrocarbons, aldehydes, pyrazines and ketones have been identified in cooked beef (Landy et al., 1996). In addition, non-volatile constituents (peptides, amino acids, sugars, organic acids and inorganic salts) of fresh meat are vital for taste of cooked meat (Koutsidis et al., 2008b, Madruga et al., 2010, Ramalingam et al., 2019). The Maillard reaction, which is the reaction between amino acids and reducing monosaccharides, is one of the most important processes for flavour generation in cooked meat. These reducing monosaccharides consist of glucose, glucose-6-phosphate, ribose, ribose-6-phosphate, fructose, fructose-6-

phosphate, mannose and mannose-6-phosphate (Farmer et al., 1989, Farmer et al., 1999, Koutsidis et al., 2008b, Lauridsen et al., 2006, Mottram and Nobrega, 2002).

1.6.2.1 Formation of beef aroma and flavour

The Maillard reaction, lipid thermal degradation, thiamine degradation, and interaction of Maillard reaction products with lipid thermal degradation product are the main reactions triggered by high temperatures that are responsible for cooked beef aroma (Figure 1.2) (Dashdorj et al., 2015, Macleod and Ames, 1986).

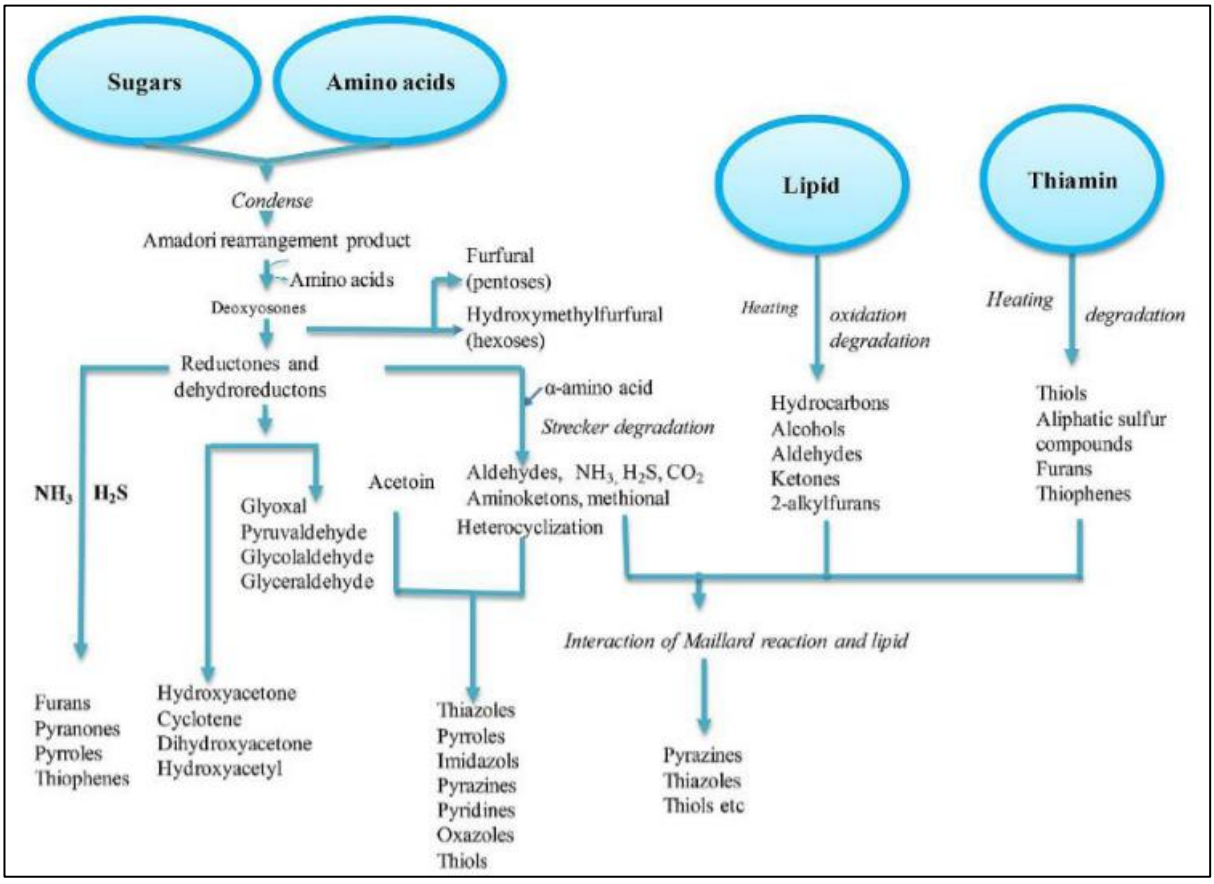


Figure 1.2 Schematic diagram illustrating the formation of volatile compounds derived from Maillard reaction, lipid thermal degradation and thiamine degradation, reproduced from Dashdorj et al. (2015).

The Maillard reaction is the process of non-enzymatic browning of beef at high temperature, which involves the degradation of proteins in the presence of reducing

sugar. Carbon, hydrogen, oxygen, the sulphur from the side chains of amino acids and nitrogen (N) from the peptide backbone are the primary elements derived from protein. The first stage of the Maillard reaction is a dehydration reaction followed by the Strecker degradation of the amino acid (Thorpe and Baynes, 2003). The deamination and decarboxylation of amino acids leads to the formation aldehydes. In addition, Strecker degradation can generate many flavour compounds, such as sulphur-containing compounds (thiazoles, thiophenes), nitrogen-containing compounds (pyrroles, pyrazines), oxygen-containing compounds (furans) and other heterocyclic volatile compounds (Thorpe and Baynes, 2003). These common compounds are shown in Table 1.2.

Lipid thermal degradation is the disassembly of polar lipids (phospholipids) and neutral lipds (triglycerides). Reactions that occur during the non-oxidative heating of lipids comprise of dehydrocyclisation, dehydrogenation, polymerisation, dehydration, decarboxylation and degradation of the carbon bond cleavage (Nawar, 1969). Fatty acids and fats have vital roles in contributing to a specific meat flavour. Shorter chain fatty acids are released to a greater extent compared to longer chain fatty acids when triglycerides are heated, due to higher water solubility of short chain fatty acids (Nawar, 1969). Thermal breakdown of lipids is responsible for the development of specific volatiles such as aldehydes, ketones and alcohols (Table 1.2) (Meinert et al., 2007, Soncin et al., 2007). Interestingly, the volatile products produced from lipid oxidation and lipid degradation are similar. However, the compounds derived from thermal lipid degradation are described as having positive, favourable aromas, while those that are derived from lipid oxidation are described as being negative, with rancid characteristics (Mottram, 1985, Mottram, 1998).

Interaction between products formed between Maillard reaction and lipid degradation have been extensively studied (Elmore et al., 2002, Xu et al., 2011). For example, aldehydes, produced from lipid degradation, could contribute to the Maillard reaction and induce the formation of thiazoles, thiophenes, pyrazines and pyridines. Shahidi et al. (2014) suggested that products from lipid degradation prevent the formation of heterocyclic compounds from Maillard reaction. However, such inhibition maintains the concentration of sulphur compounds. Overall, the volatile compounds formed from Maillard-lipid interaction generally have higher odour threshold and weaker odour intensities compared to those formed in Maillard reaction or lipid degradation.

Disulphides, sulphides and thiols are produced from the thermal degradation of cysteine, thiamine and ribose, which contribute to the development of cooked beef aroma (Kerscher and Grosch, 1998). Increased contents of bis(2-methyl-3-furyl) disulfide. 2-methyl-3-methyldithiofuran and 2-methyl-3-furanthiol were correlated with the increase of thiamine (Kosowska et al., 2017). A previous study also showed the significance of thiamine as an important precursor for aroma of cooked ham (Thomas et al., 2015).

Table 1.2 Common volatile compounds for cooked beef and their flavour characteristics.

Compound name	Detection (ppm)	Characteristic flavours/aromas
Aldehydes		
Decanal	0.002	Orange, citrus peel, waxy
Hexanal	0.005	Green, tallow, fatty, grassy
Nonanal	0.001	Citrus, floral, waxy, soapy
Octanal	0.007	Citrus, orange peel, lemon, green
Heptanal	0.003	Oily, unpleasant, fatty, rancid
Pentanal	0.01	Winey, fermented, almond, pungent, bready
Octadecanal		Oil
Undecanal	0.0004	Waxy, laundry detergent, metallic, soapy, buttery
Strecker aldehydes		
2-Methyl-butanal	0.002	Malty, mushy, fruity, green
2-Methyl-propanal	0.0004	Apple-like odour, pungent
3-Methyl-butanal	0.0005	Malty, fatty, fish, rotten
Acetaldehyde	0.02	Green, fresh
Benzaldehyde	0.35	Nutty, wood, almond
Benzeneacetaldehyde	0.004	Sweet, rosy, floral honey
Phenyl acetaldehyde	0.004	Sweet, rosy, honey
Alkenals		
2-Hexenal	0.11	Green apple, almond, bitter
(E)-2-Decenal	0.004	Mushroom, fungal, earthy
(E)-2-Heptenal	0.01	Green, apple, sweet
(E)-2-Hexenal	0.02	Almond, green apple
(E)-2-Nonenal	0.00008	Green, fatty
Alcohols		
1-Octanol	0.11	Green, waxy, orange, citrus
1-Butanol	0.5	Malty, solvent
1-Heptanol	0.003	Fragrant, apple, winey, fruity, citrus, green
1-Hexanol	2.5	Green, apple, fruity, cut grass

Compound name	Detection (ppm)	Characteristic flavours/aromas
1-Pentanol	4	Fusel, fusel oil, balsamic, fermented
1-Octen-3-ol	0.001	Mushroom, fungal, earthy
Ketones		
2-Butanone	50	Green, fruity, chemical
2-Heptanone	0.14	Cheesy, spicy, banana, cinnamon, fruity
2-Nonanone	0.2	Cheesy, soapy, green, floral, fruity, buttery
2-Pentanone	0.04	Banana, sweet, fruity,
2-Octanone	0.07	Musty, fruity
2-Undecanone		Fruity
2,3-Butanedione	0.007	Buttery
2,3-Pentadione		Fruity, sweet, lemon
Butyrolactone	20	Milky, peachy, creamy
3-Hydroxy-2-butanone	8	Strong, creamy, buttery
Sulphur-containing compounds		
Dimethyl sulfide	0.001	Putrid, asparagus
Dimethyl disulfide	0.000005	Onion, rubbery, mouldy, pungent
Dimethyl trisulfide	0.00002	Sulphurous, gassy
Methanethiol	0.0002	Eggy, creamy, vegetable oil
Benzothiazole	0.08	Metallic
2-Acetylthiazole	0.004	Corn chip, roasted
Pyrazine		
Methylpyrazine	6027	
2,3-Dimethylpyrazine	2.5	Musty, potato, meaty
Trimethylpyrazine	0.009	Raw, potato, musty
2,5-Dimethylpyrazine	1.7	Musty, cocoa, potato, green
3-Ethyl-2,5-dimethylpyrazine	15	Popcorn, peanut, coffee, caramel
2-Ethyl-3,5-dimethylpyrazine	0.001	Roasted nuts, coffee, fragnant
2-Ethyl-5-methylpyrazine	0.1	Roasted nuts, coffee, sweet
Alkanes		
Dodecane		Fragrant, floral, geranium
Hexane		Faint peculiar odour
Pentane		Oxidised, warmed-over
Furans		
2-Pentylfuran	0.006	Butter, green bean
Terpenes		
α-Pinene		Piney, citrus, fruity
β-Pinene		Pine, turpentine, citrus, fruity
α-Pinene	0.006	Woody, turpentine, pine
Limonene	0.00001	Citrus, lemon
Dienals		
2,4-Decadienal	0.00007	Deep fat flavour, grapefruit, citrus, orange
2,4-Nonadienal	0.00006	Pungent, fat, wax, green, watermelon
(E,E)-2,4-Decadienal	0.00007	Deep fried, fat

Compound name	Detection (ppm)	Characteristic flavours/aromas
2,4-Nonadienal	0.06	Green, fatty
2,6-Nonadienal	0.05	Cucumber
Acids		
Heptanoic acid	3	fruity, cheesy
Hexanoic acid	3	Lavender, fragrant, floral
Pentanoic acid	11	Fruity, sweaty
Propanoic acid	5	Fruity, acidic, dairy
3-Methyl-butanoic acid	540	Sweaty
Acetic acid	180	Sour

(Ba et al., 2012, Buttery et al., 1988, Buttery and Ling, 1995, Christlbauer and Schieberle, 2011, Farmer et al., 2013, Legako et al., 2015, Machiels et al., 2004).

1.6.2.2 Influence of reducing sugars on flavour formation

Glucose is normally present at the highest concentration in beef followed by fructose while ribose is normally at the lowest concentration (Koutsidis et al., 2008a). The quantities of reducing sugars decreased during cooking, with fructose showing the highest loss in quantity (Madruga et al., 2010). The decrease in sugar quantities is because of their involvement in the Maillard reaction process (Madruga, 1994). The effects of reducing sugars on flavour generation on meat has been studied by the addition of sugars to meat follow by volatile analysis. Addition of xylose into mutton positively modified the flavour (Hudson and Oxly, 1983). Ribose is believed to be the most vital reducing sugar as a previous study showed that a higher concentration of ribose caused an increase in the concentration of 2-furanmethanethiol which led to an enhanced roasted aroma in chicken (Aliani and Farmer, 2005). Glucose-6-phosphate, ribose and inosine monophosphate increased the roasted aroma in pork (Farmer et al., 1999). Madruga et al. (2010) reported 44% of ribose remained after cooking, which is surprising because ribose has been reported previously as the most active reducing sugar in the Maillard reaction (Mottram, 1998).

1.6.3 Factors influencing volatile compounds of beef

There are a few factors that can influence the formation of volatile compounds. Extrinsic and intrinsic factors that might influence the formation of these compounds will be discussed in this section.

The pH of beef plays a vital role in the development of flavour through the Maillard reaction. The polymeric compounds and colour increase when pH increases, and nitrogen-containing volatile compounds such as pyrazines are favoured (Mottram and Madruga, 1994). Higher ultimate pH of meat favours the generation of thiophenones and thiazoles but decreases the formation of sulfur-containing compounds, thus decreasing the overall meat flavour intensity (Mottram and Madruga, 1994). The water binding properties and heat transfer increase with increasing pH in beef. High pH meat or dark, firm and dry meat is said to have serumy, bloody, grassy, cowy, mouldy and musty flavour (Kerth and Miller, 2015). A previous study showed that the concentration of furans decreased while the concentration of thiophenes, pyrimidine and pyrazine increased when the pH increased from 4.5 to 6.5 (Meynier and Mottram, 1995).

Many studies have compared the differences in tenderness between different cuts because there is approximately three times variation compared to the differences in flavour (Shackelford et al., 1995, Wulf and Page, 2000). However, a few studies have studied the differences in beef flavour between different cuts (Jeremiah et al., 2003a, Jeremiah et al., 2003b, Rhee et al., 2004, Shackelford et al., 1995). *M. vastus lateralis, M. rectus femoris* and *M. triceps brachii* have lower rancid or metallic characteristics compared to *M. vastus intermedius* (James and Calkins, 2005). These differences might be due to the differences in beef flavour profile. For example, 2-heptanone and 2-nonanone were detected in M.vastus lateralis but not in *M. vastus intermedius*, *M. rectus femoris* and *M. triceps brachii* (Hodgen et al., 2006).

The chemical traits and physical properties of beef determine the formation of volatile compounds during the cooking process. There are two types of cooking conditions, dry conditions or moist conditions. Dry cooking conditions such as pan-frying, broiling or grilling use sufficient temperature (over 177°C) that lead to a change in surface colour and formation of Maillard products. The internal temperature of beef increases with the length of time the meat held at the high temperature. The external surface and internal portions of the samples will have distinct flavour profiles (Lorenzen et al., 1999, Lorenzen et al., 2003). On the other hand, moist cooking conditions cause the meat to be cooked at low temperature (less than 100°C), which avoid the process of Maillard reaction on the exterior surface of beef and affects the volatile profile of beef. High quantities of volatile compounds derived from lipid

oxidation were identified in beef cooked at moderate (80 °C for 6 h) and low cooking condition (60 °C for 6 and 24 h) while higher quantities of volatile compounds derived from Strecker degradation were detected in beef cooked under intense conditions (80 °C for 24 h) (Roldán et al., 2015).

Beef shows significant changes during the ageing period in the quantities of numerous compounds such as flavour precursors, inosine-5'-monophosphate and amino acids which lead to changes in volatile profile (Feidt et al., 1996, Koutsidis et al., 2008a, Mullen et al., 2000, Watanabe et al., 2004). The quantities of Strecker aldehydes and nitrogen-containing compounds increase with ageing (Watanabe et al., 2015). Free amino acids including leucine, lysine, isoleucine, phenylalanine and methionine increased with ageing (Koutsidis et al., 2008a). The process of post-mortem ageing also had positive impacts on the organoleptic properties for *M. longissimus lumborum* (Koutsidis et al., 2008a). These compounds act as a pool of intermediate and reactive flavour chemicals which react to form volatile compounds during cooking. Strecker reaction, Maillard reaction and lipid oxidation are most likely to be responsible for the changes in flavour precursors (Resconi et al., 2013). Study conducted by Jeremiah and Gibson (2003) suggested that post-mortem ageing improved tenderness, intensity of flavour and desirability. Another study also showed improved flavour characteristics and the author suggested this was due to the changes in the amount of volatile compounds (Gorraiz et al., 2002).

1.7 Measurement of volatile compounds

Flavour volatile compounds are collected by analytical instrument such as gas chromatography-mass spectrometry. Sample preparation is the first step in most of these analytical techniques. The sample preparation step is essential because some samples cannot be directly analysed by the instrument (e.g. solid samples) or the analyte cannot be directly measured by the instrument (e.g. a derivatization step is required before analysis). However, this step is also a source for uncertainty and errors due to human error. Therefore, choosing an appropriate method to collect and concentrate the volatile compounds significantly affects the sensitivity of the analytical method. The ideal sample preparation technique should be fast, simple, cost

effective, reproducible, have low sample consumption, high accuracy, high sensitivity, and preferably be automated (Ghorbani et al., 2018). Several methods that are commonly used for volatile compound collection will be discussed, focusing especially on one of the most widely used techniques, solid-phase microextraction.

1.7.1 Headspace method for collection of volatile compounds

Several collection techniques are available to collect headspace volatile compounds and the most common extraction technique for food analysis is non-exhaustive extraction techniques (Reyes-Garcés et al., 2018).

Examples of non-exhaustive extraction techniques are static headspace sampling, dynamic headspace concentration and solid phase-microextraction. For the static headspace sampling method, volatile compounds of the samples are first equilibrated with the surrounding environment in an airtight container such as a glass vial. The volatile compounds are collected by a gas-tight syringe and injected into the inlet of a gas chromatograph (GC) (Qualley and Dudareva, 2009)

Dynamic headspace concentration techniques have also been widely used to collect volatile compounds. These methods are particularly suitable for samples with ultra-trace levels of volatiles, ranging from parts-per-million to parts-per-trillion (Wojnowski et al., 2017). Purified gas is passed through the samples and the volatile compounds are collected and concentrated onto a solid adsorbent material. The volatile compounds are released from the adsorbent material using organic solvents or directly injected to the GC inlet via thermal desorption. The thermal desorption technique has higher sensitivity compared to the organic solvent flushing technique (Maafi, 2017). This technique has been employed to analyse volatile compounds of meat samples (Chen et al., 2009, El-Magoli et al., 1996, Farmer et al., 2013, Rivas-Cañedo et al., 2011).

The solid-phase microextraction (SPME) method was introduced in 1990 by Arthur and Pawliszyn (1990) as a rapid, easy and solvent-less method compared to the other volatile extraction techniques. In recent years, the SPME collection technique has become increasingly popular because of its ease of use. The preparation time for

sample collection is reduced and the technique is said to provide high selectivity extraction capacity.

1.7.2 Solid phase microextraction technique

1.7.2.1 Principle of the SPME technique

Headspace SPME methods can be employed for non-invasive and direct extraction techniques for cooked beef volatiles. An SPME fibre contains adsorbent material which acts as the SPME stationary phase (Figure 1.3). The fibre is inserted into a syringe needle and introduced into the headspace of an airtight container containing the sample of interest (Figure 1.4). The SPME fibre is exposed to absorb the volatile compounds from the headspace of the samples and thermally desorbed into the GC inlet (Figure 1.4). Separation and identification of the volatile compounds are primarily performed using gas chromatography- mass spectrometry (GC-MS). GC-MS is a very robust and highly sensitive separation method (Dickschat, 2014, Matysik et al., 2009). The molecules are fragmented once they elute from the GC column and compounds are identified using their linear retention index and mass to charge ratio (Maafi, 2017).

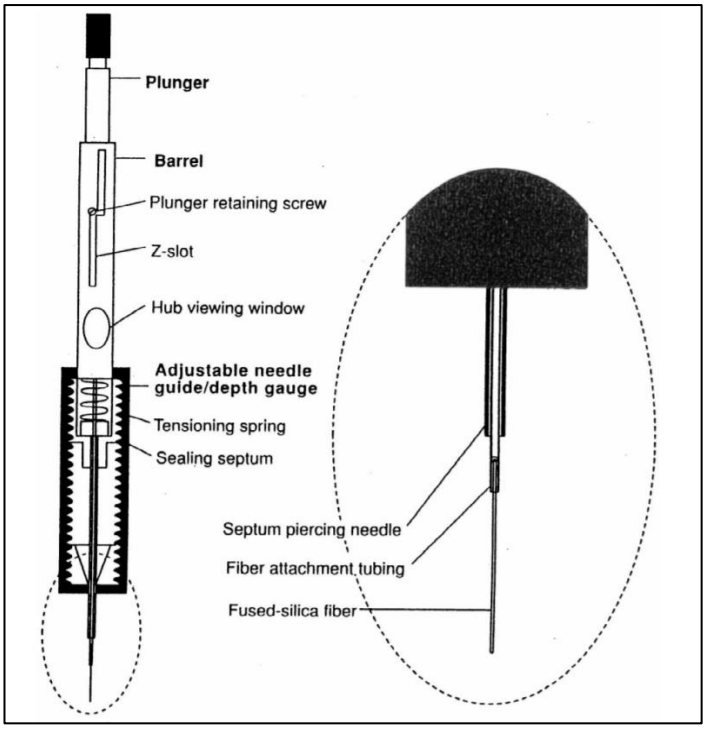


Figure 1.3 Schematic diagram of a SPME syringe equipped with SPME fibre, reproduced from Zhang et al. (1994).

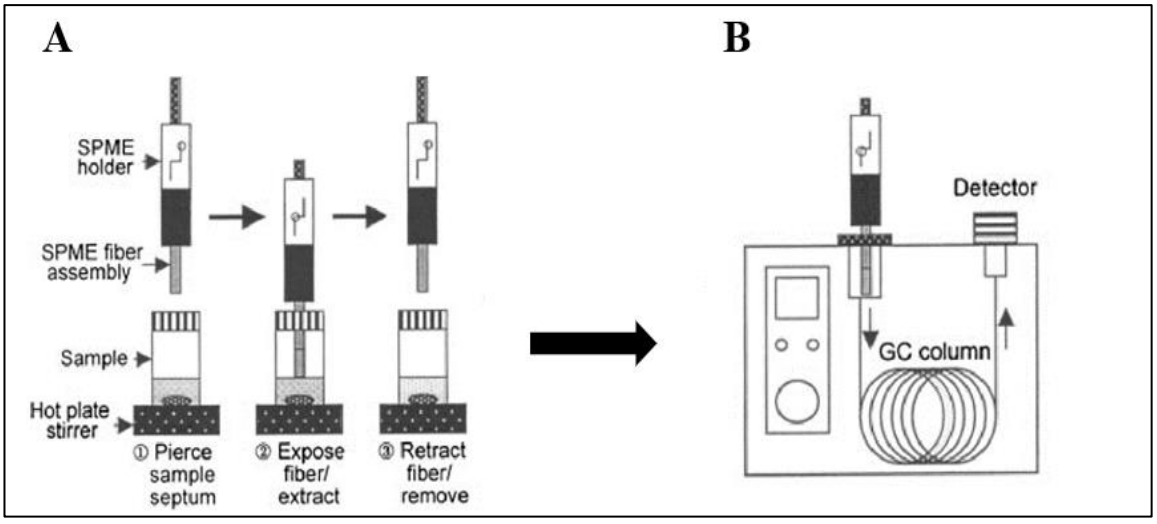


Figure 1.4 SPME process; (A) Extraction of volatile compounds using SPME fibre and (B) Thermal desorption on GC inlet, reproduced from Kataoka et al. (2000).

1.7.2.2 SPME fibre type

There are different types of SPME fibres with different stationary phases. Examples of the SPME fibres include polyethylene glycol (PEG), polyacrylate (PA),

polydimethylsiloxane (PDMS) and divinylbenzene (DVB). These stationary phases are different in their extraction mechanism (adsorbent or absorbent) and polarity (non-polar, polar and bi-polar). The affinity of the SPME fibre for the analyte type highly depends on the thickness and properties of the coating fibre using the "like dissolves like" principle (Kataoka et al., 2000). The common SPME fibres are listed in Table 1.3. The most common packing material is PDMS because of its physiochemical properties (Ulrich, 2000). SPME fibre with mixed fibre coatings (e.g. Carboxen-PDMS) extract through absorption of compounds depositing on the surface of the SPME fibre (Ouyang et al., 2005).

Thicker fibre coatings demand a longer extraction time but the recovery of thicker coatings is normally higher. In common practice, using the thinnest acceptable coating reduces the extraction time. In addition, the length of extraction is not dependent on the analyte concentration (Ulrich, 2000). During extraction, analytes equilibrate in the enclosed environment and diffuse in and out of the fibre stationary phase. Smaller analytes are retained over shorter lengths of time (Pawliszyn, 2011). The details of commercially available SPME fibre coatings were reviewed by Mani (1999).

Table 1.3 SPME fibre coating and the compounds to be analysed.

Recommended SPME fibre	Suitable analyte	Coating method	Polarity
Polyacrylate (PA)	Phenols, polar semi-volatiles	Cross-linked	Polar
Polydimethylsiloxane (PDMS)	Volatile compounds	Non-bonded	Non-polar
polyethylene glycol (PEG)	Polar compounds, alcohols	Non-bonded	Polar
Carbowax–DVB	Alcohols, polar analytes	Cross-linked	Polar
Carboxen–PDMS	Volatiles, low molecular weight compounds and gases	Cross-linked	Bipolar
PDMS-DVB	Nitro-aromatic compounds, amines and volatiles	Cross-linked	Bipolar
DVB–PDMS–Carboxen	Flavours and odours, semi-volatile and volatile	Cross-linked	Bipolar

1.7.2.3 Advancements in solid phase microextraction techniques

In recent years, instruments for volatile analysis have undergo considerable improvements and the measurement of volatile compounds with these instruments is now more efficient, more sensitive, faster, more accurate and convenient compared to older techniques. The challenge remaining for researchers and scientists is identification of methods that have the highest precision and accuracy for real samples (Płotka-Wasylka et al., 2015, Souza-Silva et al., 2015).

The heterogeneity of the sample matrix for beef samples is one of the difficulties faced by researchers wishing to identify or quantify the cooked beef volatile compounds. Most studies on beef volatile compounds utilise headspace volatile analyses, by placing the beef sample into an enclosed vial and the headspace volatile compounds are sampled with a solid phase microextraction (SPME) fibre (Legako et al., 2015, Lorenzo, 2014, Souza-Silva et al., 2015). The SPME fibre is then transferred and desorbed into a gas chromatograph/mass spectrometer (GC/MS) instrument, with the possibility of an olfactory port being equipped on the instrument enabling panellist to smell the volatile compounds as they elute from the GC. The benefit of measuring the volatile compounds in beef samples served to consumers or trained sensory panellists is to be able to correlate consumer liking with the volatile compounds.

The first reference in the scientific literature for automated SPME analysis was published by Arthur et al. (1992) prior to the commercial SPME fibres being released. Automation of the SPME extraction techniques provides a few advantages, for example, reduction of analysis time, higher reproducibility, less manual handling and faster sample throughput. Numerous studies used the automated SPME technique for a range of sample matrices (Fernando et al., 2003, Frost et al., 2003, Henriksen et al., 2001, Jiang et al., 2015). The application of automated SPME is well established and the ability of autosamplers to run 24 hours non-stop makes a manual SPME impractical and too slow (O'Reilly et al., 2005). Alongside the advantages, there are some disadvantages that require consideration. The hardware of the autosampler can be restricted, such as the size of the vial, type of trays and agitator hardware. The cost of an autosampler is also higher than the cost of consumables required for manual sampling. However, for routine SPME analysis, full automated SPME improves the

robustness of the SPME extraction method and working efficiency for researchers (Jiang et al., 2015).

1.8 Aims of the study

The overall objective of this postgraduate research project was to investigate the differences in consumers' perception of beef and gain a better understanding of meat eating quality, with a particular focus on flavour. The ultimate goal was to provide information that helps ensure the production of beef of consistent eating quality that meets consumer demands.

With this primary objective, four experiments were designed and performed to cover important topics relating to the differences between beef consumers from different regions and factors that affect the eating quality and flavour of beef. The development of new analytical techniques for cooked beef volatile compounds was conducted to identify extraction methods which were easier to use, more flexible and give higher reproducibility. These experiments have been organised into four chapters (Chapter 2 to Chapter 5).

Chapter 2 Consumer Perceptions of Beef- A comparison of consumers from Northern Ireland (NI), Republic of Ireland (ROI) and Great Britain (GB)

The goals of the work described in this chapter were, first, to identify the factors affecting the meat quality. In addition, the author wished to identify the similarities and differences in consumer perception of beef between consumers from Northern Ireland (N.I), Republic of Ireland (R.O.I) and Great Britain (G.B). The sensory characteristics of beef associated with consumers' preferences were investigated. Statistical analyses were conducted to identify the correlation between sensory evaluation and objective measurement. In addition, cluster analysis was conducted to identify consumer clusters with distinct scoring patterns and preferences of beef. Preference mapping techniques were employed to understand the differences between consumers from different regions and cluster groups.

Chapter 3 Investigation of the effect of value-added processes and muscles on beef quality

In recent years, value-added treatments have been shown to improve the eating quality of meat (Toohey et al., 2011, Marques et al., 2010, Brooks et al., 2010). This experiment was designed to understand the effect of value-added processes (tenderisation and extract solution enhancement) on objective measurements, flavour precursors and volatiles. Value-added treatments were selected from the previous studies conducted by Botinestean et al. (2018), Garmyn et al. (2012), McGilchrist et al. (2013), which have demonstrated changes in beef eating quality. This study aimed to investigate the change in flavour chemistry in samples tested by consumers in Australia. Principal component analysis was employed to understand the relationships between the volatiles, flavour precursors, objective measurements and consumer liking.

Chapter 4 Development of an accessible and robust headspace solid phase microextraction technique for the analysis of cooked beef samples

The main objective of this study was to develop and optimise a robust headspace-solid phase microextraction technique to analyse the volatile profile of cooked beef. With the availability of automated SPME, there was an opportunity for developing an alternative extraction method for cooked beef volatile compounds. Criteria such as ease of use, amount of beef samples required, flexibility of the methods, detection range of compounds, detection quantities of the compounds, reproducibility and ability to differentiate beef samples with different process condition were considered.

Chapter 5 Investigation of the effects of packaging and ageing period on the eating quality and flavour of two beef muscles

The aim of this experiment was to identify the effects of packaging method and extended ageing period on eating quality and beef flavour. To allow comparison, samples from two muscles were included. These samples were previously tested by

consumers and the results were published by Polkinghorne et al. (2018). The relationship between consumer liking of beef and chemical analyses was investigated with multivariate statistical analyses.

Chapter 6 General discussion and future directions for research

This chapter discussed the possible implications of the findings of Chapter 2 to Chapter 5 for the beef industry and for researchers in the meat science area. Additionally, future directions for research ideas were proposed with the intention to increase understanding of consumer perceptions of beef and to explore opportunities to increase consistency in the eating quality of beef.

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Chapter 2 Consumer Perceptions of Beef- A comparison of consumers from Northern Ireland (NI), Republic of Ireland (ROI) and Great Britain (GB)

2.1 Introduction and Objective

The development of the Meat Standards Australia (MSA) grading scheme focused on providing consumers in Australia with beef that was consistent in eating quality (Polkinghorne and Thompson, 2010). In order to evaluate the possibility of using MSA as an international grading scheme, studies were conducted to identify the differences in consumers between countries (Bonny et al., 2017, Bonny et al., 2018, Farmer et al., 2009a, Legrand et al., 2012, McCarthy et al., 2017), and it has been found that the MSA grading scheme is internationally translatable. As demonstrated in Chapter 1, some studies found differences between consumers from different regions or countries (Hwang et al., 2008, Neely et al., 1998, Thompson, 2002) while other studies did not (Bonny et al., 2017, Egan et al., 2001). Therefore, the author wished to identify the similarities and differences in consumer perception of beef between consumers from Northern Ireland (NI), Republic of Ireland (ROI) and Great Britain (GB). On the other hand, the beef industries in NI and ROI have exported a high proportion of beef products to many regions in GB. Therefore, the information about the consumer response from GB relative to the consumers in NI and ROI is commercially valuable to the beef industries.

MSA uses critical control points to control and predict beef eating quality. These are cooking method, breed, hanging method, muscle, ultimate pH, ageing period, electrical stimulation, low animal handling stress, marbling and maturity (Bonny et al., 2018, Polkinghorne et al., 2008). Some of these factors are not applied to United Kingdom and other European countries, such as use of hormonal growth promoters and % *Bos indicus* breed. In addition, the MSA system does not accommodate prediction of the eating quality for meat from bulls and cows. Therefore, this study will include samples from bulls and cows to investigate their eating quality.

It has been suggested that socio-economic factors such as age, gender, culture, income and education level might have impacts on consumer sensory scores or consumer

behaviour (Berry and Hasty, 1982, Thompson et al., 2005). In contrast, a previous study on Korean and Australian consumers showed limited socio-demographic effect on sensory scores (Hwang et al., 2008). Other studies showed that American, South African, Japanese and Irish consumers are prepared to pay twice as much for premium beef (Lyford et al., 2010, Thompson et al., 2010). In the present study, socio-economic status, consumer consumption habits and motivations of beef choice were investigated to thoroughly understand the consumers in NI, ROI and GB.

Differences in consumer perception of beef also occur between individual people. Cluster analysis is a useful tool to identify the variability among consumers, including consumer preferences and consumer behaviours (Schilling and Coggins, 2007, Tyron, 1939). Studies have been conducted on salami (Marino et al., 2017), organic food (Tleis et al., 2017), genetically modified food (Kaye-Blake et al., 2007) and beef (Schmidt et al., 2010). In order to understand the differences between consumers, cluster analysis will be employed in this study.

The purposes of this study are (a) to use the beef selected for this study (from different hanging methods, sexes, animal breeds and sample position on striploin) to evaluate the association between descriptive sensory attributes and consumer liking using external and internal preference mapping, (b) to assess the differences between consumers from NI, ROI and GB on their palatability traits, MSA grade boundaries, importance of palatability attributes, differences in willingness to pay and (c) identify consumer cluster groups and characterise how they differ in respect to preferences of beef and consumer scores and (d) evaluate the impact of socioeconomic and behavioural traits on sensory assessments.

2.2 Materials and Methods

2.2.1 Experimental design

The main experiment was a 3*2*2 design with three types of animals (bulls, steers, cows), two hanging methods (straight hung and aitch hung) and two breeds (continental and dairy). Beef striploins (n=72), representing 6 treatments and 12 sub-

groups (Table 2.1) were collected and animal ID number was allocated to each striploin.

Table 2.1 Details of sample treatments.

Hanging method	Bulls	Steers	Old Cows
Straight Hung (AT)	**Treatment 1a (T1a)** -Continental Breed -Animal 1-6	**Treatment 3a (T3a)** -Continental Breed -Animal 25-30	**Treatment 5a (T5a)** -Continental Breed -Animal 49-54
	Treatment 1b (T1b) -Dairy Breed -Animal 7-12	**Treatment 3b (T3b)** -Dairy Breed -Animal 31-36	**Treatment 5b (T5b)** -Dairy Breed -Animal 55-60
Aitch Hung (TS)	**Treatment 2a (T2a)** -Continental Breed -Animal 13-18	**Treatment 4a (T4a)** -Continental Breed -Animal 37-42	**Treatment 6a (T6a)** -Continental Breed -Animal 61-66
	Treatment 2b (T2b) -Dairy Breed -Animal 19-24	**Treatment 4b (T4b)** -Dairy Breed - Animal 43-48	**Treatment 6b (T6b)** -Dairy Breed -Animal 67-72

Boxes with purple background represent samples collected from NI and boxes with green background represent samples collected from ROI. The carcass weights were between 225kg to 411kg.

2.2.1.1 Animal information and collection

The animal treatments were selected to give a wide range of expected eating qualities, to ensure that consumers would perceive differences. The first batch of samples (Batch 1) was purchased from a commercial abattoir in Northern Ireland, and included T1a, T2a, T3a, T4a, T5a, T5b, T6a, T6b (Table 2.1). Sample specifications were agreed by supplier to meet the objectives of the study, these included:

1) No dark cutting meat.
2) Ensure all animal information (kill date, type, hanging method, age, animal number, EUROP, breed, HSCW) was on label.
3) Full striploin from each animal. Weight of each striploin approximately 10kg.

Continental breeds included Limousin (LIM), Simmental (SIM), Charolais (CH) and Blonde d' Aquitaine (DAQ) cattle. Dairy breeds included Holstein (HOL) and Friesian (FR) cattle. The average ages of continental bulls and continental steers were 15

months and 24 months, respectively. Older cows ranged from 35 months old to 188 months old. Six striploins were collected for each sub-group, with the AT and TS hanging method from different animals. After slaughter, the striploins were stored under 4°C and delivered to Agri-Food and Biosciences Institute (AFBI) in Belfast.

A second batch of samples (Batch 2) was collected from the Republic of Ireland, these included T1b, T2b, T3b and T4b. Dairy breed (Holstein-Friesian) calves were bought from commercial farms in Ireland. The calves were randomly assigned to 19 months bulls production system and 24 months steer production system. The animals were sent to a commercial abattoir for slaughter after they reached they intended age. After slaughtering, each hemi-carcass of the same animal was hung using TS method and AT method. The hanging method was assigned between consecutive animals. The difference in hanging method design between the NI and ROI animals was due to a misunderstanding. When the NI animals were slaughtered, it was understood that separate animals had been used for the two hanging methods for the Irish animals. This turned out to be incorrect. All carcasses were stored at 4°C for 2 days and divided into fore and hind quarters. The striploin was cut, vacuum packed and delivered to Teagasc Ashtown, where it was aged for another 19 days to achieve the total of 21 days aging period. Twenty-four striploins were collected and stored at -18°C until further sectioning. Animal ID number was allocated to each striploin. Sex, hanging method, breed, kill date, pH, conformation and fat score were recorded.

2.2.1.2 pH measurement and "cut up" protocol

A pH meter (Extech instrument, Waltham, MA) was calibrated with pH4 and pH7 solutions before taking the pH measuarement of the carcass. For samples from Batch 1, the ultimate pH (pHu) was measured using calibrated pH meter prior to the cutting process. The side muscles of the *longissimus lumborum* were removed and the anterior end was trimmed to ensure a flat surface for slice 1. Each striploin was cut into fifteen slices from anterior, each with 25mm thickness. Slice 1 and slice 6 were packed and labelled as Warner Bratzler Shear Force (WBSF) sample, aged to 21 and 7 days, respectively. Three sets of samples were cut from each striploin and labelled as anterior (A), middle (M) and posterior (P). The total number of sample sets was 216 (72 animals*3 sets). Set A was collected from slice 2 to slice 5, Set M was collected

from slice 7 to slice 10 and set P was collected from slice 12 to slice 15. Each slice was further cut into two or three small steaks depending on the size.

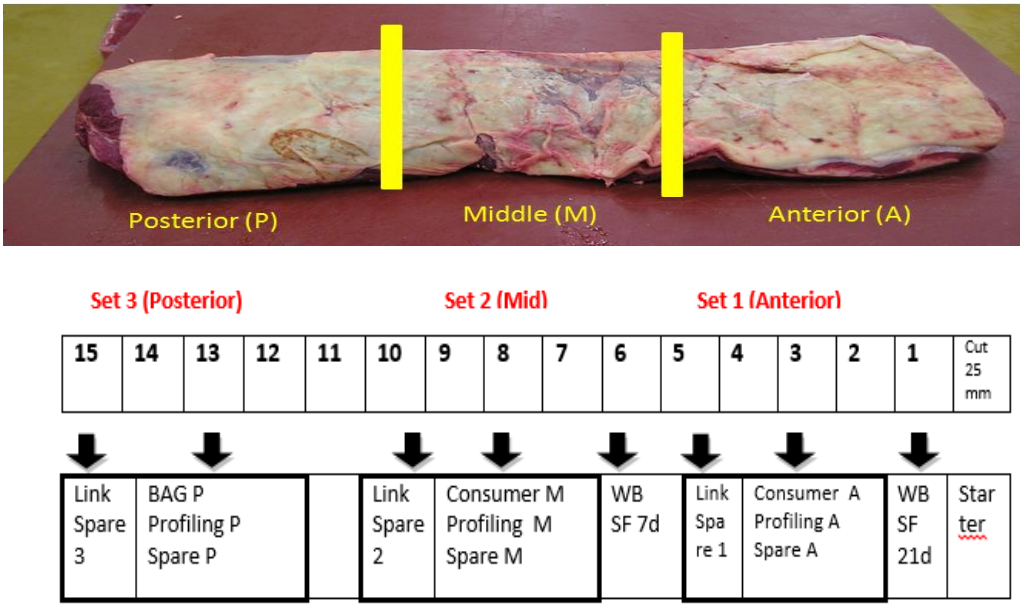


Figure 2.1 Demonstration of beef striploin cut up and collection of sample for different tests.

All samples were cut and packed in individual vacuum bag according to the test assigned (Figure 2.1). Slice 6 was labelled as WBSF Day 7 and aged for 7 days under 4°C. This sample was blast frozen and stored in -18°C commercial freezer. All other samples were aged for 21 days under 4°C, blast frozen and stored separately in -18°C commercial freezer. The temperature of the commercial freezer was monitored daily. Similar procedures were followed to cut up samples from Batch 2. However, the striploins were aged for 21 days, stored at -18°C and cut into slices using a band saw. Therefore, no WBSF 7 days samples were collected. pHu measurement was provided by Teagasc as part of the animal information.

2.2.2 Quantitative Descriptive Analysis (QDA)

2.2.2.1 Sensory profiling panel design

The sensory profiling panels consisted of eight expert trained panellists (4 females and 4 males). Extra samples were collected from the “cut up” and cooked for training

sessions. During the training sessions, these samples were tasted by the panellists and 48 attributes were generated. Definitions for each attribute were agreed by the trained panellists.

Two panellists (who were not adjacent) were paired up such that they received the same sample all the time (Table 2.2). During evaluation, panellists were seated into individual booths separated from the preparation area and samples were evaluated under fluorescent light. There were 6 samples from one striploin, with 2 samples from set A, set M and set P. The total servings for the profiling panel were 432 samples (72 animals* 6 samples). The samples were randomised for 9 sessions using a latin square design. The sensory software used to design the sessions were Biosystem Fizz Acquisition (company: Biosystem, home city: Dijon, Country: France).

Table 2.2 Panellist paired up for profiling panel.

Pair		
Pair 1	Panellist 1	Panellist 5
Pair 2	Panellist 2	Panellist 6
Pair 3	Panellist 3	Panellist 7
Pair 4	Panellist 4	Panellist 8

Two panellists from the same pair received the same samples.

2.2.2.2 Sensory profiling panel cooking protocol

Frozen steaks for assessment and scrap meat samples were tempered at 2°C for 24 hours. The grill (S-143, SILEXIA UK. Ltd, OXON, United Kingdom) was switched on 45 minutes prior to the session and the temperature was set to 180°C. Scrap meat was cooked on the grill for 4 minutes to condition the grill before the session commenced. There were 12 rounds of cooking in each session, 4 samples were cooked for 3 minutes 30 seconds on the grill at each round to achieve an internal temperature of 72°C (well done) as this cooking doneness was preferred by more than 50% of consumers in Northern Ireland (Farmer et al., 2009b) After 2 minutes of resting, the samples were cut in half and served to one pair of panellists. Internal temperature of samples was recorded. A small sample was collected from each beef steak and stored for 4 weeks for microbiological assessment, if required. Samples were identified using 3 random digit codes and served according to the sensory profiling panel design.

2.2.3 Sensory Consumer Panel Evaluation (Belfast, Cork, Reading)

2.2.3.1 Consumers recruitment

Three untrained consumer panels were conducted, which involved 360 consumers, 120 from each of Belfast, Cork and Reading. Consumers were recruited from these 3 cities so that the geographical areas Northern Ireland, Republic of Ireland and Great Britain were represented. All consumers were over 18 years old and were beef consumers who normally eat their steak cooked "medium" to "well done".

Consumers in Belfast were recruited in groups of 20 from charity groups, societies and clubs. Each group received a £200 donation upon completion of the session. Consumers in Cork and Reading were recruited individually. Methods of recruitment included posters, consumer databases provided by universities and internet recruitment (Facebook, gumtree, society pages or local forums). Consumers in Cork were rewarded with €20 cash and consumers in Reading received £10 cash on completion.

Basic personal information such as name, gender, age, email address and phone number were obtained during recruitment. All information remained confidential and was deleted after the panel. Clear instructions were given to all participants before the date of the panel. These include allocated session time, location of the study, arrival information, parking information, allergen information and a general outline of the objectives of the research project.

2.2.3.2 Consumer panel design

Seventy-two sets of samples were picked for consumer analysis at each region. The design is recorded in Table 2.3. The design was a latin square design with consideration based on the factors including sexes, breeds, sample position and hanging methods. An additional "link" sample was served to all consumers as the first round to provide a standardised benchmark and avoid any bias associated with the first sample.

Table 2.3 Samples used for consumer panels in Belfast, Cork and Reading.

BELFAST 1

Sample		PROD 1 Cow AT	PROD 2 Cow TS	PROD 3 Bull AT	PROD 4 Bull TS	PROD 5 Steer AT	PROD 6 Steer TS
1	Cont	49A	61A	1M	13M	25P	37P
2	Dairy	55A	67M	7M	19P	31P	43A
3	Cont	50M	62M	2P	14P	26A	38A
4	Dairy	56M	68P	8P	20A	32A	44M
5	Cont	51P	63P	3A	15A	27M	39M
6	Dairy	57P	69A	9A	21M	33M	45P

BELFAST 2

Sample		PROD 1 Cow AT	PROD 2 Cow TS	PROD 3 Bull AT	PROD 4 Bull TS	PROD 5 Steer AT	PROD 6 Steer TS
1	Cont	52A	64A	4M	16M	28P	40P
2	Dairy	58A	70M	10M	22P	34P	46A
3	Cont	53M	65M	5P	17P	29A	41A
4	Dairy	59M	71P	11P	23A	35A	47M
5	Cont	54P	66P	6A	18A	30M	42M
6	Dairy	60P	72A	12A	24M	36M	48P

CORK 1

Sample		PROD 1 Cow AT	PROD 2 Cow TS	PROD 3 Bull AT	PROD 4 Bull TS	PROD 5 Steer AT	PROD 6 Steer TS
1	Cont	49M	61P	1P	13A	25A	37M
2	Dairy	55P	67P	7A	19A	31M	43M
3	Cont	50P	62A	2A	14M	26M	38P
4	Dairy	56A	68A	8M	20M	32P	44P
5	Cont	51A	63M	3M	15P	27P	39A
6	Dairy	57M	69M	9P	21P	33A	45A

CORK 2

Sample		PROD 1 Cow AT	PROD 2 Cow TS	PROD 3 Bull AT	PROD 4 Bull TS	PROD 5 Steer AT	PROD 6 Steer TS
1	Cont	52M	64P	4P	16A	28A	40M
2	Dairy	58P	70P	10A	22A	34M	46M
3	Cont	53P	65A	5A	17M	29M	41P
4	Dairy	59A	71A	11M	23M	35P	47P
5	Cont	54A	66M	6M	18P	30P	42A
6	Dairy	60M	72M	12P	24P	36A	48A

READING 1

Sample		PROD 1 Cow AT	PROD 2 Cow TS	PROD 3 Bull AT	PROD 4 Bull TS	PROD 5 Steer AT	PROD 6 Steer TS
1	Cont	49P	61M	1A	13P	25M	37A
2	Dairy	55M	67A	7P	19M	31A	43P
3	Cont	50A	62P	2M	14A	26P	38M
4	Dairy	56P	68M	8A	20P	32M	44A
5	Cont	51M	63A	3P	15M	27A	39P
6	Dairy	57A	69P	9M	21A	33P	45M

READING 2

Sample		PROD 1 Cow AT	PROD 2 Cow TS	PROD 3 Bull AT	PROD 4 Bull TS	PROD 5 Steer AT	PROD 6 Steer TS
1	Cont	52P	64M	4A	16P	28M	40A
2	Dairy	58M	70A	10P	22M	34A	46P
3	Cont	53A	65P	5M	17A	29P	41M
4	Dairy	59P	71M	11A	23P	35M	47A
5	Cont	54M	66A	6P	18M	30A	42P
6	Dairy	60A	72P	12M	24A	36P	48M

Columns in yellow, green and red represented samples from anterior (Set A), middle (set M) and posterior (set P) respectively. The design does not include “link” samples.

The pick design was generated adapting the Meat Standard Australian (MSA) protocol following consultation with Rod Polkinghorne (Polkinghorne, 2006, Polkinghorne et al., 2008, Watson et al., 2008). The picked samples were further processed using NI Blue software, where each set of samples was given a specific NI Blue code. NI Blue Software is a macro designed in AFBI to facilitate consumer panels experimental design. NI Blue code, consisted of two random numbers and two random letters, representing a set of samples from specific animal and specific position (e.g. S49A represented set 49M). NI Blue software randomised these samples to 120 consumers from one region. Table 2.4 outlined an example design for the consumer panel in Cork for panellists 1 to panellist 60.

Table 2.4 Consumer experimental design for Cork panellists 1 to panellists 60.

Consumers	Round1 (Link)	Round2	Round3	Round4	Round5	Round6
1	A45R	S49A	X95X	Q32T	N97R	Y14D
2	A45R	S49A	X95X	Q32T	N97R	Y14D
3	A45R	D93L	W49Q	M49X	N77L	D23W
4	A45R	D93L	W49Q	M49X	N77L	D23W
5	A45R	Z75N	W20H	Z68Y	C14N	L47X
6	A45R	Z75N	W20H	Z68Y	C14N	L47X
7	A45R	J35G	V02B	M80H	D53L	R28N
8	A45R	J35G	V02B	M80H	D53L	R28N
9	A45R	H45Y	N65V	F88W	V17S	Z60S
10	A45R	H45Y	N65V	F88W	V17S	Z60S
11	F75C	D85N	P96M	F50V	J39X	K09K
12	F75C	D85N	P96M	F50V	J39X	K09K
13	F75C	L54R	H45Y	W49Q	Q32T	C14N
14	F75C	L54R	H45Y	W49Q	Q32T	C14N
15	F75C	R91A	D93L	N65V	F50V	D53L
16	F75C	R91A	D93L	N65V	F50V	D53L
17	F75C	J47S	J35G	W20H	F88W	J39X
18	F75C	J47S	J35G	W20H	F88W	J39X
19	F75C	X73W	S49A	P96M	M80H	N77L
20	F75C	X73W	S49A	P96M	M80H	N77L
21	W74A	W15C	D85N	X95X	Z68Y	V17S
22	W74A	W15C	D85N	X95X	Z68Y	V17S
23	W74A	V72F	Z75N	V02B	M49X	N97R
24	W74A	V72F	Z75N	V02B	M49X	N97R
25	W74A	Y14D	X73W	D85N	W20H	F50V
26	W74A	Y14D	X73W	D85N	W20H	F50V
27	W74A	L47X	V72F	J35G	N65V	M80H
28	W74A	L47X	V72F	J35G	N65V	M80H
29	W74A	R28N	J47S	Z75N	W49Q	Z68Y
30	W74A	R28N	J47S	Z75N	W49Q	Z68Y
31	G28Y	K09K	W15C	S49A	V02B	Q32T
32	G28Y	K09K	W15C	S49A	V02B	Q32T
33	G28Y	D23W	R91A	H45Y	X95X	F88W
34	G28Y	D23W	R91A	H45Y	X95X	F88W
35	G28Y	Z60S	L54R	D93L	P96M	M49X
36	G28Y	Z60S	L54R	D93L	P96M	M49X
37	G28Y	N97R	L47X	J47S	H45Y	W20H
38	G28Y	N97R	L47X	J47S	H45Y	W20H
39	G28Y	D53L	D23W	L54R	S49A	W49Q
40	G28Y	D53L	D23W	L54R	S49A	W49Q
41	E19U	N77L	Y14D	W15C	Z75N	X95X
42	E19U	N77L	Y14D	W15C	Z75N	X95X
43	E19U	V17S	K09K	X73W	J35G	P96M
44	E19U	V17S	K09K	X73W	J35G	P96M
45	E19U	C14N	Z60S	R91A	D85N	N65V
46	E19U	C14N	Z60S	R91A	D85N	N65V
47	E19U	J39X	R28N	V72F	D93L	V02B
48	E19U	J39X	R28N	V72F	D93L	V02B
49	E19U	Q32T	V17S	Y14D	J47S	D85N
50	E19U	Q32T	V17S	Y14D	J47S	D85N
51	Z09E	F50V	N77L	K09K	V72F	S49A
52	Z09E	F50V	N77L	K09K	V72F	S49A
53	Z09E	Z68Y	J39X	L47X	R91A	J35G
54	Z09E	Z68Y	J39X	L47X	R91A	J35G
55	Z09E	F88W	D53L	Z60S	X73W	D93L
56	Z09E	F88W	D53L	Z60S	X73W	D93L
57	Z09E	M49X	C14N	D23W	W15C	H45Y
58	Z09E	M49X	C14N	D23W	W15C	H45Y
59	Z09E	M80H	N97R	R28N	L54R	Z75N
60	Z09E	M80H	N97R	R28N	L54R	Z75N

Link samples were served in round 1 to all panellists. As an example, the distribution of samples “S49A” were highlighted in red.

NI Blue software, automatically produced labels for plates, labels for micro-samples and posting sheet for each cooking round. The posting sheet outlined the ten samples required for a specific cooking round (Figure 2.2). During sorting, the sample was removed from the bag and put on the posting sheet according to their NI Blue code. The bag was vacuum packed and stored in -18°C commercial freezer.

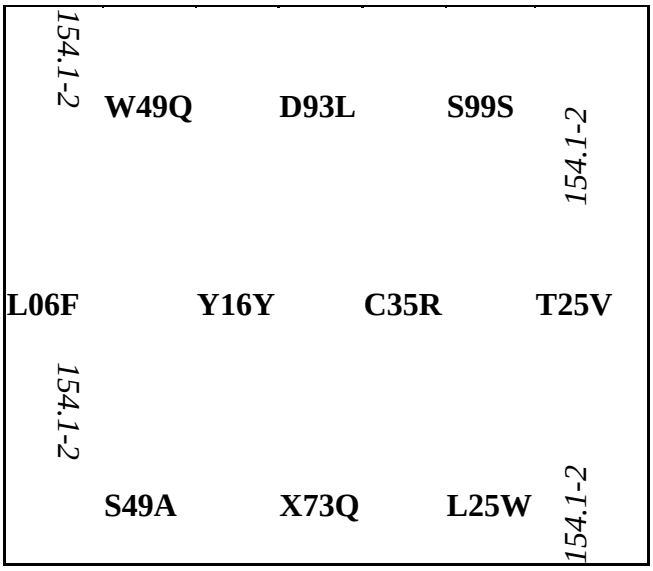


Figure 2.2 Posting sheet example.
Each posting sheet outlined 10 samples required for each cooking round.

2.2.3.3 Demographic survey and product rating

The sensory software used to design the questionnaire was Biosystem Fizz Paper (Biosystem, Dijon, France). The questionnaire was divided into two parts. First part was the socioeconomic or demographic survey and the second part consisted of product rating.

At the start of the consumer panel session, consumers were asked to fill in the first part of the questionnaire to provide the following socioeconomic information:

- Age class based on 6 categories: (a) 18-24, (b) 25-34, (c) 35-44, (d) 45-54, (e) 55-64 or (d) 65+;
- Gender based on 2 categories: (a) male or (b) female;
- Own occupation based on 10 categories:
  (a) Chief executive, legislator, manager, managing director, etc.
  (b) Science professional, doctor, engineer, teacher, legal professional, etc.

(c) Technician, nurse, IT worker, finance, admin, sales, artist, etc.
(d) Police officer, army, fireman, etc.
(e) Secretary, customer service, general clerk, etc.
(f) Shop assistant, waiter, cashier, hairdresser, personal care worker, etc.
(g) Farmer, fisherman, manual worker, etc.
(h) Machinist, electrician, carpenter, plumber, etc.
(i) Student, homemaker, etc. or
(j) Not currently in employment;

- Household income based on 4 categories: (a) below £25,000/€29,000 per year, (b) between £25,000/€29,000 and £50,000/€59,000 per year, (c) between £50,000/€59,000 and £75,000/€89,000 per year or (d) above £75,000/€89,000 per year;
- Number of children based on 4 categories: (a) none, (b) between 1 to 3, (c) between 4 to 6 or (d) more than 6;
- Number of adults based on 4 categories: (a) between 1 to 2, (b) between 3 to 4, (c) between 5 to 6 or (d) more than 6;
- Their appreciation of beef based on 4 categories: (S1) I enjoy red meat. It's important part of my diet, classified as "frequent beef consumers", (S2) I like red meat well enough. It's regular part of my diet, classified as " regular beef consumers"(S3) I do eat some red meat although it wouldn't worry me if I didn't, classified as "casual beef consumers" or (S4) I rarely/ never eat red meat, classified as "not beef consumers";
- Their preferred degree of doneness or level of cooking based on 6 categories: (a) blue, (b) rare, (c) medium rare, (d) medium, (e) medium well or (f) well done;
- Consumption frequency for different products based on 3 categories: (i) never, (ii) less than twice per month or (iii) twice or more per month. The product types including (a) brisket, (b) casserole steaks, (c) fillet, (d) frying steaks, (e) mince, (f) lean mince, (g) rib eye, (h) rump, (i) silver side, (j) sirloin and (k) topside;
- Location to buy beef based on 5 categories: (a) butcher, (b) farm shop, (c) supermarket, (d) other or (e) don't buy beef;

- Level of importance of motivation of beef choice based on 3 categories: (i) not important or little important, (ii) moderately important or (iii) very important. Consideration factors including (a) it is good value, (b) it has a good flavour, (c) it has good tenderness, (d) it looks good, (e) I know how to cook it, (f) it is easy to prepare, (g) I enjoy cooking it, (h) it is a healthy choice, (i) I enjoyed it last time, (j) animal well cared for, (k) environmentally friendly and (l) I know where it comes from;
- Most important attributes from 4 categories: (a) aroma, (b) flavour, (c) tenderness or (d) juiciness.

Information was provided to consumers participating in the trial about the quality grade of beef products. The grading system of beef was explained and consumers were asked to provide information about their willingness to pay for products ranging from (a) premium quality, (b) better than everyday quality (c) satisfactory everyday quality and (d) unsatisfactory on a line scale range from £6/€8 to £30/€32 (the currency for questionnaires provided in Cork was in euro while for questionnaires provided in Belfast and Reading it was in pounds. The assumption was made that €1 was equal to £0.85 based on the exchange rate on 4th February 2017.

In the second part of the questionnaire, consumers were instructed to rate the quality of the link product and then the trial samples on a 100-point line scale (0= low intensity/liking; 100= high intensity/liking) for *aroma liking* (AL), *tenderness* (TE), *juiciness* (JU), *flavour liking* (FL) and *overall liking* (OL). Consumers were also asked to assess the overall quality for a sample range from (a) unsatisfactory, (b) satisfactory everyday quality, (c) better than everyday quality and (d) premium.

2.2.3.4 Sensory consumer panel session and cooking protocol

Six sessions were hosted in each region, which accommodated 20 consumers per session. If extra consumers attended, they were given spare samples and the results were not statistically analysed. Each session lasted for 60 minutes.

The cooking protocol for the sensory consumer panel was adopted from the MSA cooking protocol (Watson et al., 2008). Samples were thawed 24 hours at 2°C before the session. There were 7 rounds of cooking in one session. The first round was

considered as “link” product and six samples were tested by the consumers. The grill (model: S-143, manufacturer: SILEXIA UK. Ltd, Oxford, United Kingdom) was switched on at least 45 minutes prior to the session and the temperature was set to 180°C. The starter samples were cooked on the grill for 4 minutes to condition the grill when the consumers arrived. The cooking time for the “link” product was 4 minutes and for the samples were 3 minutes and 45 seconds to achieve an internal temperature approximately of 72°C (as described for sensory profiling). Ten steaks were cooked in one cooking round and the steaks were cut in half then served to twenty consumers. The samples for the following round were loaded on the grill after the previous samples were unloaded and temperatures were measured from three random samples per cooking round. The samples were rested for 2 minutes before being cut and served to consumers. Samples were identified using a NI Blue code created by NI Blue software. Small portion of samples were collected from all ten samples for microbiological tests (if required) and stored for 4 weeks at -18°C in the freezer.
Panellists were provided a pen, questionnaire, toothpicks, packaged utensils containing napkin, plastic fork and knife. In addition, cream crackers and water were provided for consumers to serve as palate cleansers. Consumers were instructed to refresh their palate between each sample.

Questionnaires were collected according to consumer’s number after the session was completed. The forms were scanned and the results were automatically transferred into a result file stored in FIZZ database. Scan report was printed from FIZZ and all errors were checked and corrected in FIZZ. After this, the results were exported into an Excel workbook, NI Blue code was linked to animal ID number and then related to animal information (treatment, animal number, position, etc.).

2.2.4 Warner Bratzler Shear Force (WBSF)

Steaks for WBSF were assigned using a latin square design, into five batches, each of which included samples from all sexes, hanging methods and breeds. Steaks were thawed under 2°C for 24 hours and outer fat was trimmed off from the steaks. Pre-cooked weight was measured and recorded. The steak was vacuum-packed in a clean vacuum pouch with animal number and aging period labelled clearly. Waterbaths were set to 72°C two hours prior to cooking process. Steaks were cooked in a waterbath for

50 minutes to achieve an internal temperature of 72°C. A maximum of 15 steaks was cooked in one waterbath. After 50 minutes, the steaks were immersed into a basin filled with ice water for 30 minutes. Cooked weight was measured and recorded. Cooking loss was calculated using the equation: Cooking loss= [(pre-cooked weight of steak − cooked weight of steak) / pre-cooked weight of steak] ∗ 100 and recorded. The steaks were stored at 2°C overnight before coring.

The WBSF was measured following the procedure set up by Shackelford et al. (1994). A minimum of eight 1.3cm diameter cores were obtained from each steak. The number of cores depended on the size of the steak. The cores were taken parallel to the fibres in the muscle of the individual steak. Each core was sheared at a parallel angle to the muscle fibres. The shear force value was measured and recorded by an Instron device (model 2350-416, Instron Calibration Laboratory, Norwood, United States). The machine operated with software known as BlueHill 3. The crosshead speed was set as 100mm/min with V shaped blade and 550kgf load cell. The individual shear force was recorded in kilogram force (kgf) and the mean shear force was calculated as the average shear force readings for cores from the same slice of steak.

Raw data was exported in Excel file format from BlueHill 3 (Instron software). The WBSF value was compared between different aging period (7 days and 21 days) for samples from Northern Ireland abattoirs. In addition, the mean shear force was assessed for different sexes, hanging methods and breeds.

2.2.5 Statistical design and analysis

Some socioeconomic groups were combined for further statistical analysis due to the low number of consumers in some groups. These included:

1) Occupation was consolidated into 5 categories: C1 (group a and group b), C2 (group c and group d), C3 (group e), C4 (group f, group g and group h), C5 (group i and group j)
2) Presence of children in the household : None (group a) or yes (group b to group d)
3) Number of adults: One or two (group a) or more than two (group b to group d)

4) Preferred "doneness" or level of cooking: blue or rare (group a and group b), medium rare (group c), medium (group d), medium well (group e) or well done (group f);
5) Beef appreciation: S1 (group a), S2 (group b) or S3 and S4 (group c and group d)
6) Purchase habit: Supermarket (group c) or other (group a, group b, group d and group e)

CMQ4 scores were calculated by employing the MSA model: MQ4 = 0.3 *tenderness* + 0.1 *juiciness* + 0.3 *flavour liking* + 0.3 *overall liking* (Polkinghorne et al., 2008). This score enabled the assessment of overall satisfaction or acceptability of beef. The protocol described by Watson et al. (2008) was implemented to increase the precision of mean as a predictor and minimise bias of estimate by clipping or removing the sensory scores (two highest and two lowest scores) in the consumer panel to produce "clipped" sensory scores. The proportion of WTP relative to satisfactory everyday quality grade was calculated and recorded as P-WTP.

Linear discriminant analyses were conducted on four variables (*tenderness, juiciness, flavour liking, overall liking*) and three variables (*tenderness, juiciness, flavour liking)* to derive MQ4* and MQ3* formulae, separately for each region (Belfast, Cork and Reading). The average of these formulae formed a modified MSA formulae for each region (Watson et al., 2008).

Hierachical cluster analysis was conducted to divide consumers into groups according to consumer *overall liking* score and all sensory profiling attributes. Two linear discriminant analyses were conducted, the first analysis was conducted on four variables (TE, JU, FL, OL) and second analysis was conducted on three variables (TE, JU, FL). The average of these two analyses was used to generate a modified MSA formula for NI, ROI and GB, as described by Watson et al. (2008)

Consumer distribution between regions, overall cluster group and flavour cluster group in socio-economic groups, consumption habit and motivation for beef choice were analysed using chi-square test. Random Effect Model variance component (REML) analysis was conducted on the results of sensory profiling panel, consumer panel, instrumental analysis and cluster groups (Ahrens, 1974). Animal sex, hanging

method, breed, sample position, cluster groups, regions were fitted as fixed effects in the REML model. Co-variates including consumer age, gender, occupation, income, number of adults in the household, presence of children, important attributes, preferred "doneness" and purchase habit were added to the fixed REML model in turn. It was deemed appropriate to fit animal number, panellist number and consumer number as random effects, as these effects were not of primary interest in this study and they are randomly selected from general population (Gilmour et al., 1995).

Pearson's correlation was conducted to analyse the correlation between consumers' palatability scores and instrumental analyses. External and internal preference mapping were performed on consumer scores, profiling scores, cluster analyses and instrumental analyses to establish the relationship between consumer palatability traits, profiling attributes, consumer cluster groups, and WBSF (MacFie and Thomson, 1994). All statistical analysis was conducted using GenStat (GenStat 16.2.0.11713, VSN International Ltd, Hemel Hempstead, United Kingdom).

2.3 Results

2.3.1 Instrumental analysis

2.3.1.1 Instrumental analysis for samples aged for 21 days

A REML method was used to analyse the pHu, cooking loss and WBSF for samples aged for 21 days (Table 2.5). Hanging method had no effects on any instrumental measurements. Breed and sex interaction showed significant impacts on pHu ($P<0.05$) and cook loss ($P<0.001$). In addition, the first order effect showed that beef from continental breeds had significantly ($P<0.001$) higher cook loss than that from dairy breeds. Steer samples also showed significantly lower pHu ($P<0.01$) and cook loss ($P<0.001$) compared to other animal sexes.

Table 2.5 Instrumental analysis results.

	pHu	WBSF (kgf)	Cook loss (%)
Hang (H)			
AT	5.57	4.67	26.9
TS	5.52	4.47	26.7
avSED	0.030	0.151	0.45
P	0.120	0.207	0.751
Breed (B)			
Continental	5.56	4.59	27.8
Dairy	5.53	4.55	25.9
avSED	0.030	0.151	0.45
P	0.407	0.816	**<0.001**
Sex (S)			
Steers	5.47^{a}	4.29^{a}	25.5^{a}
Bulls	5.59^{b}	4.66^{ab}	27.7^{b}
Cows	5.58^{b}	4.77^{b}	27.2^{b}
avSED	0.037	0.185	0.55
P	**0.003**	**0.034**	**<0.001**
Interaction			
H x B	0.163	0.403	0.735
H x S	0.266	0.792	0.736
B x S	**0.028**	0.083	**<0.001**
H x B x S	0.377	0.948	0.604

a, b, c Numbers in the same column which do not share a common superscript are significantly different. ***P***: probability, H: hanging method, B: animal breed, S: animal sex, AT: Straight hung, TS: tenderstretch, pHu: ultimate pH, WBSF: Warner Bratzler Shear Force.

2.3.1.2 Analysis of ageing effect on WBSF

Samples collected from the Republic of Ireland were aged to day 7 and day 21 and the effect of ageing on WBSF and cooking loss is shown in Table 2.6. WBSF and cooking loss were both significantly affected (***P***<0.001) by ageing period, but there were no interactions with other factors. As might be expected, the result showed that the increased in ageing period significantly (***P***<0.001) decreased the WBSF. The average WBSF for 7 days was 5.1 kgf and WBSF for 21 days was 4.6 kgf. This suggested that ageing improved the tenderness of the sample.

The average cooking losses for samples aged for 7 days and 21 days were 28.1% and 26. 9% respectively. The lower cooking loss in the aged beef suggested the samples have higher ability to retain juice during cooking procedure.

Table 2.6 REML analysis of the effect of ageing period on WBSF and cooking loss.

	WBSF (kgf)	Cooking loss (%)
Ageing period (AP)		
7 days	5.2	28.1
21 days	4.6	26.9
avSED	0.14	0.34
P	<0.001	<0.001
Interaction		
H x AP	0.134	0.546
B x AP	0.597	0.355
S x AP	0.057	0.309
H x B x AP	0.167	0.124
H x S x AP	0.702	0.353

Only samples from Northern Ireland were included in this analysis. H: hanging method, B: animal breed, S: animal sex, AP: ageing period, ***P***: probability.

2.3.2 Quantitative Descriptive Analysis

Forty-eight attributes were agreed by trained panellists, including 10 attributes for appearance, 10 attributes for texture, 11 attributes for aroma, 12 attributes for flavour and 5 attributes for aftertaste. Definition and REML analysis of the attributes is presented in Table 2.7. The definition was discussed and agreed by all the panellists in the training session.

Table 2.7 Abbreviation and definition for sensory attributes.

Group	Attributes	Abbreviations	Definition
External appearance (EXAP)	Pale colour	PaleEXAP	Pale colour of meat
	Chestnut colour/ Brownness	ChestnutEXAP	Brown colour of cooked meat
	Juicy	JuicyEXAP	Liquid juice around the meat
	Charred	CharEXAP	Black or well-cooked on outer surface
	Bloody	BloodyEXAP	Pink and undercooked bloody outer surface
	Redness of juice	RedJuiEXAP	Red juice on surface and plate
	Brownness of juice	BrownJuiEXAP	Brown juices on surface and plate
	Greasy/ Oily/ Fatty	Greasy EXAP	Overall oily bright surface
Internal appearance (INAP)	Tight	TightINAP	Closely packed between fibres, close grain
	Lean	LeanINAP	No obvious internal fat
Aroma (AR)	Roast Beef	RstBfAR	Aroma of outside of roasted joint
	Grilled steak	GrilStkAR	Aroma reminiscent of meat browned on grill

Group	Attributes	Abbreviations	Definition
	Beefy	BeefyAR	Aroma of inner core of roast beef
	Charred	CharAR	Burnt charcoal aroma
	Fatty	FattyAR	Aroma of fatty animal or lard
	Bloody	BloodyAR	Aroma of reminiscent of metallic, blood
	Mealy	MealyAR	Aroma related to cooked animal feed
	Herby	HerbyAR	Herbs, green aroma
	Acrid	AcridAR	Pungent, acrid aroma
	Farmyard	FarmyardAR	Animal, farmyard odour
	Spice	SpiceAR	Sausage spice aroma
Texture on cutting (TXC)	Tenderness	TenderTXC	Easily cut, soft texture
	Crumbly/ Dry	CrumblyTXC	Sample separates, crumbs form during cutting
	Fibrous/ Stringy	FibrousTXC	Long strands in the meat on cutting
Mouth-feel (MOU)	Tenderness	TenderMOU	Soft and easy to chew before swallowing
	Spongy/Rubbery	SpongyMOU	Rubbery, keeps its shape, chewy
	Succulence	SucculeMOU	Juicy mouthfeel
	Sticky/ Clingy	StickyMOU	Sticks to teeth and roof of the mouth
	Forms Balls	BallsMOU	Forms balls when chewing
	Crumbly	CrumblyMOU	Sample separates after chewing
	Greasy	GreasyMOU	Oil coating on the roof of the mouth
Flavour (FL)	Intensity of Flavour	IntensityFL	Strength of flavour
	Grilled Steak	GrilStkFL	Flavour reminiscent of meat browned on grill
	Roast Beef	RstBfFL	Flavour of outer 1cm of roasted joint
	Beefy	BeefyFL	Flavour of inner core of roast beef
	Char Grilled	CharGrillFL	Burnt charcoal-like flavour
	Metallic/ Bloody	MetallicFL	Flavour of reminiscent of blood
	Saltiness	SaltyFL	Salt flavour in the mouth
	Sour/ Acidic	SourFL	Sour milk, lactic flavour
	Bitterness	BitterFL	Bitter flavour in the mouth
	Sweetness	SweetFL	Sweet beef flavour
	Earthy	EarthyFL	Root vegetable flavour
	Rancid	RancidFL	Rancid oil, distinct rancid flavour
Aftertaste (AF)	Intensity of Aftertaste	IntensityAF	Strength of aftertaste
	Roast Beef	RstBfAF	Aftertaste of outside of roasted joint
	Acidic	AcidicAF	Sour aftertaste in the mouth
	Bitterness	BitterAF	Bitter aftertaste in the mouth
	Saltiness	SaltyAF	Salty aftertaste in the mouth

A total of 35 sensory attributes showed significant differences and these are shown in Table 2.8. There were a number of interactions that significantly affected the sensory attributes, with higher number of significant interactions observed for the breed by sex interaction compared to other interactions. First order effects, including hanging

method, animal sex, breed, and sample position had significant impacts on sensory attributes. As expected, hanging method significantly affected seven sensory attributes, mostly related to texture on cutting and mouthfeel. A total of 22 sensory attributes were significantly affected by animal sex, mostly mouthfeel, texture on cutting and flavour attributes, with 15 of these attributes significant at $\boldsymbol{P}<0.001$ or $\boldsymbol{P}<0.01$. Breed had significant impacts on 22 sensory attributes, mostly aroma, appearance, flavour and mouthfeel attributes, with 18 of these sensory attributes significant at $\boldsymbol{P}<0.001$ and $\boldsymbol{P}<0.01$. Position of the sample within the muscle affected the *char grilled flavour*, *chestnut colour* and *charred external appearances* of the steak.

The sensory attributes that showed the highest intensities were *tight internal appearance, charred external appearance, grilled steak aroma, beefy flavour, tender mouthfeel, tender texture on cutting,* and *intensity of aftertaste*. These results revealed that hanging method, animal breed, animal sex and sample position had significant impacts on some of the sensory attributes.

Table 2.8 Mean and average standard deviation of the intensity scores for the sensory attributes from sensory trained panel.

	First order effects																		Interactions						
	Hang (H)				Breed (B)				Sex (S)					Sample Position (P)					H.B	H.S	B.S	S.P	H.B.P	B.S.P	H.B.S.P
	AT	TS	SED	*P*	Con	Dai	SED	*P*	Bulls	Cows	Steers	SED	*P*	An	Mi	Po	SED	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*
PaleEXAP	22	22	1.7	0.799	28	16	2.1	**<0.001**	26	20	21	2.5	0.063	22	23	21	1.5	0.305	0.951	0.859	**0.020**	0.652	0.193	0.813	0.652
ChestnutEXAP	56	55	1.7	0.621	49	62	2.0	**<0.001**	51^{a}	58^{b}	56^{b}	2.5	**0.008**	56^{b}	52^{a}	57^{b}	1.6	**0.008**	0.420	0.535	0.236	0.240	**0.047**	0.991	0.240
JuicyEXAP	19	19	1.2	0.784	16	21	1.2	**<0.001**	21^{b}	16^{a}	19^{b}	1.4	**0.002**	19	17	20	1.5	0.287	**0.005**	0.350	**<0.001**	0.370	0.317	**0.041**	0.370
CharEXAP	35	34	1.6	0.647	29	40	1.9	**<0.001**	33	33	38	2.3	0.144	33^{a}	33^{a}	37^{b}	1.7	**0.022**	0.502	0.075	0.191	0.966	0.070	0.253	0.966
BloodyEXAP	5	5	0.9	0.955	4	6	1.0	0.175	7	4	4	1.3	0.091	5	4	6	1.0	0.260	0.381	0.234	**0.004**	0.161	0.659	**0.005**	0.161
RedJuiEXAP	7	6	1.1	0.632	6	8	1.2	0.099	9^{b}	5^{a}	6^{ab}	1.5	**0.029**	6	6	8	1.2	0.261	0.344	**0.016**	**0.009**	0.468	0.619	0.092	0.468
BrownJuiEXAP	19	18	1.3	0.863	16	21	1.4	**<0.001**	18	17	20	1.7	0.191	20	17	19	1.4	0.112	0.733	0.911	**0.005**	0.530	0.241	**0.006**	0.530
GreasyEXAP	11	11	0.6	0.339	10	12	0.7	**0.011**	11	11	11	0.8	0.707	11	11	11	0.7	0.511	0.344	0.763	**0.009**	0.455	0.424	0.710	0.455
TightINAP	58	59	1.5	0.841	58	59	1.6	0.419	56^{a}	60^{b}	59^{ab}	2.0	**0.042**	58	59	58	1.8	0.583	0.733	0.109	**0.005**	0.693	0.679	0.839	0.693
RstBfAR	28	31	1.3	**0.002**	29	29	1.4	0.994	29	30	29	1.7	0.992	30	29	29	1.4	0.988	0.193	0.749	0.995	0.892	0.845	0.518	0.892
GrilStkAR	35	35	1.3	0.853	33	37	1.3	**0.009**	33^{a}	34^{ab}	37^{b}	1.6	**0.027**	36	33	35	1.6	0.232	0.399	0.369	0.718	0.226	0.945	0.761	0.226
BeefyAR	29	32	1.1	**0.011**	29	32	1.2	**0.009**	32	31	30	1.5	0.333	31	30	31	1.3	0.604	0.172	0.437	0.305	0.427	0.901	0.078	0.427
CharAR	28	26	1.4	0.268	22	32	1.6	**<0.001**	24	27	30	1.9	0.080	26	26	29	1.6	0.200	0.995	0.187	**0.023**	0.578	**0.010**	0.936	0.578
FattyAR	7	7	0.5	0.844	6	8	0.6	**0.032**	8	6	7	0.7	0.079	7	7	7	0.6	0.813	0.211	0.664	0.099	0.510	0.119	0.771	0.510
BloodyAR	7	7	0.8	0.953	7	7	0.8	0.377	9^{b}	6^{a}	6^{a}	1.0	**0.003**	7	6	8	1.0	0.414	**0.017**	0.963	**0.001**	0.444	0.351	0.207	0.444
TenderTXC	47	51	1.7	**0.006**	48	51	2.0	0.473	51^{b}	40^{a}	57^{c}	2.5	**<0.001**	50	50	48	1.6	0.629	0.213	0.534	0.314	0.217	0.691	0.438	0.217
CrumblyTXC	19	14	1.3	**<0.001**	18	16	1.5	**0.039**	18^{b}	13^{a}	20^{b}	1.8	**<0.001**	17	16	17	1.4	0.740	0.656	0.328	0.721	0.726	0.437	0.639	0.726
FibrousTXC	23	22	1.1	0.336	24	22	1.2	0.081	22^{a}	27^{b}	19^{a}	1.4	**<0.001**	24	22	22	1.3	0.182	0.906	0.578	0.311	0.078	0.157	0.938	0.078
TenderMOU	44	47	1.8	**0.009**	45	46	2.3	0.807	47^{b}	35^{a}	55^{c}	2.8	**<0.001**	45	46	45	1.6	0.749	0.585	0.498	0.303	0.085	0.799	0.465	0.085

	First order effects																	Interactions						
	Hang (H)				Breed (B)				Sex (S)					Sample Position (P)					H.B	H.S	B.S	S.P	H.B.P	B.S.P	H.B.S.P
	AT	TS	SED	***P***	Con	Dai	SED	***P***	Bulls	Cows	Steers	SED	***P***	An	Mi	Po	SED	***P***	***P***	***P***	***P***	***P***	***P***	***P***	***P***
SpongyMOU	31	27	1.3	**<0.001**	29	29	1.4	0.603	30^{b}	34^{c}	22^{a}	1.8	**<0.001**	29	29	28	1.4	0.851	0.833	0.466	0.971	0.659	0.488	0.314	0.659
SucculeMOU	26	27	1.6	0.067	23	30	1.9	**0.006**	26^{ab}	22^{a}	30^{b}	2.4	**0.011**	27	25	27	1.5	0.384	**0.022**	0.389	**<0.001**	0.509	0.574	0.465	0.509
StickyMOU	18	18	0.8	0.616	19	17	0.8	0.053	18^{b}	16^{a}	19^{b}	1.0	**0.001**	18	17	18	1.0	0.801	0.697	0.720	0.898	0.866	0.955	0.292	0.866
BallsMOU	24	25	1.2	0.806	25	24	1.2	0.162	25^{b}	27^{b}	21^{a}	1.5	**<0.001**	25	24	25	1.4	0.786	0.470	0.254	0.784	0.606	0.577	0.670	0.606
CrumblyMOU	20	20	1.6	0.992	23	18	2.0	**0.006**	20^{a}	15^{a}	26^{b}	2.5	**<0.001**	20	21	20	1.4	0.382	0.941	0.360	0.181	0.695	0.751	0.952	0.695
GreasyMOU	14	14	0.6	0.683	13	15	0.7	**<0.001**	14	14	15	0.8	0.393	14	14	14	0.6	0.794	0.426	0.325	0.081	0.340	0.886	0.304	0.340
IntensityFL	48	50	1.5	0.220	46	52	1.8	**0.001**	49^{ab}	46^{a}	51^{b}	2.5	**0.035**	49	48	49	1.5	0.605	0.556	0.053	0.484	0.140	0.467	0.597	0.140
GrilStkFL	32	31	1.3	0.389	29	34	1.3	**<0.001**	29^{a}	30^{a}	35^{b}	1.6	**0.001**	31	31	32	1.5	0.636	0.755	0.746	0.400	0.390	0.970	0.805	0.390
RstBfFL	27	29	1.0	0.057	27	29	1.0	**0.009**	28^{ab}	27^{a}	30^{b}	1.3	**0.048**	28	28	28	1.3	0.860	0.682	0.304	0.526	0.463	0.445	0.567	0.463
BeefyFL	33	35	1.1	**0.038**	32	36	1.2	**0.011**	33.1	33	36	1.5	0.088	34	34	35	1.3	0.816	0.275	0.184	0.098	0.514	**0.049**	0.068	0.514
CharGrillFL	26	26	1.3	0.940	21	30	1.4	**<0.001**	23^{a}	25^{a}	29^{b}	1.7	**0.005**	23^{a}	25^{a}	28^{b}	1.6	**0.002**	0.755	0.952	0.163	0.994	0.739	0.660	0.994
MetallicFL	12	11	0.6	0.181	11	12	0.6	0.374	13^{b}	10^{a}	11^{a}	0.8	**<0.001**	12	11	11	0.7	0.158	0.183	0.300	0.622	0.723	0.780	0.328	0.723
SweetFL	12	11	0.6	0.687	11	13	0.7	**0.004**	12	11	12	0.8	0.559	12	12	12	0.7	0.742	0.224	0.858	**0.010**	0.229	0.572	0.060	0.229
IntensityAT	28	29	1.0	0.658	26	31	1.2	**<0.001**	28^{ab}	26^{a}	31^{b}	1.4	**0.005**	29	28	28	1.1	0.301	0.209	0.446	0.103	0.893	0.292	0.288	0.893
RstBfAT	17	17	0.8	0.653	16	18.	0.9	**0.006**	17^{ab}	16^{a}	19^{b}	1.0	**0.023**	17	18	17	1.0	0.654	0.375	0.616	0.421	0.870	0.157	0.755	0.870

a,b,c: Numbers in the same row which do not share a same superscript are significantly different. Sensory attributes' abbreviation and definition are listed in Table 2.7. H: hanging method, B: breed, S: sex, P: sample position AT: straight hung, TS: tenderstretch, Cont: continental, Dai: dairy, An: anterior, Mi: middle, Po: posterior, avSED: averagestandard error, ***P***: probability. Significance interaction results are recorded in annex 2.1 to 2.5 (p 300-310).

2.3.3 Consumer Panels

2.3.3.1 Demographic and treatment effects on consumer palatability traits

A comparison between consumer groups and treatment effects is shown in Table 2.9. The overall liking score in the consumer panel was used as a general indicator of all sensory scores because *aroma liking, tenderness, juiciness* and *flavour liking* generally followed similar trends, unless otherwise specified. The two highest and two lowest scores were "clipped" for each set of samples. Probability of significance of clipped results increased and decreased in different cases. However, the average standard errors consistently increased for clipped results compared to unclipped results and there was little change in mean values (Table 2.9). Thus, the author has focused on the unclipped results, unless otherwise specified.

Three interactions between animal effects and two interactions with regional effects significantly affected some sensory scores. In total, there were 13 significant results for interaction, with eight interactions were observed at $\boldsymbol{P}<0.05$ and five were significant at $\boldsymbol{P}<0.001$. The highest significant ($\boldsymbol{P}<0.001$) differences were observed for breed by sex interaction for *tenderness, juiciness, flavour liking, overall liking* and MQ4 socres.

Hanging method significantly affected the *overall liking* score and MQ4 score as well as other sensory scores (Table 2.9). Thus, tenderstretch improve the overall eating quality of the striploin. Direct comparison of continental beef and dairy beef showed that the *overall liking* score of dairy beef was 5 points higher than continental beef. Significant differences were observed between continental and dairy breeds for all sensory scores except *tenderness*. This result suggested that dairy beef had higher eating quality.

Interestingly, the results indicated that there were significant differences between consumer groups. Region significantly affected all palatability traits or sensory scores, including *aroma liking, tenderness, juiciness, flavour liking, overall liking* and MQ4 score. The Reading consumers gave a higher satisfaction score, with an *overall liking* mean of 59.7 compared to Cork (55.0) and Belfast (55.2). Overall, the consumer panel results showed that consumers consistently differentiated the quality of beef based on the factors evaluated.

Table 2.9 The effect of hanging, breed, sex, sample position and region on the mean of consumer palatability traits of beef.

	AL	TE	JU	FL	OL	MQ4	Cl_ AL	Cl_ TE	Cl_ JU	Cl_ FL	Cl_ OL	CMQ4
Region(R)												
Belfast	55.9[a]	53.2[a]	54.3[b]	55.7[a]	55.2[a]	54.7[a]	55.8[a]	53.2[a]	54.6[b]	56.7[a]	55.7[a]	55.1[a]
Cork	57.5[a]	51.6[a]	50.9[a]	55.7[a]	55.0[a]	53.8[a]	58.5[b]	52.1[a]	51.4[a]	56.5[a]	55.7[a]	54.3[a]
Reading	62.4[b]	56.9[b]	55.3[b]	59.7[b]	59.7[b]	58.4[b]	63.0[c]	58.1[b]	56.3[b]	60.9[b]	61.0[b]	59.4[b]
avSED	1.15	1.44	1.34	1.36	1.27	1.23	1.31	1.59	1.49	1.44	1.36	1.30
P	**<0.001**	**0.002**	**0.004**	**0.005**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.005**	**0.004**	**<0.001**	**<0.001**
Hanging (H)												
AT	57.5	51.2	51.9	54.9	54.3	53.3	57.8	51.4	52.1	55.9	54.9	53.9
TS	59.7	56.6	55.1	59.1	59.0	57.9	60.5	57.6	56.1	60.2	60.0	58.6
avSED	1.08	1.63	1.44	1.19	1.30	1.28	1.15	1.83	1.63	1.34	1.42	1.38
P	**0.022**	**<0.001**	**0.010**	**<0.001**	**<0.001**	**<0.001**	**0.013**	**<0.001**	**0.003**	**0.001**	**<0.001**	**<0.001**
Breed (B)												
Continental	57.2	52.4	49.6	54.5	54.2	53.3	57.6	52.8	49.9	55.4	55.1	53.8
Dairy	60.0	55.4	57.4	59.6	59.0	58.0	60.7	56.1	58.3	60.7	59.9	58.7
avSED	1.20	1.98	1.71	1.27	1.53	1.50	1.24	2.24	1.96	1.48	1.67	1.64
P	**0.047**	0.544	**<0.001**	**0.001**	**0.022**	**0.028**	**0.029**	0.635	**<0.001**	**0.005**	**0.039**	**0.036**
Sex(S)												
Bulls	59.5[b]	57.4[b]	54.7[b]	58.0[b]	58.5[b]	57.6[b]	60.0[b]	58.2[b]	55.4[b]	59.0[b]	59.6[b]	58.4[b]
Cows	55.0[a]	40.0[a]	46.2[a]	49.6[a]	47.3[a]	45.7[a]	55.3[a]	39.0[a]	46.0[a]	49.9[a]	47.3[a]	45.6[a]
Steers	61.3[b]	64.4[c]	59.6[c]	63.5[c]	64.1[c]	63.5[c]	62.1[b]	66.1[c]	60.9[c]	65.2[c]	65.5[c]	64.8[c]
avSED	1.47	2.42	2.09	1.56	1.87	1.84	1.51	2.74	2.40	1.81	2.04	2.00
P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Position(Po)												
Anterior	60.0[b]	56.4[b]	54.6	58.7	58.4[b]	57.5[b]	60.7	57.3[b]	54.9	59.8	59.4[b]	58.4[b]

	AL	TE	JU	FL	OL	MQ4	Cl_ AL	Cl_ TE	Cl_ JU	Cl_ FL	Cl_ OL	CMQ4
Middle	57.1[a]	52.3[a]	53.5	55.7	55.3[a]	54.3[a]	57.5	52.7[a]	54.0	56.3	55.8[a]	54.6[a]
Posterior	58.6[ab]	53.0[a]	52.4	56.7	56.2[ab]	55.0[a]	59.2	53.4[a]	54.0	58.0	57.2[ab]	55.8[a]
avSED	1.15	1.44	1.34	1.36	1.27	1.23	1.31	1.59	1.49	1.44	1.36	1.30
P	**0.049**	**0.011**	0.277	0.089	**0.043**	**0.028**	0.054	**0.009**	0.593	0.059	**0.033**	**0.016**
Interaction (*P*)												
H.B	**0.034**	0.388	0.129	0.401	0.399	0.310	**0.044**	0.410	0.068	0.342	0.252	0.246
H.S	0.945	0.144	0.190	0.319	0.165	0.148	0.677	0.055	0.098	0.116	**0.028**	**0.037**
B.S	0.058	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.047**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
H.Po	0.545	**0.048**	0.215	0.507	0.182	0.136	0.526	**0.048**	0.256	0.397	0.134	0.087
S.Po	0.749	0.069	0.491	0.346	0.064	0.138	0.756	**0.016**	0.504	0.183	**0.049**	0.061
H.B.S.Po	0.500	0.127	0.646	0.084	0.225	0.102	0.347	0.064	0.502	**0.012**	0.058	**0.031**
R.B.S	0.299	0.073	**0.027**	0.244	**0.029**	0.054	0.328	0.074	**0.018**	0.237	0.017	**0.041**
R.B.Po	**0.027**	0.053	0.068	**0.025**	**0.032**	**0.020**	**0.015**	**0.036**	0.079	**0.013**	**0.048**	**0.017**

a, b, c: Numbers in the same column which do not share a common superscript are significantly different.
avSED: average standard error of difference, ***P***: probability, AT: straight hung, TS: tenderstretch, AL: *aroma liking*, TE: *tenderness*, JU: *juiciness*, FL: *flavour liking*, OL*: overall liking*, Cl_: Clipped score
*Note that interactions which had no significant effect on sensory scores are included in Annex 2.6 (p 310-311).

2.3.3.2 The relationship between consumer palatability traits and quality grade

Sensory scores were obtained from all consumers on *aroma liking, tenderness, juiciness, flavour liking* and *overall liking* on a line scale of 0-100. They also assigned a quality grade ('unsatisfactory', 'satisfactory everyday quality', 'better than everyday quality' or 'premium') to each piece of meat they tasted. Table 2.10 and Figure 2.3 below showed the variability of consumers' MQ4 score for what they determine to be 'unsatisfactory', 'satisfactory everyday quality', 'better than everyday quality' and 'premium' quality. This is based on the results from 360 consumers across three sites.

Table 2.10 Number of sample (n), mean, standard deviation, minimum and maximum of consumers' individual MQ4 scores for different quality grades.

Quality Grade	Region/ Demographic	n	Mean	Minimum	Maximum	Standard Deviation
Fail	Belfast	133	25.9	0.0	78.5	13.36
	Cork	126	23.0	0.0	80.2	13.06
	Reading	94	25.1	0.1	58.4	13.03
3*	Belfast	272	47.0	13.0	82.4	14.71
	Cork	291	47.0	13.1	77.5	13.27
	Reading	268	49.2	16.6	88.6	13.30
4*	Belfast	212	67.5	33.2	96.2	11.25
	Cork	205	68.4	39.1	97.8	11.34
	Reading	235	68.7	26.9	99.8	12.87
5*	Belfast	102	85.7	62.5	100.0	9.66
	Cork	95	83.6	58.2	100.0	8.58
	Reading	121	84.5	57.5	100.0	10.52

Quality grades: 'unsatisfactory' (fail), 'satisfactory everyday quality' (3*), 'better than everyday quality' (4*) and 'premium' quality (5*).

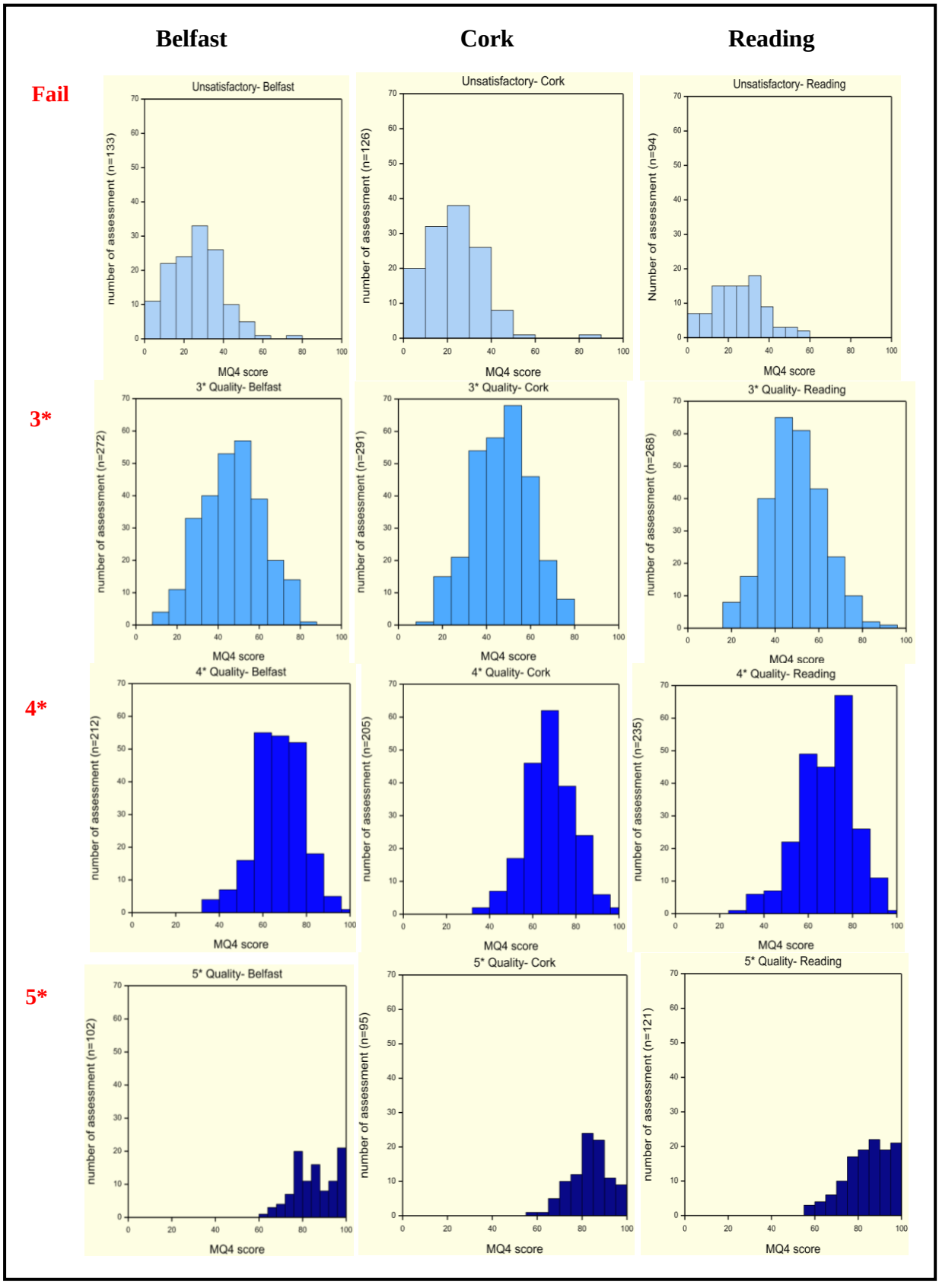


Figure 2.3 Distribution of consumers' individual MQ4 score for unsatisfactory (fail), satisfactory everyday quality (3*), better than everyday quality (4*) and premium quality (5*).

There was a steady increase of approximately 20 points in the mean MQ4 score between fail, 3*, 4* and 5*. A total of 831 samples out of 2160 samples were classified as satisfactory everyday quality, followed by 652 samples classified as better than everyday quality. Only 318 samples were classified as premium quality grade, with average MQ4 score of 84.6 across three regions. In addition, more samples were classified as better than everyday quality (4*) or premium quality (5*) in Reading compared to Belfast and Cork.

2.3.3.3 Willingness to pay

In this study, the willingness to pay (WTP) for products at different grades is recorded for three regions in Table 2.11. Generally, the willingness to pay followed similar trends in the three regions. However, the willingness to pay for premium product was significantly (***P***<0.05) lower in Cork compared to Belfast and Reading, while the willingness to pay for unsatisfactory product was significantly lower in Reading compared to Belfast and Cork.

Table 2.11 Consumer willingness to pay for products at different grades.

Quality Grade	**Unsatisfactory (fail)**	**Satisfactory everyday (3*)**	**Better than everyday (4*)**	**Premium (5*)**
Regions				
Belfast, £/kg (€/kg)	7.71[b] (9.07)	14.08 (16.56)	18.22 (21.44)	22.05[b] (25.94)
*Cork, £/kg (€/kg)	8.03[b] (9.44)	13.46 (15.84)	17.22 (20.26)	20.71[a] (24.36)
Reading, £/kg (€/kg)	6.93[a] (8.15)	13.98 (16.45)	17.71 (20.84)	21.99[b] (25.87)
avSED, £/kg	0.289	0.491	0.539	0.591
P	**<0.001**	0.444	0.193	**0.041**
Ratio (P-WTP)				
Belfast	0.55	1.00	1.29	1.57
Cork	0.60	1.00	1.28	1.54
Reading	0.50	1.00	1.27	1.58

P-WTP: Proportion relative to satisfactory everyday quality, avSED: average standard error of difference, avSED: average standard error of difference, ***P***: probability. The assumption was made that €1 was equal to £0.85.

2.3.4 Consumers' socioeconomic survey in the three regions

2.3.4.1 Socioeconomic background

The distribution of socioeconomic status was analysed with chi-squared test and REML analysis showed the effect of demographic status on palatability traits and WTP (Table 2.12 and Table 2.13).

A significant (***P***<0.05) difference was reported in consumers' age distribution between regions (Table 2.12). However, consumer's age had no significant effect on consumer perception of all sensory scores or palatability traits (Table 2.13).
Occupations were broken down into 10 categories. Due to a low number in some occupation categories, the category groups were combined in order to analyse the distribution and effect on consumer scores using REML analysis. There were more students and homemakers participating in Cork and Reading compared to Belfast. However, the analysis showed that occupation had no effect on sensory scores (Table 2.13). A significant difference was reported in consumers' distribution for household composition, where more consumers in Reading had more than two adults in a household.

The household income of the participants spanned a broad range with most of the participants having a household income level between £25,000 and £50,000 (€29,000-€59,000) per annum (Table 2.12). There was an income effect on the *aroma liking* score (***P***<0.05) but this was not observed for the other sensory scores (Table 2.13).

Table 2.12 Distribution of consumers in various socioeconomic groups.

Region	**NI** (n=120)	**ROI** (n=120)	**GB** (n=120)	χ^2	***P***
Age Group					
18-24	23	19	21	22.71	**0.012**
25-34	19	28	16		
35-44	9	25	16		
45-54	20	19	23		
55-64	24	22	27		
65+	25	7	17		
Gender					
Female	61	62	66	0.47	0.791
Male	59	58	54		
Income					
Below £25,000	27	27	36	8.80	0.185
£25,000- £50,000	61	46	53		
£50,000- £75,000	20	32	19		
Above £75,000	12	15	12		
Occupation					
C1 + C2	36	40	37	24.94	**0.002**
C3 + C4	33	31	28		
C5	9	9	13		
C6 + C7 + C8	27	8	9		
C9 + C10	15	32	33		
Children					
Yes	89	79	87	1.95	0.378
No	31	40	33		
Number of adult					
Less than 2	70	79	91	7.46	**0.014**
More than 2	48	41	29		

χ^2 : chi-square test, ***P***: probability.

Table 2.13 Effects of socioeconomic status on consumer sensory scores and WTP.

	Sensory score						WTP (£)			
	AR	TE	JU	FL	OL	MQ4	Fail	3*	4*	5*
Age Group										
18-24	59.4	55.0	54.8	56.8	57.8	56.4	7.6	14.0	18.4	22.3
25-34	59.2	53.6	51.8	56.1	56.3	55.0	7.5	14.2	18.4	22.3
35-44	60.1	54.6	53.5	59.6	58.2	57.1	7.3	12.4	16.6	21.3
45-54	55.7	53.0	53.2	55.5	55.4	54.5	7.7	13.8	17.4	21.2
55-64	58.5	54.8	54.2	57.8	56.6	56.2	7.4	14.2	17.6	21.5
65+	59.2	54.4	54.3	58.5	57.6	56.6	7.4	13.4	16.8	20.4
avSED	2.686	2.39	2.69	2.49	2.40	2.24	0.43	0.74	0.80	0.89
P	0.666	0.864	0.927	0.929	0.960	0.970	0.795	0.191	0.251	0.270
Gender										
Female	59.7	55.0	53.4	57.1	56.9	56.0	7.6	13.6	17.5	21.5
Male	57.3	52.6	53.6	56.8	56.3	55.1	7.5	14.1	17.9	21.7
avSED	1.447	1.30	1.47	1.36	1.31	1.22	0.24	0.40	0.44	0.48
P	0.104	0.068	0.921	0.868	0.625	0.434	0.882	0.175	0.430	0.604
Income										
Below £25,000	57.2[ab]	54.7	53.4	57.2	56.8	56.0	7.5	13.3[a]	17.1[a]	21.1[a]
£25,000-£50,000	60.1[b]	54.3	54.7	57.0	57.0	55.9	7.5	13.8[a]	17.7[a]	21.4[a]
£50,000-£75,000	60.3[b]	53.4	52.7	57.9	57.0	55.8	7.5	13.8[a]	17.6[a]	21.9[ab]
Above £75,000	53.8[a]	52.8	51.5	55.7	55.2	54.3	8.1	15.8[b]	19.8[b]	23.5[b]
avSED	2.306	2.08	2.33	2.18	2.09	1.96	0.37	0.63	0.69	0.76
P	**0.041**	0.709	0.494	0.818	0.856	0.811	0.409	**0.004**	**0.006**	**0.039**
Occupation										
C1	58.1	53.1	52.5	56.2	56.1	54.9	7.8	14.5	18.1	21.5
C2	58.2	53.1	53.6	57.2	56.0	55.3	7.5	13.3	17.2	21.7
C3	59.1	53.6	51.1	55.5	54.3	54.1	7.5	13.5	16.5	20.8
C4	61.0	55.7	56.4	59.7	59.4	58.1	7.8	13.5	17.6	21.2
C5	59.6	55.8	55.3	57.6	58.6	57.1	7.2	13.9	18.2	21.9
avSED	2.702	2.43	2.71	2.52	2.42	2.27	0.44	0.74	0.81	0.89
P	0.945	0.614	0.436	0.700	0.426	0.560	0.560	0.200	0.127	0.789
Children										
None	57.6	53.7	53.4	56.1	56.1	55.1	7.6	14.0	17.9	21.8
Yes	61.2	54.5	53.9	59.4	58.3	57.0	7.5	13.4	17.2	21.2
avSED	1.61	1.45	1.63	1.49	1.44	1.36	0.26	0.44	0.49	0.53
P	**0.026**	0.601	0.778	0.029	0.137	0.161	0.695	0.177	0.150	0.285
Number of adults										
Less than 2	58.3	53.7	53.2	56.8	56.1	55.3	7.8	13.8	17.6	21.4
More than 2	59.0	54.0	53.8	57.1	57.4	55.9	7.3	13.9	17.9	22.0
avSED	1.58	1.41	1.58	1.47	1.41	1.32	0.25	0.44	0.48	0.52
P	0.657	0.757	0.676	0.742	0.269	0.540	**0.029**	0.833	0.447	0.279

a, b: Numbers in the same column which do not share a common superscript are significantly different. AL: aroma liking, TE: *tenderness*, JU: *juiciness*, FL: *flavour liking*, OL: *overall liking*, WTP: Willingness to pay, ***P***: probability, avSED: average standard error, Fail: unsatisfactory, 3*: satisfactory everyday quality, 4*: better than everyday quality, 5*: premium quality.

2.3.4.2 Behavioural factors

Analysis by chi-square test showed that consumers in the three locations had similar preference for "doneness" (Table 2.14). Relatively low percentages of consumers preferred their steaks blue or rare with a higher preference for medium rare, medium to well done for consumers in Cork, Belfast and Reading. As expected, the preferred degree of doneness significantly affected (***P***<0.05) *tenderness* and *overall liking* scores. The *tenderness* and *overall liking* scores for consumers who preferred blue or rare steak were significantly lower (***P***<0.05) compared to consumers who preferred medium to well-done steak (Table 2.15). This result was expected as all the samples were presented with internal temperature of 72°C. The differences were more likely caused by individual preference rather than differences in meat quality.

A large proportion of consumers rated flavour (53%) and tenderness (41%) as the most important attributes when they consumed beef (Table 2.14). There were significantly (***P***<0.05) more consumers who rated juiciness to be the most important attributes in Reading compared to Cork and Belfast. However, this factor had no effects on sensory scores or WTP (Table 2.15).

Regular consumption of beef was a prerequisite of participation. It was, therefore, as expected that more than 80% of consumers stated that beef was part of their regular diet and the rest of the consumers were considered as "casual beef consumers" (Table 2.14). Approximately half of the consumers from Belfast purchased their beef products from the butcher, farm shops or other shops while most of the consumers from Cork and Reading purchased from the supermarket. Consumers who bought beef from supermarkets had lower WTP.

Table 2.14 Distribution of consumers in various behavioural factor groups.

	Region			χ^2	*P*
	NI	**ROI**	**GB**		
Preferred "Doneness"					
Blue+ Rare	6	17	10	11.84	0.158
Medium Rare	24	29	34		
Medium	36	26	35		
Medium Well	32	25	25		
Well done	22	23	16		
Most important attributes					
Tenderness	54	51	41	11.14	**0.025**
Juiciness	1	8	12		
Flavour	65	60	67		
Frequency of consumption					
S1	51	50	48	3.27	0.513
S2	46	54	56		
S3 and S4	23	15	16		
Purchase habit					
Supermarket	49	75	102	46.33	**<0.001**
Others	70	45	20		

χ^2: chi-square test, ***P***: probability.

Table 2.15 Effects of consumer behaviour and preferences on sensory socres and WTP.

	Sensory score						WTP (£)			
	AL	TE	JU	FL	OL	MQ4	Fail	3*	4*	5*
Preferred Doneness										
Blue+ Rare	55.2	48.1[a]	47.8	54.0	50.7[a]	50.6	7.3	13.3	17.5	23.4[c]
Medium Rare	58.3	52.8[ab]	53.4	56.2	55.3[ab]	54.6	7.5	13.9	17.9	22.2[bc]
Medium	57.8	54.3[b]	53.5	57.6	57.4[b]	56.1	7.9	14.3	17.8	21.7[bc]
Medium Well	59.6	56.3[b]	55.2	57.5	58.2[b]	57.1	7.4	13.6	17.7	21.4[ab]
Well done	60.6	54.6[b]	54.0	58.4	58.2[b]	56.7	7.5	13.4	16.9	19.9[a]
avSED	2.55	2.24	2.56	2.37	2.26	2.12	0.41	0.70	0.77	0.83
P	0.422	**0.022**	0.273	0.599	**0.047**	0.091	0.447	0.650	0.548	**0.008**
Most important attribute										
Tenderness	58.7	54.1	53.4	56.6	56.1	55.4	7.6	13.7	17.4	21.4
Juiciness	53.1	47.5	49.8	50.7	51.3	49.8	9.1	15.7	20.0	21.0
Flavour	58.5	54.0	53.3	57.4	57.1	55.9	7.4	13.9	18.0	21.8
avSED	3.96	3.55	3.98	3.68	3.56	3.33	0.63	1.09	1.19	1.31
P	0.961	0.666	0.923	0.814	0.718	0.833	0.205	0.343	0.255	0.729
Frequency of consumption										
S1	58.5	54.9	53.2	57.2	57.1	56.1	7.5	13.9	17.8	22.1
S2	59.2	53.2	53.8	57.4	56.5	55.5	7.4	13.7	17.6	21.4
S3 and S4	57.4	53.5	53.5	55.7	56.1	54.9	8.0	14.4	18.0	21.3
avSED	1.99	1.78	2.01	1.85	1.79	1.67	0.32	0.55	0.60	0.66
P	0.640	0.402	0.942	0.600	0.806	0.718	0.371	0.572	0.813	0.267
Purchase habit										
Supermarket	58.3	58.6	53.9	54.1	57.3	56.5	7.6	13.8	17.4	21.4
Butcher+ Farm Shop+ Other	58.6	58.3	52.9	51.5	56.3	56.0	7.5	14.5	18.7	22.5
avSED	1.69	1.69	1.52	1.70	1.58	1.52	0.27	0.46	0.50	0.55
P	0.947	0.947	0.711	0.242	0.592	0.920	0.858	0.292	**0.018**	0.104

a, b: Numbers in the same column which do not share a common superscript are significantly different. AL: *aroma liking*, TE: *tenderness*, JU: *juiciness*, FL: *flavour liking*, OL: *overall liking*, WTP: Willingness to pay, ***P***: probability, avSED: average standard error, Fail: unsatisfactory, 3*: satisfactory everyday quality, 4*: better than everyday quality, 5*: premium quality.

2.3.4.3 Consumption frequency of beef muscles

Consumers were asked which types of products they consumed and the corresponding frequency (never, less than twice a month and more than twice a month). The results were recorded in Table 2.16 below, including consumer numbers who failed to answer the question. The consumption frequencies of brisket, casserole steak, rump, silverside, sirloin and topside were significantly different between regions. Consumers in Belfast and Cork consumed less brisket, casserole steak, rump and topside while most consumers in Cork never consumed silverside.

Sensory scores were significantly affected by the consumption frequencies of mince, lean mince, rump and silverside (Table 2.17). Consumers who consumed mince, lean mince, rump and silverside more than twice a month rated the striploin steaks significantly higher than consumers who never consumed these muscles (Table 2.17). On the other hand, WTP was significantly affected by the consumption frequencies of frying steak, which consumers who consumed this muscle more than twice a month had lower WTP for satisfactory everyday quality and better than everyday quality beef (Table 2.17).

Table 2.16 Distribution of consumer consumption frequency of each product.

Product	Frequency	Region			χ^2	*P*
		Belfast (n=120)	Cork (n=120)	Reading (n=120)		
Brisket	Never	64	93	63	21.84	**<0.001**
	<2 per month	45	22	54		
	≥2 per month	5	3	3		
	No answer	6	2	0		
Casserole steak	Never	35	44	23	10.53	**0.032**
	<2 per month	52	50	70		
	≥2 per month	27	25	27		
	No answer	6	1	0		
Fillet	Never	24	19	14	3.81	0.433
	<2 per month	71	74	81		
	≥2 per month	22	27	25		
	No answer	3	0	0		
Frying Steak	Never	37	27	26	8.44	0.077
	<2 per month	56	53	66		
	≥2 per month	22	38	28		
	No answer	5	2	0		
Mince	Never	13	20	13	3.35	0.502
	<2 per month	22	25	29		
	≥2 per month	81	74	78		
	No answer	4	1	0		
Lean Mince	Never	17	16	10	3.45	0.486
	<2 per month	26	33	32		
	≥2 per month	74	69	78		
	No answer	3	2	0		
Rib Eye	Never	34	48	43	6.70	0.152
	<2 per month	63	55	68		
	≥2 per month	18	15	9		
	No answer	5	2	0		
Rump	Never	44	68	20	46.41	**<0.001**
	<2 per month	57	47	82		
	≥2 per month	14	3	18		
	No answer	5	2	0		
Silverside	Never	26	77	57	66.07	**<0.001**
	<2 per month	59	37	56		
	≥2 per month	32	3	7		
	No answer	3	3	0		
Sirloin	Never	5	13	15	10.29	**0.036**
	<2 per month	85	69	81		
	≥2 per month	28	37	23		
	No answer	2	1	1		
Topside	Never	51	63	44	16.86	**0.002**
	<2 per month	49	50	71		
	>2 per month	15	5	5		
	No answer	5	2	0		

<2/ month: less than twice per month, ≥2/ month: twice or more per month, χ^2 : chi-square test, ***P***: probability, ns: not significant,

Table 2.17 Effects of consumer consumption frequency on sensory scores and WTP.

		Sensory Scores						WTP (£)			
		AR	TE	JU	FL	OL	MQ4	Fail	3*	4*	5*
Brisket	Never	58.4	53.6	53.7	57.3	57.1	55.8	7.5	14.0	17.8	21.7
	<2/ mth	58.5	53.6	52.1	55.7	55.0	54.5	7.6	13.7	17.8	21.9
	≥2/ mth	65.3	56.9	58.2	58.4	60.5	58.6	6.9	12.4	16.5	20.7
	avSED	3.47	3.12	3.53	3.22	3.12	2.92	0.57	0.97	1.05	1.14
	P	0.365	0.573	0.402	0.621	0.246	0.459	0.449	0.397	0.409	0.694
Casserole steak	Never	56.4	53.2	52.2	55.8	55.3	54.5	7.5	14.0	17.9	21.9
	<2/ mth	58.9	54.1	54.6	57.7	57.3	56.2	7.6	13.5	17.4	21.2
	≥2/ mth	60.2	53.5	52.2	56.8	56.8	55.4	7.4	14.0	18.2	22.3
	avSED	1.89	1.70	1.91	1.76	1.71	1.59	0.31	0.53	0.58	0.64
	P	0.179	0.826	0.237	0.446	0.339	0.450	0.692	0.458	0.311	0.174
Fillet	Never	54.7	52.4	51.6	55.0	55.8	54.1	7.3	13.1	17.0	20.8
	<2/ mth	59.0	54.2	53.8	57.6	57.1	56.0	7.6	14.0	17.8	21.7
	≥2/ mth	59.7	54.5	53.5	56.8	56.4	55.7	7.6	14.1	18.2	22.2
	avSED	2.13	1.90	2.15	1.99	1.91	1.79	0.35	0.59	0.64	0.71
	P	0.129	0.526	0.591	0.397	0.722	0.507	0.482	0.267	0.273	0.191
Frying steak	Never	57.3	52.8	51.5	56.0	54.9	54.2	7.6	13.7^{ab}	17.6^{ab}	21.6
	<2/ mth	59.1	54.0	53.8	57.2	56.9	55.8	7.6	14.4^{b}	18.4^{b}	21.9
	≥2/ mth	59.8	55.4	55.2	58.3	58.7	57.2	7.5	13.1^{a}	16.8^{a}	21.2
	avSED	1.89	1.70	1.92	1.76	1.71	1.59	0.31	0.52	0.57	0.64
	P	0.492	0.450	0.291	0.570	0.141	0.287	0.856	**0.013**	**0.013**	0.418
Mince	Never	55.5	49.4	47.0^{a}	53.6	52.0^{a}	51.2^{a}	7.4	14.1	17.5	22.1
	<2/ mth	58.9	55.1	54.9^{b}	57.0	57.2^{b}	56.3^{b}	7.7	14.5	18.0	21.4
	≥2/ mth	59.1	54.3	54.2^{b}	57.7	57.3^{b}	56.2^{b}	7.5	13.6	17.7	21.7
	avSED	2.23	1.95	2.21	2.05	1.96	1.83	0.36	0.62	0.68	0.74

		Sensory Scores						WTP (£)			
		AR	TE	JU	FL	OL	MQ4	Fail	3*	4*	5*
	P	0.316	0.072	**0.005**	0.184	**0.042**	**0.044**	0.802	0.178	0.758	0.684
Lean Mince	Never	53.4[a]	53.4	52.0	53.8	55.3	54.0	7.3	14.9	18.3	21.6
	<2/ mth	59.4[b]	52.6	53.1	55.9	55.3	54.4	7.5	13.8	17.6	21.5
	≥2/ mth	59.2[b]	54.6	53.9	58.2	57.5	56.5	7.6	13.6	17.7	21.7
	avSED	2.19	1.99	2.24	2.04	1.99	1.85	0.36	0.61	0.67	0.74
	P	**0.043**	0.374	0.642	0.051	0.244	0.180	0.673	0.092	0.612	0.858
Rib Eye	Never	58.5	52.9	53.8	56.8	56.6	55.3	7.2	14.2	18.0	21.6
	<2/ mth	58.9	54.2	53.1	57.1	56.9	55.7	7.7	13.6	17.7	21.9
	≥2/ mth	57.2	55.2	53.2	56.6	55.4	55.5	7.6	13.9	17.9	21.3
	avSED	2.17	1.92	2.17	2.01	1.94	1.81	0.35	0.61	0.67	0.73
	P	0.590	0.821	0.678	0.879	0.584	0.861	0.182	0.493	0.808	0.533
Rump	Never	57.4	53.0	51.6	54.7[a]	54.8	53.9	7.3	13.9	18.0	22.0
	<2/ mth	58.3	53.4	53.6	57.6[ab]	56.8	55.7	7.7	14.2	18.0	21.8
	≥2/ mth	62.7	57.4	56.2	61.0[b]	60.0	59.2	7.1	11.8	16.8	21.8
	avSED	2.79	2.47	2.79	2.57	2.49	2.31	0.45	0.76	0.83	0.91
	P	0.156	0.185	0.116	**0.034**	0.142	0.079	0.351	0.162	0.657	0.996
Silverside	Never	56.9[a]	53.7	52.9	55.8	55.7	54.8	7.4	13.5	17.7	21.5
	<2/ mth	59.5[b]	53.5	53.3	57.8	57.2	55.9	7.8	14.3	18.0	22.2
	≥2/ mth	63.5[b]	54.3	52.8	58.8	56.6	56.2	7.2	12.8	16.5	21.4
	avSED	2.85	2.58	2.90	2.66	2.58	2.41	0.47	0.79	0.86	0.95
	P	**0.041**	0.768	0.815	0.275	0.588	0.596	0.411	0.212	0.694	0.266
Sirloin	Never	55.8	52.2	51.4	58.1	56.2	55.1	7.2	13.1	17.4	20.8
	<2/ mth	59.0	53.5	53.5	56.6	56.4	55.3	7.6	13.8	17.6	21.4
	≥2/ mth	58.7	55.6	53.9	57.8	57.1	56.5	7.7	14.0	18.2	22.4
	avSED	2.55	2.29	2.58	2.38	2.30	2.15	0.41	0.70	0.77	0.84
	P	0.388	0.444	0.799	0.687	0.914	0.759	0.614	0.893	0.678	0.299

		Sensory Scores						WTP (£)			
		AR	TE	JU	FL	OL	MQ4	Fail	3*	4*	5*
Topside	Never	57.2	54.3	54.1	56.0	56.3	55.4	7.6	13.8	17.6	21.3
	<2/ mth	58.9	53.2	52.8	57.6	56.9	55.6	7.5	14.0	18.0	22.0
	≥2/ mth	65.1	56.5	52.2	58.6	56.3	56.7	7.4	13.5	17.5	21.5
	avSED	2.70	2.43	2.75	2.53	2.45	2.29	0.44	0.75	0.82	0.90
	P	0.117	0.686	0.656	0.486	0.862	0.986	0.965	0.445	0.485	0.326

a, b: Numbers in the same column which do not share a common superscript are significantly difference. AL: *aroma liking*, TE: *tenderness*, JU: *juiciness*, FL: *flavour liking*, OL: *overall liking*, WTP: Willingness to pay, <2/ mth: less than twice per month, ≥2/mth: twice or more per month, ***P***: probability, avSED: average standard error, Fail: unsatisfactory, 3*: satisfactory everyday quality, 4*: better than everyday quality, 5*: premium quality.

2.3.4.4 Motivation for beef choice

Consumers were asked to fill in a survey on how important were the 12 factors that might affect their motivation of beef choice; the results are shown in Table 2.18. REML analysis was conducted to identify the effects of importance level on consumer palatability traits and WTP; the results are shown in Table 2.19.

As expected, all consumers agreed that flavour and tenderness were very important factors to motivate them to choose beef (Table 2.18). The importance level of "I know how to cook it", "it's a healthy choice" and "I know where it comes from" were significantly different between regions. More consumers from Cork and Reading rated "I know how to cook it" at the very important level. Reading consumers were less concerned about the origin of beef product (Table 2.18). In addition, higher proportions of Reading consumers listed "it's a healthy choice" as not or little important factor to motivate them to choose beef.

Consumers for whom value was important scored significantly higher for *juiciness* (***P***<0.05), *flavour liking* (***P***<0.01), *overall liking* (***P***<0.01) and MQ4 (***P***<0.01). As expected, they had significantly lower willingness to pay to better than everyday beef (***P***<0.05) and premium beef (***P***<0.001). Consumers for whom animal welfare was important scored higher for most sensory attributes (Table 2.19).

The factors "it is good value", "I enjoyed it last time", "it is a healthy choice", " it is easy to prepare" and "animal well cared for" significantly impacted sensory scores (Table 2.19). On the other hand, the factors "it is good value" and "it is easy to prepare" significantly impacted WTP for some beef products (Table 2.19).

Table 2.18 Importance level for consumer motivation of beef choice.

Factor	Importance level	Region			χ^2	*P*
		Belfast (n=120)	Cork (n=120)	Reading (n=120)		
It is good value.	Not/ Little	9	10	6	1.68	0.795
	Moderately	58	53	61		
	Very	52	55	53		
It has a good flavour.	Not/ Little	1	2	3	1.47	0.833
	Moderately	20	24	23		
	Very	97	93	94		
It has good tenderness.	Not/ Little	1	5	6	5.58	0.233
	Moderately	24	31	32		
	Very	92	82	82		
It looks good.	Not/ Little	17	7	12	4.90	0.297
	Moderately	39	46	44		
	Very	61	65	64		
I know how to cook it.	Not/ Little	23	18	27	12.24	**0.016**
	Moderately	60	48	38		
	Very	34	52	55		
It is easy to prepare.	Not/ Little	29	28	32	4.43	0.351
	Moderately	63	58	50		
	Very	25	32	38		
I enjoy cooking it.	Not/ Little	26	29	27	3.59	0.465
	Moderately	61	48	54		
	Very	30	41	39		
It is a healthy choice.	Not/ Little	19	23	36	14.02	**0.007**
	Moderately	46	56	57		
	Very	50	39	27		
I enjoyed it last time.	Not/ Little	11	10	17	3.39	0.494
	Moderately	49	48	41		
	Very	55	60	62		
Animal well cared for.	Not/ Little	19	33	24	5.01	0.287
	Moderately	50	43	51		
	Very	47	42	45		
Environmentally friendly.	Not/ Little	33	33	32	1.48	0.831
	Moderately	53	48	50		
	Very	29	36	38		
I know where it comes from.	Not/ Little	10	15	29	16.57	**0.002**
	Moderately	59	44	52		
	Very	50	58	39		

χ^2 : chi-square test, ***P***: probability.

Table 2.19 Effects of consumer motivation on beef choice on sensory scores and WTP

		Sensory Scores						WTP (£)			
		AL	TE	JU	FL	OL	MQ4	Fail	3*	4*	5*
It is good value.	Not/ Little	56.7	52.5	49.4[a]	55.3[ab]	54.6[a]	53.7[a]	8.6	14.7	19.5[b]	23.7[b]
	Moderately	57.8	52.7	52.5[ab]	55.3[a]	54.9[ab]	54.1[ab]	7.5	14.0	17.9[ab]	22.1[b]
	Very	60.0	55.6	55.4[b]	59.4[b]	58.9[a]	57.7[a]	7.5	13.5	17.2[a]	20.68[a]
	avSED	2.50	2.23	2.51	2.27	2.21	2.06	0.41	0.68	0.757	0.816
	P	0.224	0.063	**0.036**	**0.007**	**0.006**	**0.007**	0.081	0.179	**0.019**	**<0.001**
It has a good flavour.	Not/ Little	59.4	55.1	52.7	53.6	53.6	54.0	7.6	14.1	18.0	22.7
	Moderately	56.2	52.0	52.3	56.7	56.5	54.8	7.6	13.3	16.9	20.5
	Very	59.2	54.4	53.8	57.1	56.7	55.8	7.6	14.0	18.0	21.9
	avSED	4.874	4.377	4.933	4.562	4.402	4.122	0.79	1.34	1.47	1.60
	P	0.278	0.372	0.702	0.724	0.727	0.683	0.889	0.393	0.180	0.058
It has good tenderness.	Not/ Little	56.3	57.2	51.4	53.5	54.4	54.6	7.8	14.3	18.6	24.0
	Moderately	59.1	53.7	54.5	58.2	57.7	56.3	7.7	13.5	17.2	20.9
	Very	58.6	54.1	53.2	56.8	56.5	55.5	7.5	14.0	17.9	21.8
	avSED	4.216	3.786	4.269	3.914	3.797	3.55	0.69	1.17	1.29	1.40
	P	0.413	0.966	0.491	0.377	0.452	0.633	0.695	0.518	0.466	0.204
It looks good.	Not/ Little	57.5	53.9	47.7	55.3	55.7	54.2	7.6	13.8	17.4	21.6
	Moderately	58.3	52.9	54.0	56.5	55.8	54.9	7.5	14.1	18.2	22.3
	Very	59.1	54.7	54.0	57.8	57.4	56.4	7.6	13.7	17.4	21.1
	avSED	2.314	2.072	2.309	2.132	2.07	1.933	0.38	0.64	0.70	0.77
	P	0.677	0.417	0.134	0.362	0.398	0.377	0.897	0.773	0.299	0.077
I know how to cook it.	Not/ Little	56.6	52.8	52.6	55.7	55.2	54.4	7.4	14.0	17.7	22.4
	Moderately	58.6	54.4	54.5	57.0	56.8	55.9	7.7	13.9	18.0	21.9
	Very	59.5	53.7	52.6	57.7	57.1	55.8	7.5	13.9	17.6	21.1
	avSED	1.906	1.711	1.918	1.757	1.71	1.595	0.31	0.52	0.58	0.64
	P	0.293	0.553	0.436	0.365	0.366	0.437	0.498	0.975	0.672	0.217
It is easy to prepare.	Not/ Little	55.6[a]	51.3[a]	50.7	54.2[a]	53.3[a]	52.7[a]	7.59	14.08	18.1[b]	22.5[b]
	Moderately	60.0[b]	54.5[b]	54.9	58.0[b]	57.5[b]	56.5[b]	7.55	14.06	18.1[b]	21.8[ab]

		Sensory Scores						WTP (£)			
		AL	TE	JU	FL	OL	MQ4	Fail	3*	4*	5*
	Very	59.0[ab]	55.9[b]	53.6	58.0[b]	58.7[b]	57.1[b]	7.48	13.22	16.8[a]	20.7[a]
	avSED	1.85	1.67	1.88	1.71	1.65	1.55	0.302	0.510	0.562	0.618
	P	**0.046**	**0.033**	0.073	**0.045**	**0.005**	**0.014**	0.917	0.124	**0.024**	**0.011**
I enjoy cooking it.	Not/ Little	57.0	52.3	51.9	56.6	55.6	54.5	7.3	13.8	17.4	21.5
	Moderately	59.9	54.9	55.2	57.2	57.4	56.4	7.7	14.1	18.2	22.0
	Very	58.3	53.8	52.4	57.6	56.6	55.6	7.5	13.4	17.3	21.3
	avSED	1.844	1.669	1.863	1.712	1.657	1.553	0.30	0.51	0.57	0.62
	P	0.279	0.288	0.111	0.843	0.591	0.514	0.373	0.284	0.167	0.441
It is a healthy choice.	Not/ Little	56.0	51.5[a]	52.4	54.4[a]	54.3[a]	53.3[a]	7.6	14.0	18.11	21.91
	Moderately	58.2	53.2[a]	53.3	56.4[a]	56.1[a]	55.0[a]	7.5	13.8	17.59	21.58
	Very	60.7	56.8[b]	53.9	60.0[b]	59.3[b]	58.2[b]	7.5	13.7	17.56	21.55
	avSED	1.90	1.68	1.93	1.75	1.70	1.58	0.31	0.53	0.59	0.64
	P	0.083	**0.007**	0.820	**0.013**	**0.023**	**0.014**	0.886	0.834	0.641	0.928
I enjoyed it last time.	Not/ Little	57.2	53.4[ab]	52.8	56.5	56.0	55.1[ab]	7.1	13.5	16.7	20.7
	Moderately	57.2	52.1[a]	52.0	55.3	55.1	54.0[a]	7.6	13.6	17.7	21.7
	Very	59.9	55.8[b]	54.8	58.6	58.0	57.2[b]	7.7	14.0	17.9	21.8
	avSED	2.21	1.97	2.23	2.04	1.99	1.85	0.36	0.61	0.68	0.74
	P	0.187	**0.024**	0.190	0.070	0.109	**0.041**	0.376	0.603	0.304	0.378
Animal well cared for.	Not/ Little	56.2	53.8	53.3	56.5[ab]	55.8[ab]	55.1[ab]	7.5	13.55	17.33	21.33
	Moderately	57.8	52.5	52.1	54.9[a]	55.0[a]	53.9[a]	7.7	13.62	17.74	21.60
	Very	60.5	55.7	54.9	59.6[b]	59.0[b]	57.8[b]	7.4	14.16	17.89	21.90
	avSED	1.86	1.67	1.87	1.72	1.67	1.56	0.30	0.52	0.58	0.63
	P	0.078	0.086	0.269	**0.008**	**0.020**	**0.018**	0.629	0.414	0.660	0.623
Environmentally friendly.	Not/ Little	57.5	53.0	52.9	55.6	55.1	54.4	7.4	13.4	17.5	21.3
	Moderately	58.7	53.4	53.9	56.8	56.6	55.4	7.7	13.7	17.8	21.8
	Very	59.7	55.7	53.8	58.9	58.5	57.3	7.4	14.3	17.8	21.8
	avSED	1.82	1.644	1.838	1.697	1.642	1.538	0.29	0.51	0.56	0.61
	P	0.548	0.243	0.847	0.191	0.153	0.204	0.319	0.314	0.820	0.622

		Sensory Scores						WTP (£)			
		AL	TE	JU	FL	OL	MQ4	Fail	3*	4*	5*
I know where it comes from.	Not/ Little	57.5	52.4	53.6	55.4	54.0	53.9	8.4	14.4	18.0	21.9
	Moderately	58.1	53.9	53.7	57.1	57.2	55.8	7.3	13.4	17.4	21.2
	Very	60.4	54.8	53.2	57.9	57.3	56.3	7.6	14.2	18.1	22.1
	avSED	2.085	1.876	2.114	1.944	1.878	1.756	0.34	0.58	0.64	0.70
	P	0.125	0.418	0.916	0.457	0.231	0.428	0.072	0.172	0.288	0.262

a, b: Numbers in the same column which do not share a common superscript are significantly different. AL: *aroma liking*, TE: *tenderness*, JU: *juiciness*, FL: *flavour liking*, OL: *overall liking*, WTP: Willingness to pay, ***P***: probability, avSED: average standard error, Fail: unsatisfactory, 3*: satisfactory everyday quality, 4*: better than everyday quality, 5*: premium quality.

2.3.5 Cluster analysis of the consumer panel

Cluster analysis was conducted and identified 4 cluster groups for *overall liking*. The total number of consumers in group 1, group 2, group 3 and group 4 were 121, 85, 96 and 58 respectively with 60% of similarity for *overall liking* score.

2.3.5.1 Socio-economic status, motivation of beef choice and consumption habit of consumers in cluster groups

The distribution of consumers in cluster groups (CG) for socioeconomic background, behavioural factors, consumption habits and motivation of beef choice were analysed. There were no significant differences between the cluster groups for these factors except for the consumption frequency of mince beef, but the significance was low (***P***<0.05), which are recorded in Annex 2.7 (p 311-314). As the total number of consumers was different in each group, comparisons between groups were showed in percentage (Figure 2.4). As shown in Figure 2.4, fewer consumers from Cork were classified in CG1 compared to Belfast and Reading. More than one third of consumers from Cork were classified in CG 3, and the number was much higher compared to Belfast and Reading. CG2 and CG4 appeared to have similar number of consumers from the three regions. There were no differences between consumers for the sociodemographic background, behavioural factors, consumption frequency for different muscle and motivation of beef choice, except for a small difference in consumption frequency of mince (Annex 2.7, p 311-314).

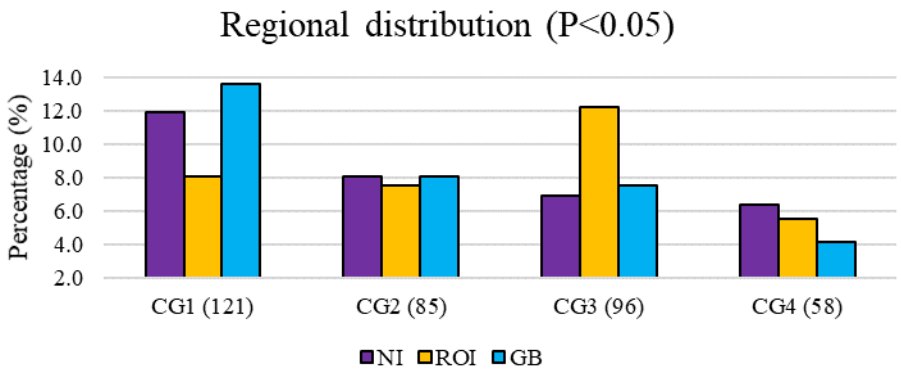


Figure 2.4 Regional distribution of members of cluster groups (CG) in percentage.

2.3.5.2 Comparison of overall liking scores between consumer cluster groups

The four cluster groups can be grouped by beef preferences and scoring pattern that they exhibit (Table 2.20). Although all consumers tasted portions of each treatment of samples, the mean sensory scores of CG1 was significantly highest (***P***<0.001) while CG4 was significantly lowest (***P***<0.001) amongst four cluster groups (Table 2.20). The mean *overall liking* scores for CG2 and CG3 were not significantly different (***P***>0.05).

Consumers in the four cluster groups exhibited differences in beef preferences. There were significant third order effects, with the highest significance identified for cluster group by hanging method by sex (Table 2.20). Other third order effects involving cluster group are of lower significance and can be interpreted with the following second order effects: cluster group by sex interaction, cluster group by hanging method interaction and cluster group by sample position interaction significantly affected consumer sensory scores.

Table 2.20 Mean differences in consumer overall liking across various cluster groups and significant interaction associated with overall cluster groups.

	AL	**TE**	**JU**	**FL**	**OL**	**MQ4**
Cluster group (CG)						
CG1 (121 consumers)	66.5^{c}	61.8^{c}	62.6^{c}	66.7^{c}	66.8^{c}	64.8^{c}
CG2 (85 consumers)	57.9^{b}	52.3^{b}	52.4^{b}	56.3^{b}	55.4^{b}	54.4^{b}
CG3 (96 consumers)	56.8^{b}	52.3^{b}	51.8^{b}	55.0^{b}	54.9^{b}	53.8^{b}
CG4 (58 consumers)	46.7^{a}	41.2^{a}	39.4^{a}	40.8^{a}	39.7^{a}	40.5^{a}
SED	1.88	1.66	1.82	1.54	1.39	1.35
P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Second order effects						
CG.H						
CG1 - AT	65.9^{d}	60.2^{de}	62.2^{e}	65.0^{d}	65.0^{d}	63.3^{de}
CG1 - TS	67.2^{d}	63.4^{e}	63.0^{e}	68.4^{e}	68.6^{e}	66.4^{e}
CG2 - AT	58.5^{c}	52.6^{c}	52.4^{c}	57.7^{c}	57.0^{c}	55.4^{c}
CG2 - TS	57.2^{bc}	52.0^{c}	52.4^{c}	54.9^{c}	53.8^{c}	53.5^{c}
CG3 - AT	53.3^{b}	45.1^{b}	46.1^{b}	48.2^{b}	47.5^{b}	46.9^{b}
CG3 - TS	60.3^{c}	59.5^{d}	57.4^{d}	61.8^{d}	62.3^{d}	60.8^{d}
CG4 - AT	45.7^{a}	39.6^{a}	39.9^{a}	39.8^{a}	38.6^{a}	39.4^{a}
CG4 - TS	47.7^{a}	42.8^{ab}	38.8^{a}	41.9^{a}	40.9^{a}	41.6^{a}
avSED	2.12	2.36	2.29	2.02	1.95	1.89
P	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

	AL	TE	JU	FL	OL	MQ4
CG.S						
CG1 - Bulls	66.5^{f}	67.1^{f}	65.9^{f}	68.6^{g}	69.9^{f}	68.3^{h}
CG1 - Cows	61.2^{e}	43.8^{c}	51.0^{cd}	56.3^{de}	53.8^{d}	51.3^{c}
CG1 - Steers	71.8^{g}	74.4^{g}	70.9^{f}	75.3^{h}	76.6^{g}	75.0^{i}
CG2 - Bulls	60.5^{e}	56.9^{de}	54.6^{de}	59.9^{ef}	60.4^{e}	58.6^{dfg}
CG2 - Cows	57.3^{cde}	44.8^{c}	51.3^{cd}	54.0^{d}	52.2^{d}	50.4^{c}
CG2 - Steers	55.7^{cd}	55.2^{d}	51.4^{cd}	55.0^{de}	53.7^{d}	54.3^{cdef}
CG3 - Bulls	57.5^{cde}	54.6^{d}	50.6^{cd}	53.6^{cd}	54.5^{d}	53.9^{cde}
CG3 - Cows	53.7^{c}	39.1^{b}	46.6^{c}	48.9^{c}	46.7^{c}	45.1^{b}
CG3 - Steers	59.2^{de}	63.1^{ef}	58.1^{e}	62.6^{f}	63.5^{e}	62.6^{g}
CG4 - Bulls	47.2^{b}	41.6^{bc}	38.9^{b}	40.8^{b}	38.8^{b}	40.2^{b}
CG4 - Cows	41.1^{a}	25.6^{a}	29.4^{a}	30.2^{a}	27.7^{a}	28.0^{a}
CG4 - Steers	51.8^{bc}	56.4^{d}	49.9^{cd}	51.6^{cd}	52.7^{d}	53.2^{cd}
avSED	2.47	3.23	2.88	2.57	2.58	2.53
P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
CG.Po						
CG1 - A	65.4	61.8^{f}	61.0	67.1	66.6	64.7^{e}
CG1 - M	67.3	61.9^{f}	64.6	67.1	67.2	65.3^{e}
CG1 - P	66.9	61.6^{f}	62.2	66.0	66.6	64.5^{e}
CG2 - A	59.8	53.9^{de}	52.3	57.2	56.0	55.3^{cd}
CG2 - M	55.7	51.4^{cd}	53.2	55.7	55.6	54.1^{cd}
CG2 - P	58.0	51.6^{cd}	51.8	55.9	54.6	53.8^{cd}
CG3 - A	57.7	56.6^{e}	54.3	56.1	57.0	56.4^{d}
CG3 - M	56.1	49.2^{cd}	50.7	53.0	52.9	51.6^{c}
CG3 - P	56.6	51.0^{cd}	50.3	56.0	54.6	53.5^{cd}
CG4 - A	49.5	46.3^{bc}	42.5	44.5	44.1	44.7^{b}
CG4 - M	46.9	41.0^{ab}	41.4	41.8	39.2	40.7^{ab}
CG4 - P	43.7	36.4^{a}	34.2	36.2	35.9	36.0^{a}
avSED	2.40	2.53	2.59	2.33	2.20	2.09
P	0.180	**0.031**	0.054	0.072	0.090	**0.029**
Third order effects						
CG.H.S	0.112	**<0.001**	**0.012**	**0.004**	**<0.001**	**<0.001**
CG.H.Po	**0.033**	0.348	**0.046**	0.277	**0.030**	0.161
CG.B.Po	0.984	**0.017**	0.175	0.774	0.171	0.112
CG.S.Po	0.583	0.241	**0.035**	**0.008**	0.054	**0.038**

a, b, c..k: Numbers in the same category which do not share a common superscript are significantly different. AL: *aroma liking*, TE: *tenderness*, JU: *juiciness*, FL: *flavour liking*, OL: *overall liking*, avSED: average standard error of difference, ***P***: probability, H: hanging method, B: animal breed, S: animal sex, Po: sample position, AT: straight hung, TS: aitch hung, A: Anterior, M: Middle, P: Posterior, CG: cluster group.

2.4 Discussion

2.4.1 Factors affecting the eating quality of beef

2.4.1.1 Factors affecting the cook loss and Warner-Bratzler Shear Force

Ageing significantly decreased the WBSF and cook loss in Batch 2 samples (Table 2.6). A decrease in WBSF was previously observed by Colle et al. (2015), who reported that the WBSF value of *longissimus lumborum* decreased significantly (***P***<0.001) from day 2 to 14 and also from day 21 to day 63 of ageing. However, most studies reported an increase in cook loss due to ageing (Jayasooriya et al., 2007, Shanks et al., 2002). The differences in the effect of ageing on cook loss might probably be due to differences in cooking temperature (65°C in this study, over 70°C in other studies), and Purslow et al. (2016) suggested that longitudinal shrinkage of myofibrils and muscle fibres occurred over 70°C to 75°C.

Samples were analysed for WBSF and cook loss. A second order effect of breed by sex significantly (***P***<0.05) affected the pHu and cook loss (Table 2.5). Continental bulls had higher pHu than continental and dairy steers while continental steers had significantly lower pHu than all beef samples except dairy steers (Figure 2.5). High pH meat results from low glycolytic capacity and insufficient glycogen content (England et al., 2018). Higher pHu of continental bulls may due to higher sensitivity of bulls towards sexual activity and other stress factors (Field et al., 1966, Katz, 2007). In our study, higher pHu of cows (compared to continental steers) were reported, probably due to the older age of cows (35 months to 188 months). This agrees with the results reported by Yim et al. (2015), however, Ahnström et al. (2012a) and Runowska et al. (2017) did not report any difference in pHu due to animal age. Cooking loss of dairy steers were the lowest compared to all other treatment (Figure 2.5). This agrees with the results reported by Cafferky et al. (2019), Moran et al. (2017) and Ozawa et al. (2000) in which cooking loss of steers were lower than bulls. A previous study also showed that the cooking loss of dairy (Holstein) beef was lower than for continental (Charolais) beef (Lively et al., 2005a). The differences in results might have resulted from the breed included in the study, where a number of breeds were combined into “continental breed”.

Tenderstretch decreased the WBSF, although the effect was not significant (***P***>0.05). Other studies have reported that tenderstretch significantly (***P***<0.05) reduced the WBSF of beef (Ahnström et al., 2012b, Lively et al., 2005b, Oltra, 2010).

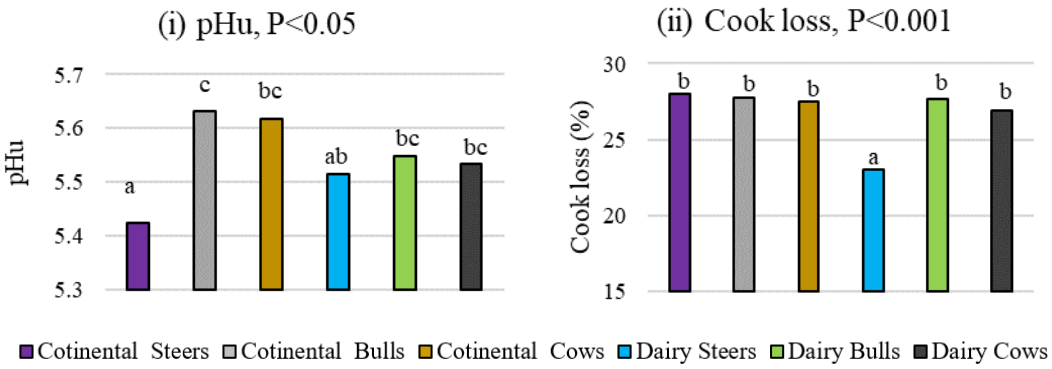


Figure 2.5 Second order effect of breed by sex interaction on pHu and cook loss.

2.4.1.2 Understanding the sensory characteristics and palatability traits of beef

Consumer results were analysed in two ways: unclipped (all results were included) and clipped (two highest and two lowest consumer results were removed for each set of samples). However, the significance levels were increased and decreased in different cases. More interaction effects were observed for the clipped results, however these effects were only significant at ***P***<0.05, which was not the main focus as the first order effects (hanging method, sex and breed) were significant at higher levels. Clipping of the scores reduced the variation within each set of 10 assessments (for a specific animal and cut/position) but did not necessarily reduce the variation within treatment due to the fact that multiple sets of samples were used for one treatment. In fact, the results (Table 2.9) showed that the differences between the unclipped and clipped sensory scores were less than 2 points in all consumers palatability traits. Thus, the discussion section focuses only on the unclipped results.

There were a number of significant interactions observed, with most palatability traits significant at ***P***<0.001 (Table 2.9). Interactions where ***P***>0.01 are shown in Table 2.9 and will not be discussed further. The decision was made based on the fact that 1 in 20 of significant interaction at ***P***<0.05 will be observed by chance. In addition, the

significant interactions with $\boldsymbol{P}<0.05$ generally had lower significance level compared to the first order effects.

Beef from dairy bulls and steers had significantly higher *red-juice external appearance* compared to dairy cows and continental steers and had higher *juicy external appearance* compared to beef from other treatments (Table 2.8, Figure 2.6). Beef from dairy steers had higher intensity on *succulence mouthfeel* compared to beef from other treatments and had higher *greasy external appearance* compared to beef from continental bulls, steers and dairy cows (Table 2.8, Figure 2.6). Meat from continental bulls had lower intensity on *tight internal appearance* compared to beef from other treatments, except for dairy steers (Table 2.8, Figure 2.6). Breed by sex interaction also had significant ($\boldsymbol{P}<0.001$) impacts on all palatability traits in consumer study except for aroma liking (Table 2.9, Figure 2.7). Meat from dairy breeds received higher scores *for beefy flavour, grilled steak flavour, intensity of aftertaste, charred aroma and greasy mouthfeel* (Table 2.8). In an agreement with this result, consumers' aroma liking, juiciness, flavour liking, overall liking and MQ4 scores were higher for meat from dairy breeds (Table 2.9). Sex also had significant impacts on sensory attributes and consumers' palatability traits. Meat from steers received higher scores for *crumbly mouthfeel, tender mouthfeel, grilled steak flavour, intensity of flavour, intensity of aftertaste* and lower scores *spongy* and *fibrous mouthfeel*, compared to bulls and cows (Table 2.8). The consumer results also supported these findings, as the meat from steers generally received higher scores.

These differences emphasised the differences in sensory quality between treatments. It was possibly that the partially thawed process for some treatments (T1b, T2b, T3b, T4b) prior to further portioning might induce juice loss but appears to have had limited effect on beef samples because these beef samples had higher intensities of *juicy external appearance* (Table 2.9), indicating that the partially thawed process had minimal changes on the sample. Within the same sex, the dairy breed generally had higher *tenderness, juiciness, flavour liking, overall liking* scores. However, this was not the case for bulls. Continental bulls performed better compared to dairy bulls. This disparity was caused by the older age of the dairy bulls (19 months) compared to the continental bulls (14-15 months).

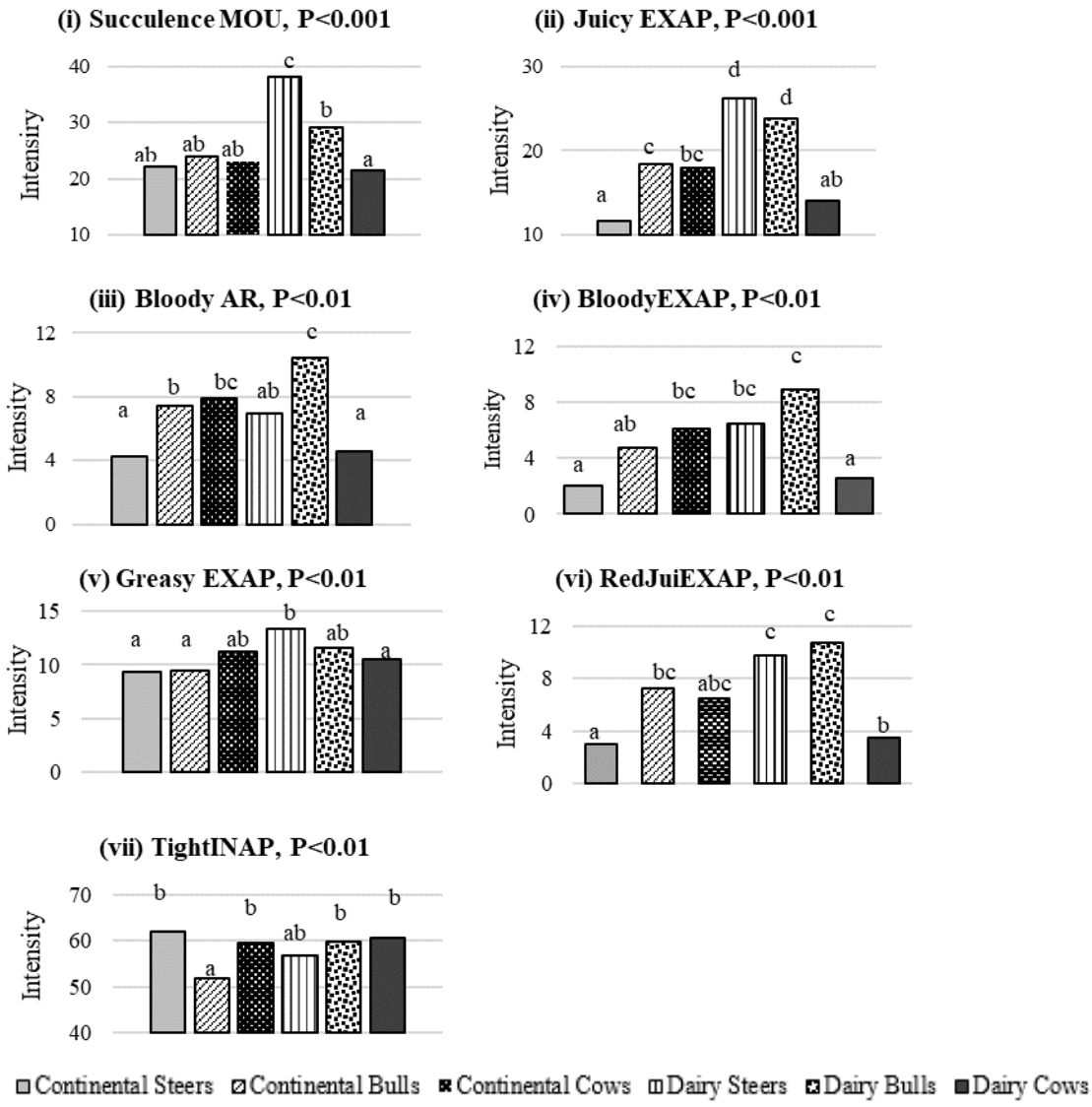


Figure 2.6 Effect of breed x sex interaction on sensory trained panel attributes, (i) *succulence mouthfeel*, (ii) *juicy external appearance*, (iii)*bloody aroma*, (iv) *bloody external appearance*, (v) *greasy external appearance*, (vi) *red juice external appearance* and (vii) *tight internal appearance.*
Columns that do not share a common superscript are significantly different (***P***<0.05).

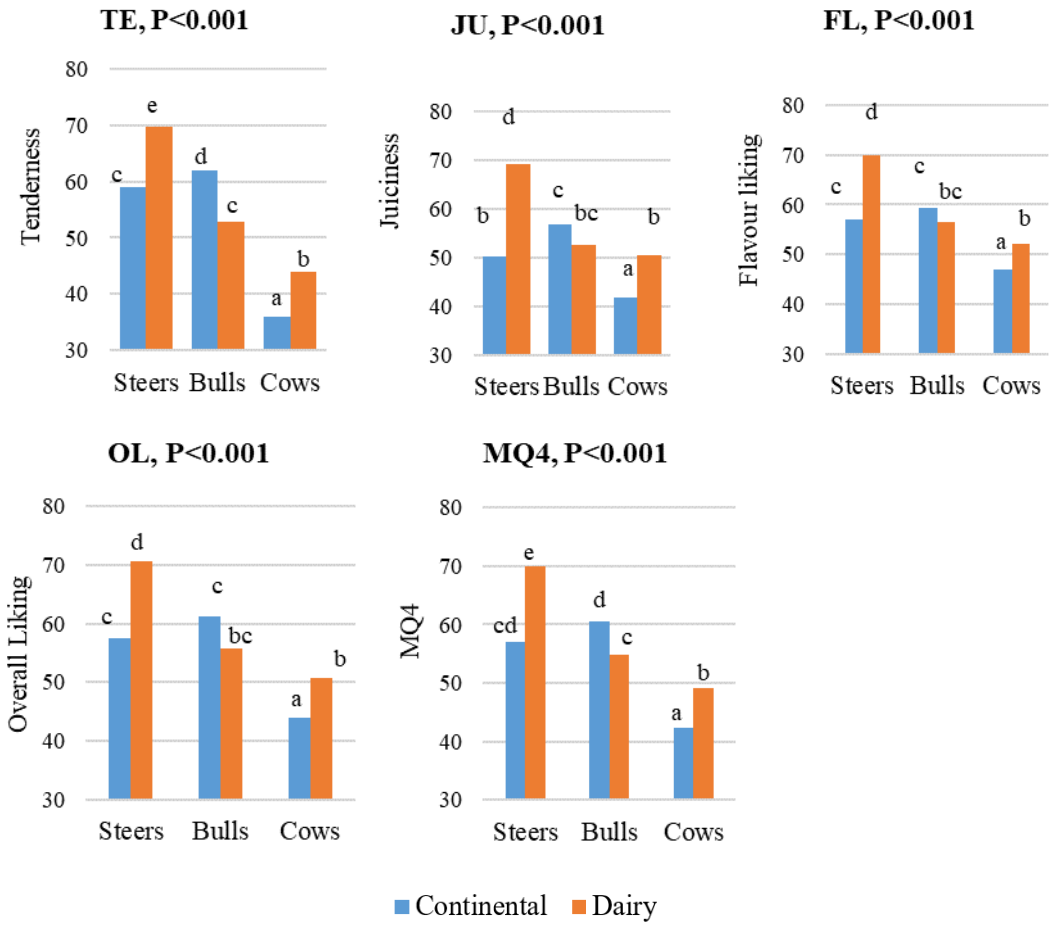


Figure 2.7 Significant (P<0.001) effect of breed by sex interaction on consumer *tenderness* (TE), *juiciness* (JU), *flavour liking* (FL), *overall liking* (OL) and MQ4 scores of consumer panel. For significance, see Table 2.9.

Columns that do not share a common superscript are significantly different (***P***<0.05).

Two types of hanging methods were compared, including straight hung (AT) and tenderstretch (TS). The consumers perceived higher eating quality in TS compared to AT striploin (Table 2.9). This agrees with the significant increases (***P***<0.01, Table 2.8) found by the trained panellists for *tender mouthfeel* and *tender texture on cutting* and lower scores for *crumbly TXC* and *spongy mouthfeel* in TS compared to AT beef. Surprisingly, *roast beef aroma* (***P***<0.01) and *beefy aroma* (***P***<0.05) were also reported higher in TS than AT beef (Table 2.8). One might question whether the significance of these effects was influenced by the mouthfeel or texture. However, aroma was assessed before the panellists consumed the sample and, therefore, this effect on aroma appears to be real. A previous study had reported differences in meat flavour due to suspension method but there was no clear indication where the meat flavour was

assessed prior or after the tenderness attribute (Ahnström et al., 2012a). In addition, consumers also scored aroma liking of the samples prior to other palatability traits, the results again showed that the aroma liking of TS beef rated higher compared to AT beef (Table 2.9). Therefore, the effects of carcass hanging method on flavour and aroma appeared to be real and justifies further investigation.

Tenderstretch hanging was introduced into the beef global market due to the tenderness of the steak being improved from 15% to 40% (Ahnström et al., 2012b). It is also used as a specific tenderness specification by some food processors and supermarkets in Northern Ireland and Republic of Ireland (D. McDonnell, pers. comm.). Ahnström et al. (2012b) concluded that pelvic suspension improved tenderness, muscle yield and decreased variation within beef muscles. For safety reasons, some abattoirs prefer to hang carcass using traditional method AT and age the meat longer. However, both factors should be considered to ensure cost effectiveness and maximise the eating quality of beef.

Anterior (A), middle (M) and posterior (P) samples were collected from each striploin. Three attributes were affected by sample position, including *char grilled flavour, chestnut colour and charred external appearances*. This concurred with the data collected from the consumer panel, where *aroma liking, tenderness, overall liking* and MQ4 score of anterior samples were higher compared to middle and posterior samples. This finding supported the results reported by a MSA study, where the consumers' palatability scores were higher for meat from anterior *longissimus dorsi* samples compared to posterior samples (Thompson, 2002). This was probably due to the differences in the levels of intramuscular fat and collagen in the muscles, although such analyses were not included in this study.

2.4.2 Understanding the differences between regions

2.4.2.1 Effects of region on consumers' palatability traits

The mean sensory scores for consumer data were significantly affected by the region where the panels were conducted (Figure 2.8). The same beef samples were tested in Belfast, Cork and Reading. Interestingly, Reading consumers consistently gave higher satisfaction for the same beef samples (Table 2.9). A previous study reported there

were significant differences in consumer palatability traits for beef samples between consumers from Houston, San Francisco, Chicago and Philadephia, suggested there were differences between regions within a country (Neely et al., 1998). However, many studies discovered the differences between countries. For example, 21 day-aged striploins were scored 5 points higher in MQ4 score by French consumers compared to Australian consumers (Legrand et al., 2012). A study conducted by Hwang et al. (2008) showed no significance difference (***P***>0.05) between Korean and Australian consumers for grilled steak but significant differences (***P***<0.05) were observed for barbeque samples. Although there were differences in the overall score, there were no distinct differences in preferences as only two interaction effects (Table 2.9) with region showed significant effects on sensory scores at ***P***<0.05 (Annex 2.6, p 310-311).

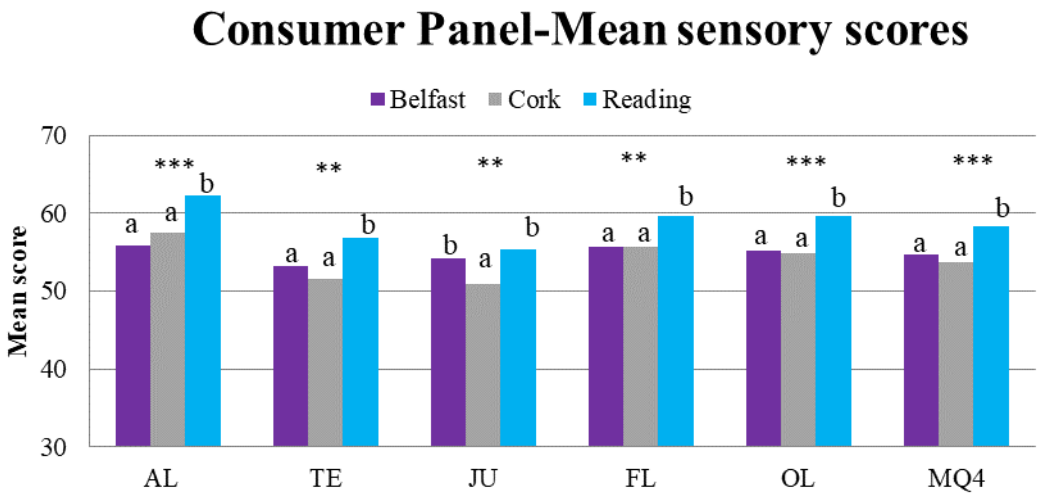


Figure 2.8 Significant (P<0.001) effect of breed by sex interaction on consumer tenderness (TE), juiciness (JU), flavour liking (FL), overall liking (OL) and MQ4 scores of consumer panel. For significance, see Table 2.9.
Columns that do not share a common superscript are significantly different (***P***<0.05).

2.4.2.2 Effects of region on consumers' willingness to pay

The results show that consumers in Belfast and Reading were willing to pay slightly more (***P***<0.05) for "premium", "better than every day" and "satisfactory everyday" products compared to Cork consumers (Table 2.11, Figure 2.9). The WTP of each product grade was converted to the proportion of willingness to pay to satisfactory everyday quality (P-WTP). The results showed that the P-WTP Belfast, Cork and

Reading for 5* beef products were 1.57, 1.54 and 1.58 respectively. This was lower compared to a study conducted by Lyford et al. (2010) on 960 Irish consumers, where the consumers P-WTP was 1.96 for 5* beef products. In addition, French consumers expressed themselves willing to pay from €5 to €23 from unsatisfactory to premium quality while previous work in Northern Ireland showed similar trends for unsatisfactory and satisfactory everyday quality, with P-WTP of 1.49±0.43 for premium quality beef (Bonny et al., 2017).

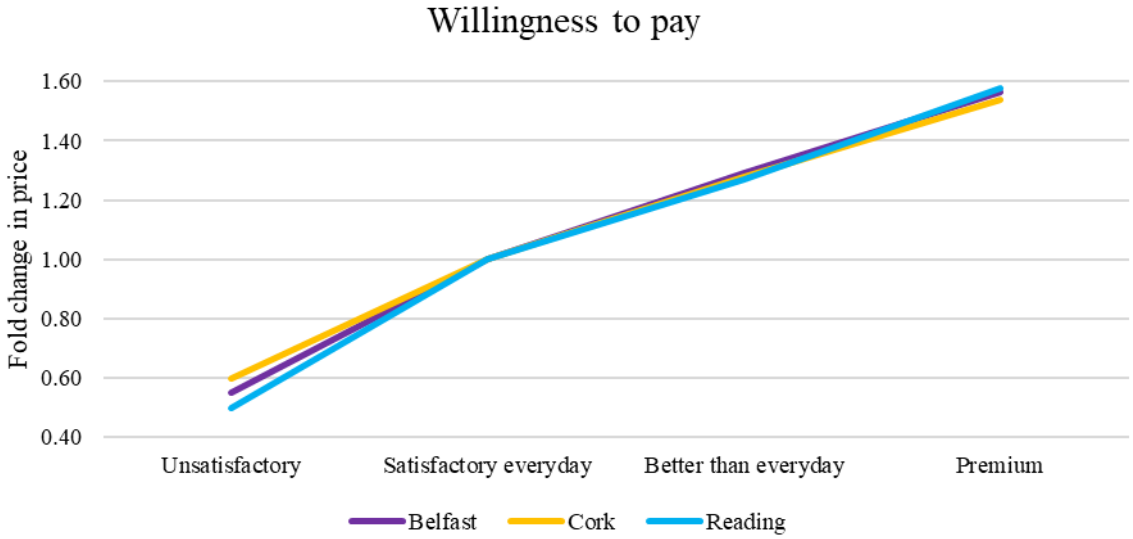


Figure 2.9 Comparison of proportion relative to satisfactory everyday quality (P-WTP) in Belfast, Cork and Reading.

2.4.2.3 Understanding the effects of consumers' socio-economic factors on region

Six questions were recorded for each consumers' socio-economic status, and the results presented in Table 2.12 showed that three factors differed significantly in distribution between the regions. The factors are consumers' age, occupation and number of adults in the household.

Differences in consumer age group and occupation group distribution differed significantly between regions (Table 2.12). However, neither consumer age group nor occupation had any effect (***P***>0.05) on palatability traits (Table 2.13). On the contrary, previous literature has demonstrated that sensory acuity was inversely proportional with the age of consumers (Baugreet et al., 2017) while another study showed that

consumers in the professional group showed an expenditure pattern which indicated a higher desire for convenience compared with normal households and they also showed a 'bourgeoisie' and 'snob' preference towards traditional cuts of beef when they want to cook at home (Newman et al., 2001).

Consumers' income level had a significant impact on WTP for "satisfactory everyday", "better than everyday" and "premium" beef, in which consumers with higher income had higher WTP than those with lower income (Table 2.12). There has also been a robust relationship reported between meat protein consumption and income level (Bruinsma, 2003). It is important to consider that consumers with lower income may perceive beef as a luxury treat and rate the palatability higher compared to consumers with a higher income. Implementation of the MSA grading system may enable all consumers from all income ranges to be supplied with beef of the overall quality they expect.

Household composition differed significantly ($P<0.05$) between the three regional consumer groups, with fewer consumers in Reading having more than two adults in a household (Table 2.12). However, number of people in the household had little impact on consumer score (Table 2.13).

2.4.2.4 Understanding the effects of consumers' behavioural factors on region

Of the four behavioural questions, two of the behavioural factors differed between regions. These included the most important attributes for the consumers and the purchase habit of the consumers (Table 14).

More consumers in Reading rated flavour as the most important sensory attribute for beef eating quality (Table 2.14). However, there was no significant impact of the factor "most important attributes" on consumers' palatability traits and WTP (Table 2.15). Thus, this factor did not explain the significant differences observed in the consumers' palatability traits between regions.

Higher proportions of consumers from Belfast purchased beef products from a butcher and other locations while more consumers in Reading purchased beef products from a supermarket (Table 2.14). This was probably due to lower availability of local

butchers or farm shops in Reading. It was suspected that consumers who purchased beef from a local butchers or farm shops may expect higher quality product, which might explain higher WTP for beef with "better than everyday quality" (Table 2.15).

Although there was no significant difference in distribution, consumers in all three regions showed a high preference for cooking level from "medium-rare", "medium" or to "medium – well done" (Figure 2.10). This contrasts with the results of a previous study conducted in ca. 2003 by Farmer et al. (2009b), in which more than 50% of NI consumers preferred "well-done" steak. This indicated that over a period of 14 years, consumer preferred cooking endpoint or "doneness" has shifted, with a greater number of NI consumers accepting a degree of pink colour in their beef. As expected, preference on doneness or cooking level had a significant effect (***P***<0.05) on sensory scores, including *tenderness* and *overall liking* as all samples were prepared "well-done". This result agreed with a study conducted by McCarthy et al. (2017), who reported that Irish consumers who preferred "rare" steaks scored *tenderness, juiciness, overall liking* and MQ4 score significantly lower compared to Irish consumers with preference for "medium" to "well-done" steak, when served a "medium" steak.

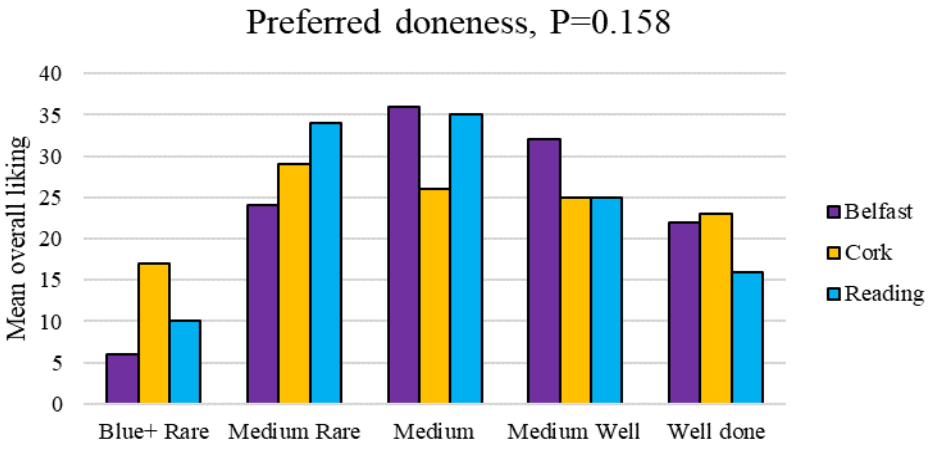


Figure 2.10 Consumers' preferred cooking endpoint in three regions.

2.4.2.5 Understanding the effects of consumers' consumption habits on region

Figure 2.11 shows the difference in consumption habits between Belfast, Cork and Reading. Mince and lean mince were the most frequently consumed products in Belfast, Cork and Reading. Consumers in Cork appeared to be more restricted when they purchased beef product as more participants never consumed brisket, silverside and rump.

The highest consumption frequency was observed in mince, followed by lean mince and sirloin. A study conducted by McCarthy et al. (2017) showed that striploin was most frequently consumed by Irish consumers amongst other cuts (blade, outside, rump, tenderloin, topside). Frequency of mince consumption significantly ($\boldsymbol{P}$<0.05) affected sensory scores, where consumers who never consumed mince scored *juiciness, overall liking* and MQ4 lower compared to other consumers (Table 2.17). The results also showed that consumers who frequently consumed mince scored significantly higher for juiciness, overall liking and MQ4 score (Table 2.17). Minced beef is normally the lowest price beef product to purchase, which might explain why the consumers awarded higher scores for striploin samples.

Frequency of consumption for different products had no effect on willingness to pay except for frying steak. Participants who consumed frying steak more than twice a month have lower willingness to pay for "satisfactory everyday beef" and "better than everyday beef", but the effect was not observed for "premium" beef.

Consumers in Reading consumed significantly more topside ($\boldsymbol{P}$<0.01) and rump ($\boldsymbol{P}$<0.001) compared to consumers in Cork and Belfast (Table 2.16). Previous research showed that 25% of grilled rump and 53% of roast topside were classified unsatisfactory (Farmer et al., 2016). Thus, this might explained the reason why consumers in Reading scored significantly higher in this study as higher quality meat, striploin steaks, were provided to all consumers. This was further proved by REML analysis in Table 2.17, where consumers who consumed more rump scored significantly ($\boldsymbol{P}$<0.05) higher in *flavour liking*.

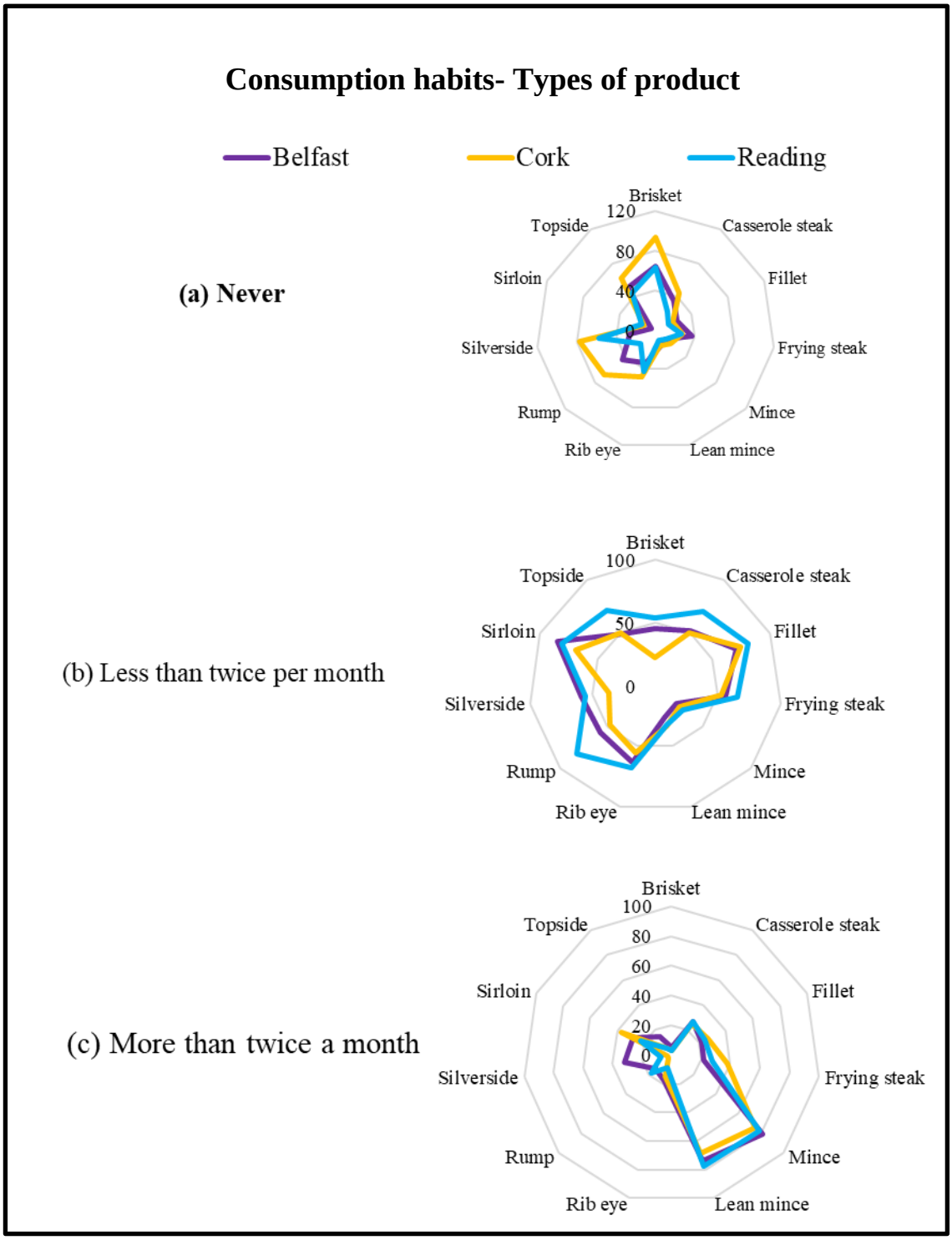


Figure 2.11 Spider diagram showing the consumer consumption frequency of different products.

The consumption for different beef products were showed in three frequencies, including (a) never, (b) less than twice per month and (c) more than twice a month (data refer to Table 2.18).

2.4.2.6 Understanding the effects of consumers' motivation for beef choice on region

Many factors can affect WTP, including production system, origin, environmental issues and others. Lewis et al. (2017) reported that German consumers had the lowest P-WTP for beef products from Great Britain while British consumers had the lowest P-WTP for Argentinian beef. Therefore, in this study, the authors investigated the motivation of beef choice for consumers in this section. Corcoran et al. (2001) discovered that low price product was more important for English and Italian consumers compared to French, Spanish and Scots.

Most of the factors that might affect the motivation for beef choice were listed as moderately important or very important (Figure 2.12). This suggested the growing awareness of consumers about the details of beef production, including eating quality, environmental issues, nutrition, or product source. Verbeke et al. (2010) discussed the increased awareness of extrinsic cues at the consumer levels among European citizens, including the interest between food and health, and consumers' interest regarding food origin and production. For example, a study conducted in Poland successfully segmented consumers into three groups according to their interest level in beef information. These three groups were ultra-conservative (18.4%), conservative (43.1%) and enthusiastic (38.5%) (Żakowska-Biemans et al., 2017).

Good flavour and tenderness were listed as very important factors to motivate consumers (Figure 2.12). This concurred with the findings of Egan et al. (2001), where the eating quality of beef was the most important factor that affected repurchase intent. Environmental issues, animal welfare, convenience and nutrition were also considered as important factors (Figure 2.12). A focus group study showed that the acceptance of consumers was low for excessive manipulation or new technologies although the innovations were aimed to improve eating quality, safety and healthiness (Barcellos et al., 2010). Convenience also becomes important as the lifestyle of consumers changes (Grunert, 2006).

Consumers who rated "ease of preparation" as an important factor scored significantly ($\boldsymbol{P}<0.05$) higher for most sensory scores except juiciness and had lower WTP for premium and better than everyday beef (Table 2.19). Grunert (2006) suggested that

the desire for products that only require little preparation is due to time pressures faced by the current generation.

Furthermore, Henchion et al. (2014) suggested that extrinsic cues such as sustainability, health or nutrition were possible to convey quality or eating quality. Interestingly, consumers in Reading were less concerned about the healthfulness of beef products compared to consumers in Belfast and Cork. Results showed that consumers who scored this factor as very important scored significantly higher for tenderness ($\boldsymbol{P}$<0.01), flavour liking ($\boldsymbol{P}$<0.05), overall liking ($\boldsymbol{P}$<0.05) and MQ4 score ($\boldsymbol{P}$<0.05).

Origin of the product was also an important factor with, out of 360 consumers, 155 consumers and 147 consumers rating it as "moderately important" and "very important", respectively (Table 2.18). However, more consumers in Reading listed the source of beef product as not an important factor. Realini et al. (2013) stated that origin was the most important factor compared to price and finishing diet with the preference being for beef that was produced locally. A consumer panel conducted in Spain found that consumers with higher income and higher education level showed preference for US beef if they were unaware of the source or origin (Sánchez et al., 2012).

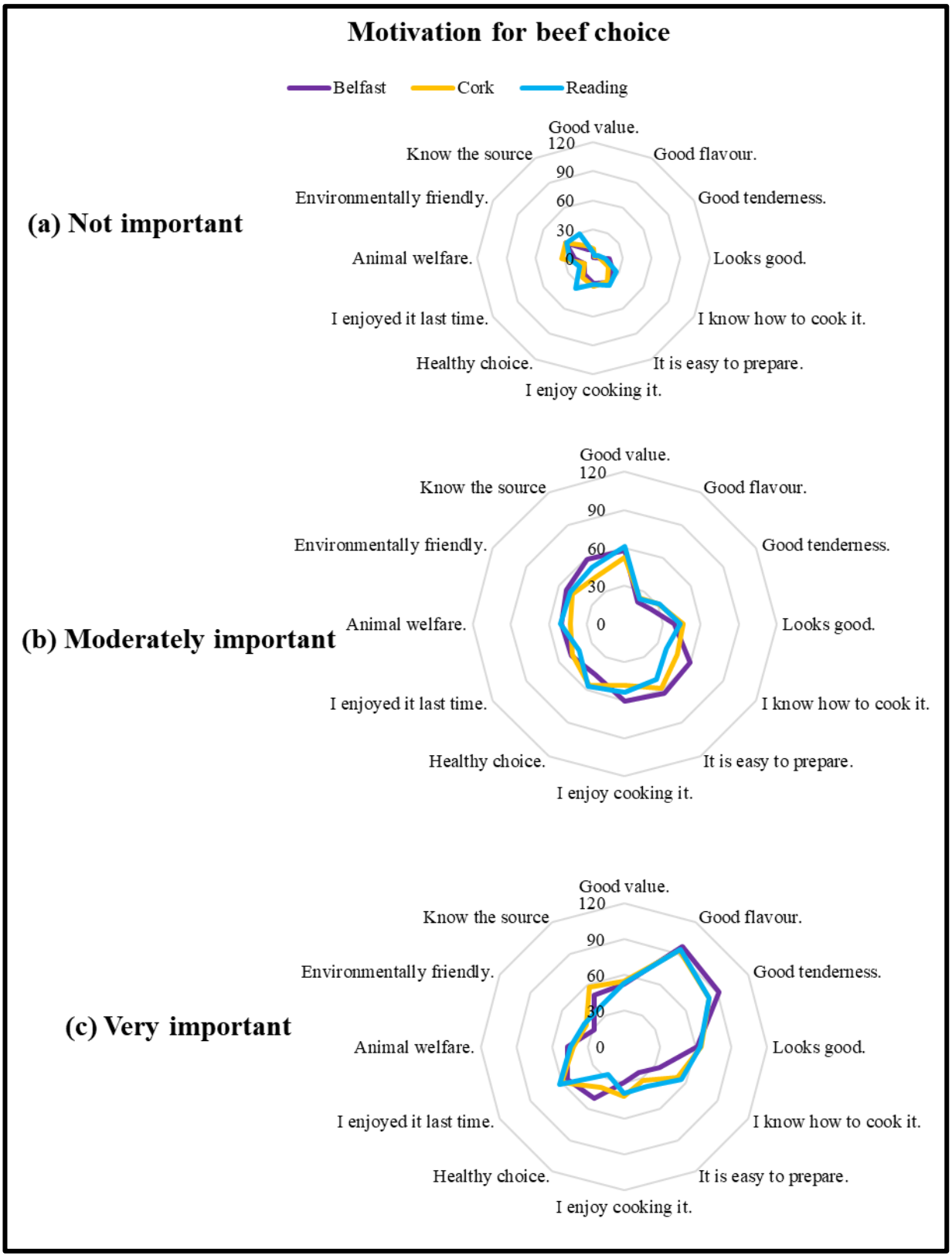


Figure 2.12 Factors that affect consumer motivation for beef choice.
The levels shown are (a) not important, (b) moderately important and (c) very important.

2.4.2.7 Understanding the differences in the MSA formula between regions

The weightings of *tenderness, juiciness, flavour liking* and *overall liking* were set to predict the ultimate rating from the Australian MQ4 equation (MQ4 = 0.3 *tenderness*+ 0.1 *juiciness*+ 0.3 *flavour liking* + 0.3 *overall liking*). However, different countries developed their own unique MQ4 model that use different weightings *of tenderness, juiciness, flavour liking* and *overall liking* scores. For example, Poland MQ4 models put more weighting on *flavour liking* and less weighting on *tenderness*. The final MQ4 model for Poland was MQ4 = 0.2 *tenderness*+ 0.1 *juiciness*+ 0.4 *flavour liking* + 0.3 *overall liking* (Hocquette et al., 2007). It is therefore interesting to identify the differences in coefficients between Northern Ireland (NI), Republic of Ireland (ROI) and Great Britain (GB) consumers using discriminant analysis (Table 2.21 and Table 2.22).

As described above, the coefficients varied for *tenderness, juiciness, flavour liking* and *overall liking*. To develop this equation, the coefficients for MQ4 were determined from the datasets from three regions, using the method described by Watson et al. (2008). In this method the coefficients are derived from two models based on all attributes and all omitting *overall liking*. As described by Watson et al. (2008), a heavy weighting was put on *overall liking*. The coefficients of *overall liking* for NI, ROI and GB were 0.61, 0.72 and 0.56 respectively (Table 2.21). Therefore, MQ3* model was developed to reduce the heavy weighting on *overall liking* (Table 2.21). The average between MQ3* and MQ4* was used to produce the final MQ4 model. The weightings and coefficients in the MQ4 model were an average between three and four variables approaches (Table 2.22).

Table 2.21 Coefficients for MQ4* and MQ3* models.

		Coefficient		
		NI	**ROI**	**GB**
MQ4* model	Tenderness	0.30	0.22	0.16
	Juiciness	-0.01	-0.02	0.16
	Flavour liking	0.11	0.08	0.12
	Overall liking	0.60	0.72	0.56
MQ3* model	Tenderness	0.48	0.40	0.30
	Juiciness	0.09	0.08	0.26
	Flavour liking	0.43	0.52	0.44

Northern Ireland consumers put higher weighting on *tenderness* compared to Republic of Ireland and Great Britain consumers. On the other hand, Great Britain had higher weighting on *juiciness* compared to Northern Ireland and Republic of Ireland. Northern Ireland consumers had slightly higher coefficient on *tenderness* compared to *flavour liking*, whereas Great Britain consumers put more weighting on *flavour liking* and Irish consumers put similar weighting on *tenderness* and *flavour liking*. Watson et al. (2008) suggested that the MQ4 equation varied with the datasets, therefore small differences between datasets were expected. Interestingly, the combined MQ4 model for 360 consumers (Table 2.22) is very similar to the Australian model (Polkinghorne et al., 2008). Watson et al. (2008) suggested that the MQ4 equation can vary from one panel to the other, thus small changes in the attribute's weightings have only small effects on the MQ4 score. The higher the number of consumers in the trial, the better the equation optimisation. The MQ4 model provides an easy and straightforward application for industry, while ensuring acceptable accuracy in predicting eating quality (Bonny et al., 2018).

Table 2.22 Final MQ4 model for different regions and countries.

Regions	**MQ4 Model**			
	Tenderness	Juiciness	Flavour Liking	Overall Liking
Current study				
NI	0.39	0.04	0.27	0.30
ROI	0.31	0.03	0.30	0.36
GB	0.23	0.21	0.28	0.28
NI+ROI+GB	0.31	0.10	0.28	0.32
Other studies				
Australia	0.3	0.1	0.3	0.3
Japan (Grill)	0.3	0.2	0.2	0.3
Japan (Yakuniku)	0.2	0.2	0.4	0.2
ROI (Previous study)	0.2	0.1	0.4	0.3
NI (Previous study)	0.2	0.1	0.4	0.3
USA	0.3	0.1	0.3	0.3
France	0.3	0.1	0.3	0.3
Poland	0.2	0.1	0.4	0.3

(Farmer et al., 2009b, Hocquette et al., 2011, Hocquette et al., 2014, Legrand et al., 2012, McCarthy et al., 2017, Polkinghorne et al., 2008).

In this study, consumers were asked to categorise beef samples into 'unsatisfactory' (fail), 'satisfactory everyday quality' (3*), 'better than everyday quality' (4*) and 'premium quality' (5*). The results are recorded in Table 2.23. The fail/3*, 3*/4/, 4*/5* boundaries in NI, ROI and GB were lower compared to the results from other studies (Table 2.23). For example, Legrand et al. (2012) found that the differences between extreme classes were important for French consumers as the boundaries between fail/3*, 3*/4* and 4*/5* were reported to be 38, 61 and 80. This suggested that consumers from these three regions had lower demand on eating quality compared to Japan, USA and France.

Table 2.23 MSA boundaries between fail, 3*, 4* and 5*.

Country/ Region	**Cut off score**		
	Fail/3*	**3*/4***	**4*/5***
Current study			
NI	36.0	57.5	76.5
ROI	35.0	58.0	76.5
GB	37.0	58.5	76.5
Other studies			
Japan (Grill)	40.4	66.8	83.1
Japan (Yakiniku)	43.4	68.5	83.9
USA (Grill)	41.0	65.0	82.0
USA (Roast)	43.0	66.0	83.0
France	38.0	61.0	80.0

(Farmer et al., 2009b, Hocquette et al., 2014, Legrand et al., 2012, McCarthy et al., 2017).

2.4.3 Multivariate analysis of consumer preference with corresponding sensory characteristics and instrumental measurements

2.4.3.1 Identifying consumer cluster groups

Four cluster groups were identified with 60% of similarity, which exhibited differences in beef preferences. There was no significant difference in the socio-economic factors or consumption habits between consumers in these cluster group except the consumption frequency of minced beef (Annex 2.7, p 311-314). Cluster group significantly ($P<0.001$) impacted all consumer sensory scores. Interestingly, although consumers received portions of sample from each treatment, consumers from CG4 were significantly lower compared to CG2 and CG3, followed by CG1 scored

the highest. There were significant third order effects, with the highest significance identified for cluster group by hanging method by sex (Table 2.20, Figure 2.13). Other third order effects involving cluster group are of lower significance and will be interpreted with the following second order effects. Cluster group by sex interaction was significantly affected on all consumer sensory scores. Consumers in CG1, CG3 and CG4 rated meat from steers significantly higher, followed by bulls and cows (Figure 2.13, 2.14). CG1 and CG4 mainly differed in the overall score, in which CG1 consumers giving mean scores over 60 while GC4 consumers giving mean scores below 50 (Table 2.20). CG2 was the only cluster group that liked beef from bulls as much as or (for tenderstretch beef) better than beef from steers. Meat from bulls was not accepted by MSA grading system in Australia, but the results from the consumer studies showed that meat from bulls received MQ4 score of over 50 from GC1, GC2 and CG3 consumers (76% of consumers), suggesting that these samples had acceptable eating quality (Table 2.20). CG3 was the only group of consumers that consistently perceived a significant difference for hanging method for meat from all sexes, though the consumers in CG1 perceived differences for meat from steers (Figure 2.13 and Figure 2.14). Cluster group by sample position had significant impacts in CG3 and CG4, for which anterior samples scored higher than middle samples or posterior samples, respectively (Figure 2.14).

These differences highlighted the variation between cluster groups and the fact that there is no “ordinary” consumer. CG1 was considered as “easy pleased” consumers, where consumers had a high degree of liking for all samples. On the other hand, CG4 was consider as “fastidious” consumers, which consumers consistently scored lower for all palatability traits on all samples. CG2 was considered as “bull beef likers”, as they preferred TS bulls the most. CG3 preferred TS beef and steer beef. Therefore, CG3 was considered as “tender beef likers”.

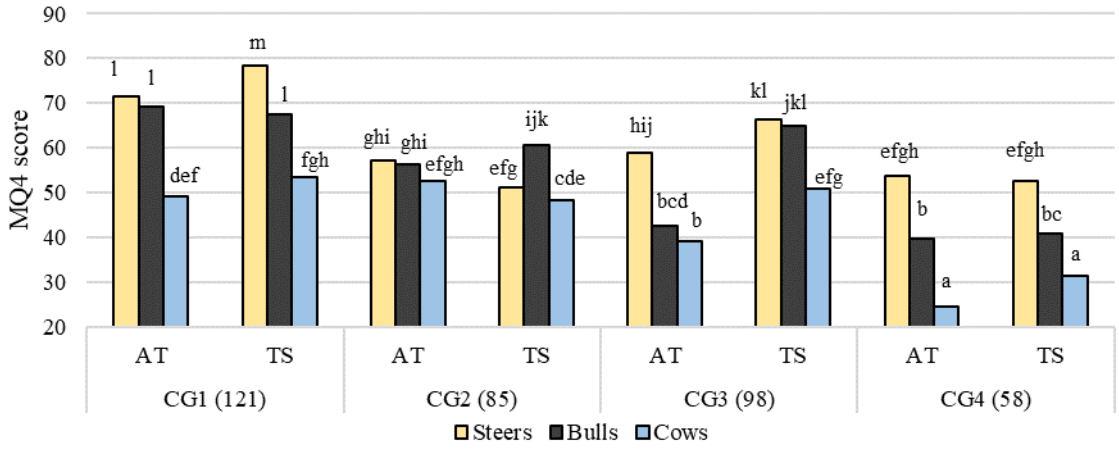


Figure 2.13 Effect of cluster group x hanging method x animal sex on consumer MQ4 score.
Number of consumers in each cluster group is included in bracket. For significance, see Table 2.5. Columns that do not share a common superscript are significantly different (***P***<0.05).

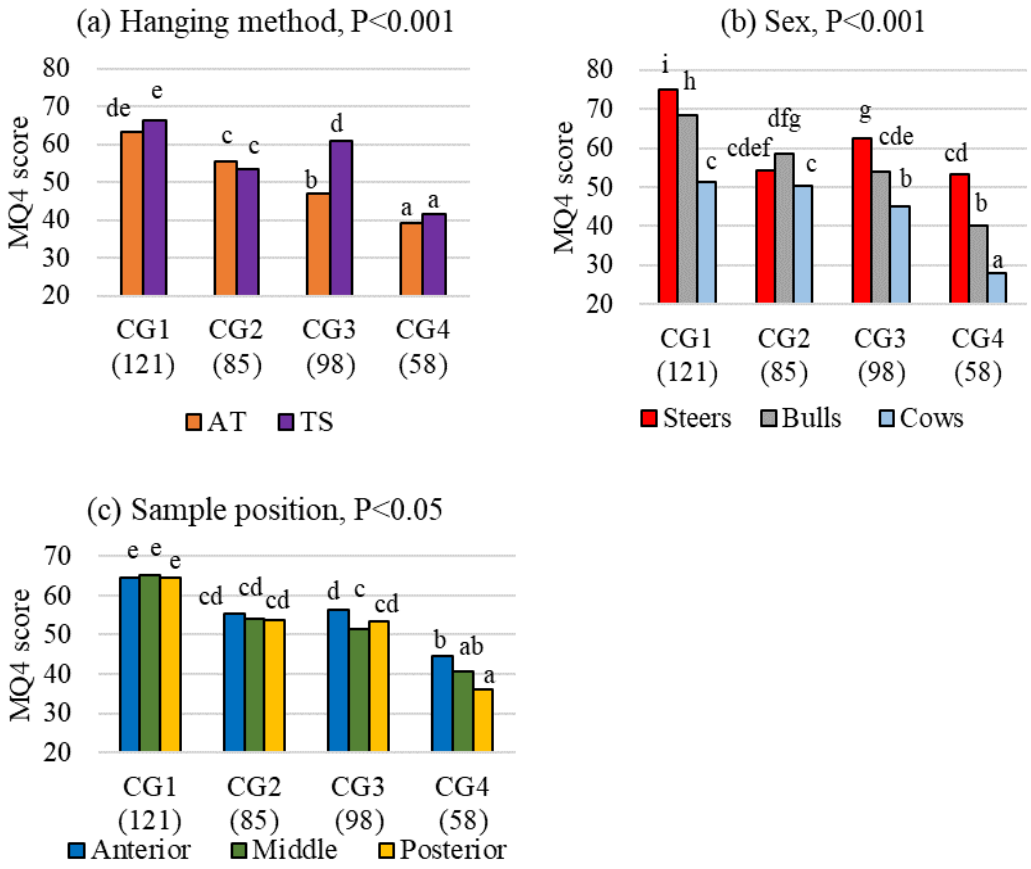


Figure 2.14 Mean consumer MQ4 score of (a) hanging method, (b) animal sex and (c) sample position among four overall cluster groups.
Columns that do not share a common superscript are significantly different (***P***<0.05).

2.4.3.2 Understanding the relationship between instrumental analysis and sensory evaluation of tenderness

The inverse relationship between WBSF with *tenderness* and cooking loss with *juiciness* was clearly observed from the external preference map for texture attributes in Figure 2.15. PC1 accounted for 75.6% of variation and the textures of samples were well separated with negative attributes (*form balls mouthfeel, fibrous mouthfeel*, etc.) at the left side of the axis and positive attributes (*tender mouthfeel, succulence mouthfeel*, etc.) at the right side of the axis. PC2 accounted for 15.3% of variation and the mouthfeel of beef samples were separated with the *succulence mouthfeel* at the top region and *crumbly mouthfeel* at the bottom region. The WBSF was negatively correlated with *tenderness* and cooking loss was negatively correlated with *juiciness* (Figure 2.15). Beef from dairy breeds was more *succulent* compared to that from continental breeds (Table 2.8) and illustrated by the association in Figure 2.15. This may be associated with a higher fat content level. Although this was not measured in this study, dairy breeds (Holstein, Danish Red, Highland, Casina, Jersey) have been showed to have higher fat content and lower muscle percentage in their meat compared to other breeds, such as Simmental, Charolais, Aberdeen Angus, Limousin (Albertí et al., 2008).

The relationships between consumer sensory measurements and meat quality measurements were investigated using Pearson's correlation. Correlation coefficients for palatability traits and instrumental analysis are presented in Table 2.24. There were strong correlations ($\boldsymbol{P}<0.001$) between the different palatability traits, with the largest between MQ4 score and *overall liking* (r=0.99), suggesting that MQ4 score was a suitable indicator to measure eating quality of beef. The correlation of *overall liking* with *tenderness* (r=0.91) was lower than that with *flavour liking* (r=0.95). This suggests that *flavour liking* had a slightly greater impact on *overall liking* than *tenderness*. This agrees with a study which also reported that *overall liking* was most highly correlated with *flavour liking*, with coefficient of 0.85 (Hunt et al., 2014). Oliver et al. (2006) reported similar correlation of *tenderness* and *flavour liking* with *overall acceptability*.

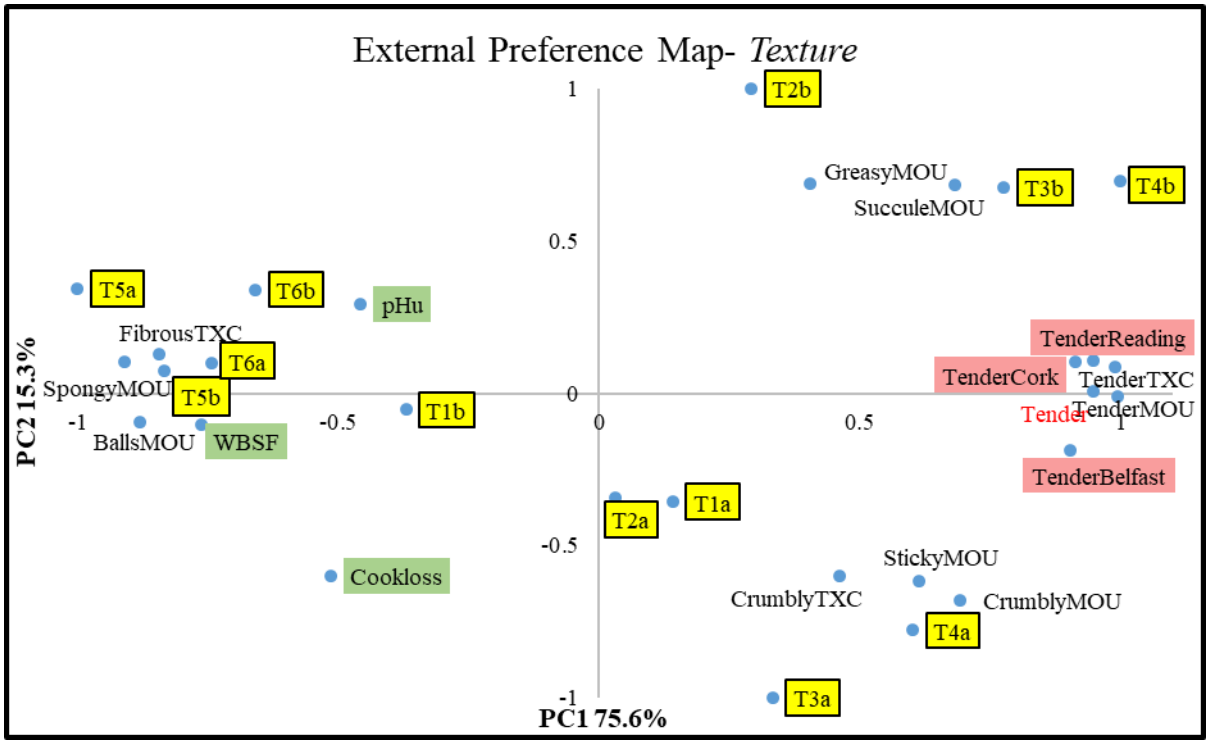


Figure 2.15 External preference map for texture on cutting and mouthfeel.
Consumer tenderness scores (red), sensory profiling panel (black) and instrumental analysis (green background). The mean tenderness scores for consumer panels in three regions shown with red background. Treatments highlighted with yellow background (details refer to Table 2.1). Abbreviations: TXC=Texture on cutting, MOU= Mouthfeel.

Table 2.24 Pearson's correlation coefficients (r-value) between consumer panel and instrumental analysis.

	AL	TE	JU	FL	OL	MQ4	pHu	WBSF21	Cook loss21
AL	-								
TE	0.58***	-							
JU	0.63***	0.78***	-						
FL	0.72***	0.84***	0.85***	-					
OL	0.69***	0.91***	0.88***	0.95***	-				
MQ4	0.68***	0.95***	0.89***	0.95***	0.99***	-			
pHu	-0.10^{ns}	-0.17^{ns}	0.00^{ns}	-0.09^{ns}	-0.09^{ns}	-0.11^{ns}	-		
WBSF 21	-0.31***	-0.44***	-0.33***	-0.39***	-0.40***	-0.42***	0.12^{ns}	-	
Cook loss 21	-0.17^{ns}	-0.26***	-0.36***	-0.28***	-0.29***	-0.29***	0.01^{ns}	0.08^{ns}	-

AL: *Aroma liking*, TE: *Tenderness*, JU: *Juiciness*, FL: *Flavour liking*, OL: *Overall liking*, pHu: ultimate pH, WBSF21: Warner Bratzler Shear Force 21 days.
ns: not significant, ***$\boldsymbol{P}$<0.001.

In this study, the correlations between WBSF 21 days with palatability traits were analysed, with the highest correlation coefficient observed for *tenderness* (r= -0.44), followed by MQ4 score (r= -0.42) and overall liking (r= -0.40). A similar study conducted by Hunt et al. (2014) reported that the correlation between consumer *tenderness* score and WBSF was only -0.22 (***P***<0.01), 0.15 (***P***<0.05), -0.18 (***P***<0.01) for longissimus, semimembranosus and Serratus ventralis, respectively. Caine et al. (2003) also reported that WBSF accounted for 36% (r=0.36) and 31% (r=0.31) of overall tenderness and overall palatability in the trained sensory panel. A higher correlation was observed in another study, where the correlation coefficient of tenderness rating and WBSF was -0.72 (Destefanis et al., 2008). As reviewed by Holman et al. (2016), the variability between studies can be derived from measurement procedure, sample preparation, cooking method and muscle type. Whilst WBSF is a valuable indicator of meat tenderness, it doesn't predict other sensory scores such as *flavour liking* or *juiciness* (Perry et al., 2001).

Cooking loss was calculated during the WBSF analysis. The result was correlated with palatability traits, with the highest correlation coefficient observed in *juiciness* score (r= -0.36). The correlation coefficients were lower compared to another study which reported the correlation between cook loss with consumer *juiciness* score, *initial juiciness* and *sustained juiciness* from trained panel were -0.51, -0.75 and -0.73 respectively (Loni, 2014). The low association between consumer score and cooking loss might be because by the different cooking methods were used for cook loss and WBSF measurement and the consumer study. The cooking method for WBSF analysis was selected because it was the standard method for WBSF measurement (Holman et al., 2016, Lively et al., 2005b).

2.4.3.3 Understanding the relationship between sensory profiling panel, instrumental measurements and consumer liking of beef using external preference mapping

Consumers' preferences have been proved to influence and predict consumers' behaviour (Font and Guerrero, 2014). Food choices can be affected by many factors and result in disliking or liking of a certain food product. In this section, the consumers' preference for grilled beef will be explored and related to specific sensory characteristics by employing internal and external preference maps.

Table 2.25 Percentage variation of principal components for external and internal preference mapping of overall liking.

Percentage Variation (%)- *Overall Liking*				
PCA - External	1	2	3	4
Percentage	50.7	27.8	8.5	3.7
PCA- Internal	1	2	3	4
Percentage	36.7	19.8	18.1	13.2

The percentage variation for the first four principal components for external and internal preference maps are recorded in Table 2.25. As stated earlier, external preference mapping relies on the sensory characteristics of the samples, which are obtained from the sensory profiling panel. Consumer data and instrument analysis data were correlated on to the same axes. In contrast, internal preference mapping is based on the consumer palatability traits, while the results from sensory profiling panel and instrumental results were then correlated.

Principal component 1 (PC1) accounted for 50.7% of total variation while PC2 accounted for 27.8% of the variation (Figure 2.16). PC1 demonstrated the separation between texture attributes, with *spongy mouthfeel* and *fibrous texture on cutting* at one extreme and *tender mouthfeel* and *succulence mouthfeel* at the other end. PC1 also showed separation between flavour attributes, with *sweet flavour* and *roast beef flavour* at one extreme and *sour flavour* at the other. Appearance attributes were also well separated by PC1, with *char external appearance* at one extreme and *pale external appearance* at the other end. PC2 mainly separated texture attributes, with *spongy mouthfeel* at one end and *tender mouthfeel* and *tender texture on cutting* at the other extreme.

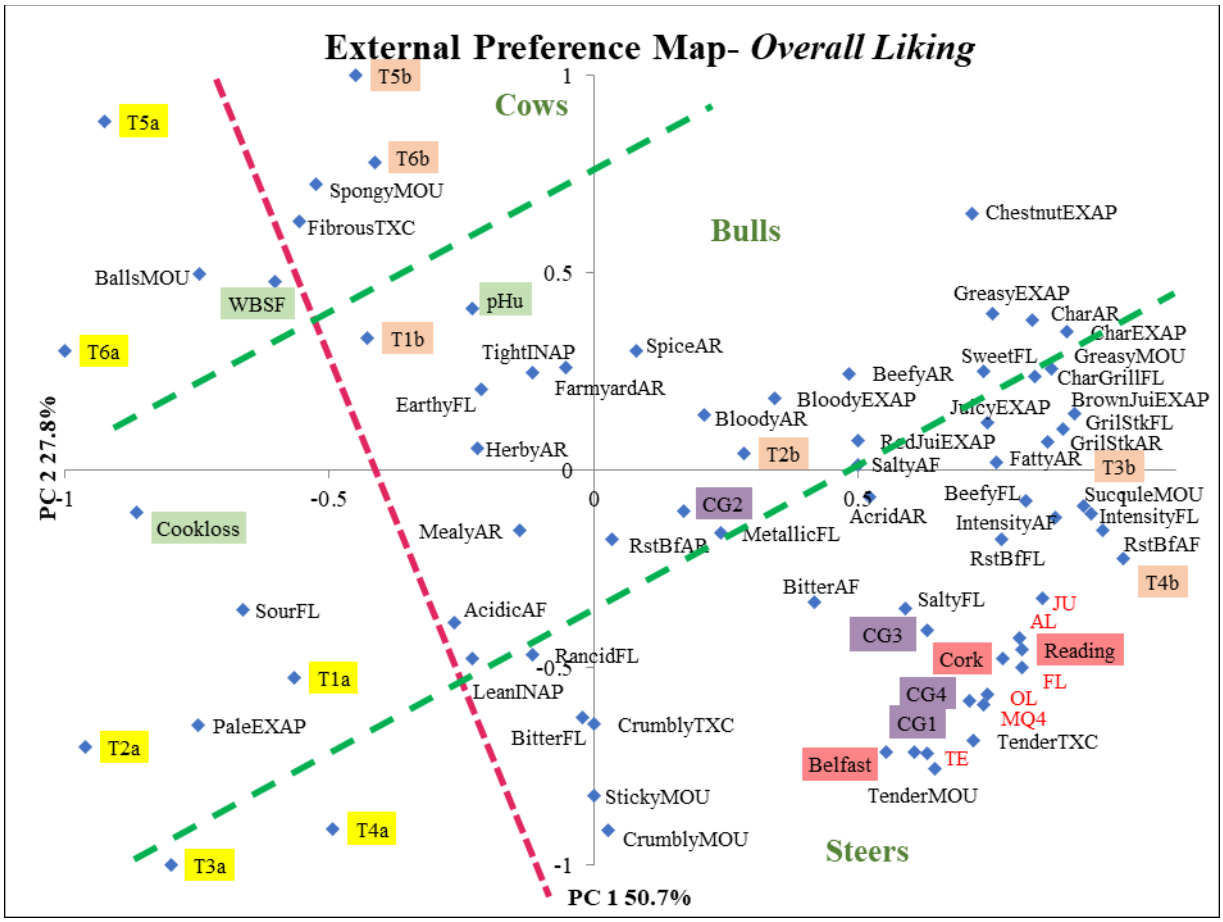


Figure 2.16 External preference mapping for overall liking.
Consumer sensory scores (red), sensory profiling panel (black) and instrumental analysis (green background) are shown on the map. The mean overall liking scores for consumer panels in three regions are shown with red background. Cluster analysis identified 4 cluster groups (purple background). Dairy breed treatments are labelled with orange background and continental breed treatments are labelled with yellow background (for treatment details refer to Table 2.1). Abbreviations: AR= Aroma, FL= Flavour, EXAP= External appearance, INAP= Internal appearance, TXC=Texture on cutting, MOU= Mouth-feel, AF= Aftertaste, CG=cluster group, AL=aroma liking, FL= flavour liking, TE= tenderness, JU= juiciness, OL= overall liking.

T4b and T3b were closest to the consumers *overall liking* score, suggesting that T4b and T3b were the most favoured product compared to other treatments. T5a and T6a were least favoured by all consumers, as they were at the extreme end of the axis. Consumer sensory scores also closely associated with *roast beef flavour, intensity of flavour, sweet flavour, beefy flavour, tender texture on cutting, tender mouth-feel, succulence mouth-feel* and *intensity of aftertaste*. This concurred with the study

conducted by Oltra et al. (2010) where consumer overall liking score was associated with *juiciness, sweet flavour* and *tender texture*.

Interestingly, the external preference map was able to distinguish the distribution of treatments as follows. In Figure 2.16, the two green lines separated the external preference map into three segments. The top segment consisted of cows (T5 and T6), middle segment consisted of bulls (T1 and T2) and the bottom segment consisted of steers (T3 and T4). Treatments were further separated by the red line, where all treatment b (dairy breed) were on the right side and all treatment a (continental breed) were on the left side on the map.

Internal preference maps were plotted, which gave priority to consumer scores rather than quantitative descriptive analysis. However, the restriction to plot such a map was that all consumers required to taste at least one sample from each treatment. Thus, in order to meet this requirement, T1a and T1b (continental and dairy breeds) were combined to form T1, similarly for T2, T3 T4, T5 and T6 (Figure 2.17). PC1, PC2 and PC3 of internal preference map accounted for 36.7%, 19.8% and 18.1% of variation. Two internal preference maps were plotted, including PC1 versus PC2 and PC1 versus PC3. PC1 separated the texture of the sample, with *tender mouthfeel* at one extreme and *fibrous texture on cutting* at the other extreme. The direction of the green arrow on the internal preference map suggested that hanging method improved the tenderness of the sample for cows and bulls but didn't affect steers, which were already very tender (Figure 2.17). In addition, the hanging method was also separated by PC2 and this PC separated some flavour attributes. This concurred with the discussion in section 2.4.1, which showed that hanging method affected some flavour and aroma attributes.

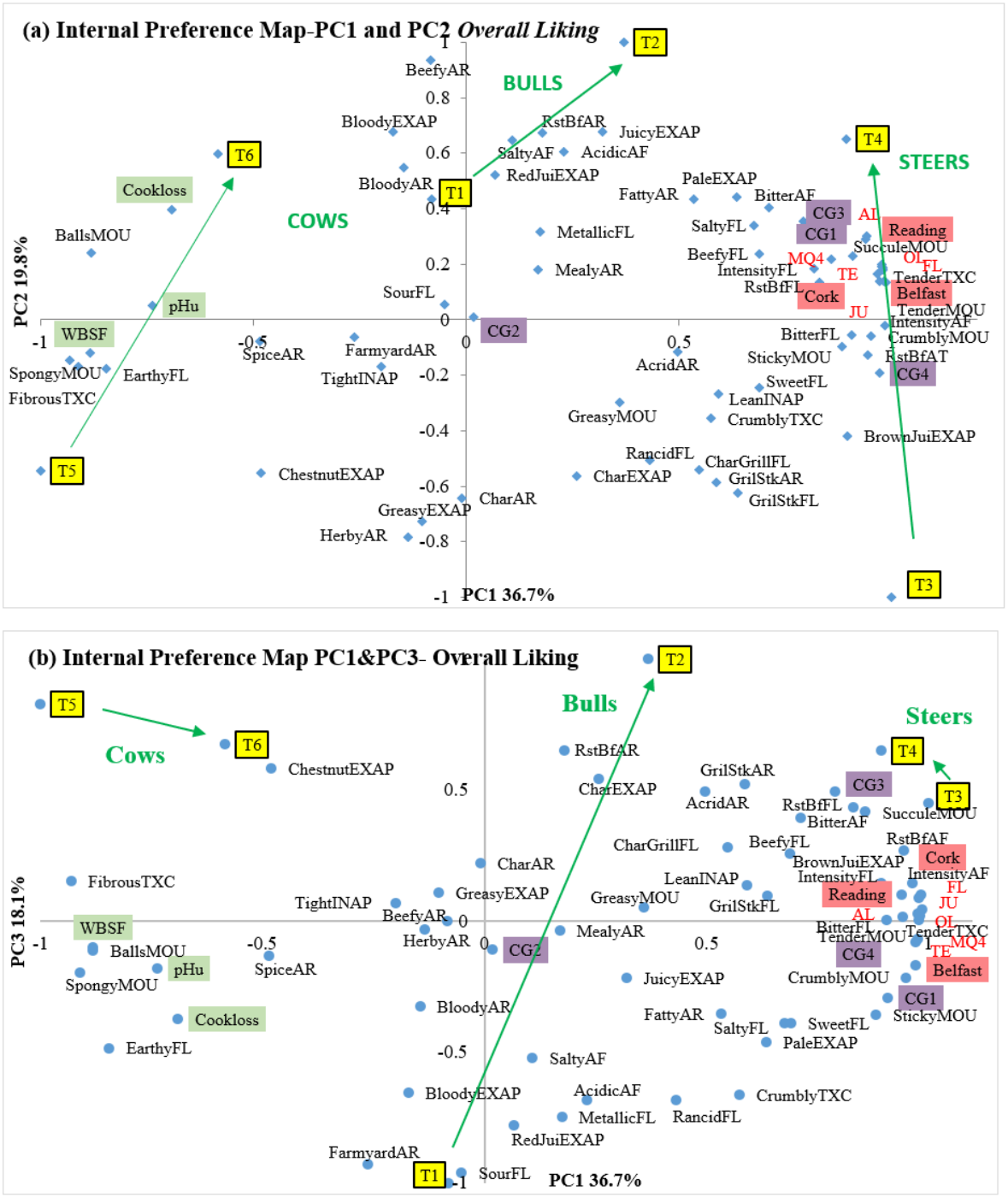


Figure 2.17 Internal preference maps for overall liking.
Consumer sensory scores (red), sensory profiling panel (black) and instrumental analysis (green background) listed on the map. The mean *overall liking* scores for consumer panels in three regions shown with red background. Cluster analysis identified 4 cluster groups in consumer (purple background). Treatments are highlighted with yellow background (details refer to Table 1). Abbreviations: AR= Aroma, FL= Flavour, EXAP= External appearance, INAP= Internal appearance, TXC=Texture on cutting, MOU= Mouth-feel, AF= Aftertaste, CG=cluster group, AL=aroma liking, FL= flavour liking, TE= tenderness, JU= juiciness, OL= overall liking.

Flavour attributes were better separated by PC3 compared to PC2. Negative attributes such as *rancid flavour* and *sour flavour* were located at one extreme and positive attributes such as *flavour liking* and *intensity of flavour* were located at the other extreme. Interestingly, the positions of treatments were slightly different. T1 and T2 (bulls) were well separated by PC3 but not cows and steers.

Figure 2.16 and Figure 2.17 consistently showed that CG2 was separated at a distance from CG1, CG3 and CG4. This cluster group can be intuitively interpreted as consumers that preferred bulls compared to steers or cows, which concurs with the results shown in Table 2.20. Furthermore, CG1, CG3 and CG4 preferred steers the most, followed by bulls and cows. This concurs with the position of CG1, CG3 and CG4 on the internal preference map (Figure 2.17), in which the position of these three overall cluster groups is closer to the end of steers (T3 and T4) on PC1 and separated away from cows (T5 and T6) at the other end. Oltra et al. (2015) identified cluster groups for lamb loin steaks and reported that these cluster groups were discriminated by their sensory preferences but not socio-demographic factors. The results in this study on beef agree with this finding, where no difference was observed for any socio-demographic factor except the consumption habit for mince. Similar results were observed by Oliver et al. (2006), who showed that the identified cluster groups in Spain, Germany and United Kingdom preferred different types of beef. A study conducted by Prescott et al. (2001) on 123 Japanese consumers and 125 New Zealand consumers identified 3 cluster groups on flavour among all consumers. CG1 and CG2 showed distinct preferences for beef products with different levels of volatile branched chain fatty acid while differences in cluster group 3 was less clear (Prescott et al., 2001). One theory is that preference in beef flavour varies due to consumer preference or background (Daley et al., 2010). Beef marketers are advised to market the beef based on consumer preferences and desirable attributes to increase the profitability to meet industry requirements (Purcell and Lusk, 2003).

The results from the preference mapping suggested that consumer liking of beef was associated with sensory attributes. Cluster analysis could be useful for categorising consumers' liking and understanding consumers' behaviour or habits towards beef attributes, which could be particularly helpful to implement product concepts.

2.5 Conclusion

This study provides findings on the differences and similarities between consumers from Belfast, Cork and Reading regarding their liking and perceptions of beef. Regions significantly impacted all consumers' palatability traits. The mean sensory score was significantly higher ($\boldsymbol{P}$<0.001) in Reading compared to Belfast and Cork. However, there were few differences between consumers from different regions on which samples they preferred.

In the present study, modified MSA models with different weighting on *tenderness, juiciness, flavour liking and overall liking* were created for each of the regions. Given that the results demonstrated there were differences between consumers from different regions, this opens up numerous potential opportunities for marketers. WTP for premium quality beef was also transferable across different regions, which indicated that the star system used by MSA would provide benefits to the NI, ROI and GB beef markets. Overall, NI, ROI and GB consumers are consistent and have a good understanding in determining the factors that affect the eating quality of beef.

Socioeconomic factors, behavioural factors, consumption frequency of different muscle and motivation of beef choice influenced some consumers' palatability traits. These factors including the consumers' income level, number of children in the household, preferred "doneness", consumption frequency of some muscles and a few factors for motivation of beef choice. WTP of beef product was affected by the consumers' income level, ease of preparation, importance of value, preferred "doneness" and the frying steak consumption frequency.

Reading and Cork consumers had higher WTP for premium beef compared to consumers from Belfast. Consumers in Reading were less cautious about the health and origin of beef products. In addition, Reading consumers had higher consumption frequency for rump and topside, which were lower quality meats. These factors might explain why consumers in Reading scored higher on all sensory scores for the same beef sample (striploin steaks).

Four overall cluster groups were identified. CG1, CG2, CG3 and CG4 were defined as "easy-pleased" consumers, "bull beef liker", "tender beef liker" and "fastidious" consumers, respectively. There were distinct differences in the distributions of

consumers from Belfast, Cork and Reading, with more consumers from Cork categorised in CG3 and a higher proportion of consumers from Reading categorised in CG2. Categorisation of consumer liking of beef and understanding consumers' attitudes towards beef sensory attributes could be very useful to implement product development and marketing strategies for meat products.

2.5.1 Future Direction

In this trial, consumer panels were conducted in Belfast, Cork and Reading to represent Northern Ireland, Republic of Ireland and Great Britain. Extension to other counties or regions would enable better representation of consumers in NI, ROI and GB, though evidence suggests that there would be little difference in preferences. However, extension of this work to other countries, e.g. those importing Irish/Northern Irish beef could be further investigated. Another possibility would be to investigate the effect of extrinsic cues and market factors on consumers' motivation of beef choice, which may provide opportunities for beef market supply-side strategies.

QDA was conducted in this study, which allowed the identification of sensory attributes affecting consumer perception of beef. This would help the beef industry to develop quality products, identifying emerging markets and improve overall meat quality. However, to ensure that eating quality meets consumer expectations, it is vital to understand the pre- and post-slaughter factors that might affect the eating quality of beef. It ixs also fundamental to thoroughly analyse the effect of flavour precursors and sugar in the production of flavour volatiles.

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Chapter 4 Development of an Accessible and Robust Headspace Solid Phase Microextraction Technique for the Analysis of Cooked Beef Samples

4.1 Introduction and Objective

Beef aroma and flavour are very important parameters that affect consumers' choices. Therefore, the ability to characterise, identify, quantify and evaluate the aromatic fraction of beef product is very important. The headspace volatile compounds for beef aroma have been extensively studied for beef but the results have been difficult to compare because different extraction methods were employed between studies (Celia Resconi et al., 2012, Gardner and Legako, 2018, Insausti et al., 2002, Legako et al., 2015, Rivas-Cañedo et al., 2011, Tansawat et al., 2013). Solid-phase microextraction (SPME) is a recognised technique to investigate the semi-volatile and volatile compounds for beef, due to its cost effectiveness, high efficiency, high sensitivity and ease of use (Saraiva et al., 2015). A method developed in 2009 between Agri-Food Bioscience Institute (AFBI) and Texas Tech University (TTU) for beef volatile analysis using manual SPME has been intensively used in many studies and the method is published in Legako et al. (2015). Therefore, this method was selected to analyse the headspace volatile compounds in Chapter 3. However, the method employed was labour intensive and time consuming. Variation was observed between trials. With the advance in new technologies and increasing availability of automatic SPME, there is an opportunity to develop alternative methods for beef volatile analysis to minimise the variation between analysis and create a less labour intensive process.

Competition between volatile compounds during SPME fibre adsorption is one of the limitations for SPME, which can affect quantification of volatile compounds (Met and Şahin Yeşilçubuk, 2017, Oliver-Pozo et al., 2015). There are several classes of fibre coating, such as polar (carbowax/ templated resin, carbowax/ divinylbenzene (DVB), polyacrylate), non-polar polydimethylsiloxane (PDMS) and mixed polarity (PDMS/DVB, carboxen (CAR)/PDMS, DVB/CAR/PDMS) (Lorenzo, 2014). Due to the physico-chemical properties of the fibre stationary phase, different fibre coatings attract compounds with different volatility and polarity. A small variation in the coating difference has a big impact on the quantity of volatile compound adsorbed on

the fibre. Therefore, this study will investigate the suitability of two SPME fibres; CAR/ PDMS SPME fibre and DVB/ CAR/ PDMS SPME fibre as they have been widely used to collect volatile compounds from cooked beef (Machiels and Istasse, 2003, Rivas-Cañedo et al., 2011, Watanabe et al., 2015).

External standards such as alkanes and bromobenzene were used in the original method developed by AFBI and TTU to monitor the daily instrument performances (Legako et al., 2015). An internal standard (IS) was not included because it had been found to give ineffective standardisation. However, in general, the addition of an IS can be very useful to monitor the variation and to allow correction of the instrumental response drift with IS analyte. The affinity of the IS analyte for the extraction phase must be considered during IS selection (Souza-Silva et al., 2015). Further consideration such as analyte volatility, identical distribution coefficient and co-elution of other native analytes were vital for IS selection (Machiels and Istasse, 2003). In this trial, 1,2-dichlorobenzene will be included as the IS for SPME extraction to review the efficiency of the IS pairing with the new method. This standard is selected as it was previously used in another study (Elmore et al., 2001b)

The integrity of the beef sample, heterogeneity of sample matrix, and the generation, acquisition and quantification of instrumental response required in-depth investigation. Therefore, the aim of this study was to develop a robust HS-SPME combined with gas chromatography/ mass spectrometry (GC/MS) method for cooked beef sample. Two trials were conducted. Trial A aimed to investigate the variability of the four methods selected. Trial B was conducted to use the effect of extended ageing period on beef volatile compounds to compare methods.

4.2 Materials and Methods

4.2.1 Experimental design

Four methods (M1 to M4) were selected to identify the suitability for headspace volatile analysis of grilled beef samples (Figure 4.1). These methods were adapted from the method developed by AFBI and TTU and described in Legako et al. (2015).

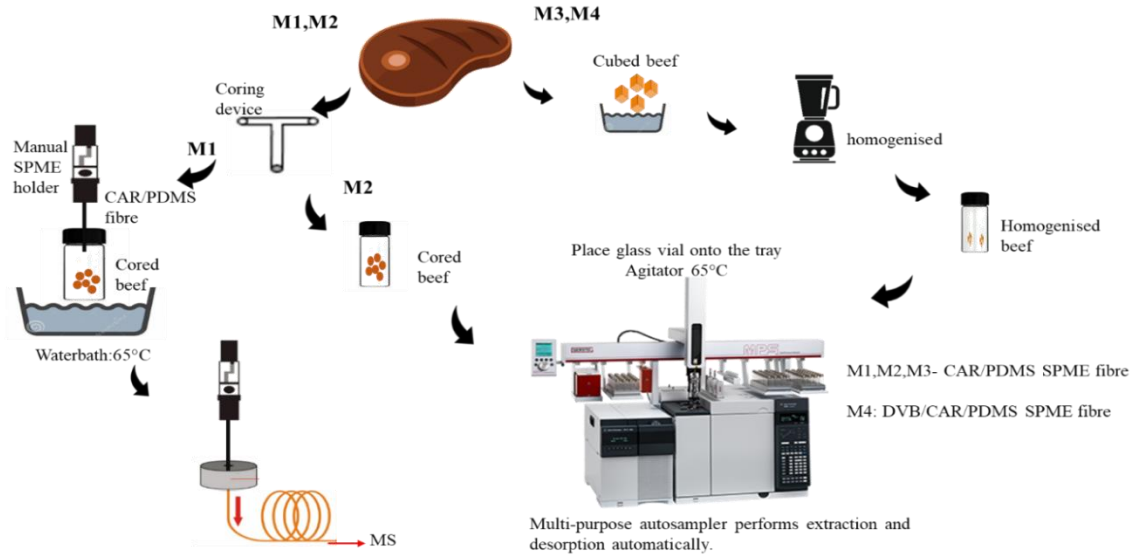


Figure 4.1 Illustration of methods selected for the trials.

Two trials were conducted, namely Trial A and Trial B. For Trial A, 48 samples from the same striploin were randomly divided to four batches and analysed with four headspace-solid phase microextraction (HS-SPME) methods (Table 4.1).

Trial B was conducted to investigate the effects of extended ageing period on headspace volatile compounds. To achieve this, sample steaks collected were allocated to 4 days, 7 days, 21 days, 49 days and 120 days of post-mortem ageing at 2°C using latin square design. In Trial B, the manual method M1 with lowest performance in Trial A was excluded and only three methods (M2-M4) were used (Table 4.1).

Table 4.1 Number of samples used for each method in Trial A and Trial B.

Method	Trial A	Trial B			
	A21	A4	A7	A21	A49
M1	12	0	0	0	0
M2	12	6	6	6	6
M3	12	6	6	6	6
M4	12	6	6	6	6

A: Ageing period (days).

4.2.2 Product procurement and preparation

One boneless striploin was collected from an animal (16 months old, Holstein breed) and delivered to AFBI Newforge. The ultimate pH of the striploin was recorded (pHu=5.46). The anterior end of the striploin was trimmed to achieve a smooth surface. Twelve steaks (25mm thick slices) were collected from the striploin. The steaks were further cut into 4 smaller samples (ca. 50mm x 50mm), and each was analysed using four different methods (M1 to M4).

For Trial B, two boneless striploins from the same animal (23 months old, Simmental breed) were collected from a commercial beef processing facility in Northern Ireland and delivered to AFBI Newforge. The ultimate pH of the striploins was recorded (pHu= 5.44). External fat of the striploins was removed. The striploins were sliced into 25mm thick steaks. Fifteen slices of steak were collected, which separated into three sets; anterior (slice 1 to slice 5), middle (slice 5 to slice 10) and posterior (slice 11 to slice 15). The samples were allocated into five batches with designated ageing periods; 4 days, 7 days, 21 days, 49 days and 120 days within each set using latin-square design. The steaks were further cut into three smaller samples (ca. 50mm x 50mm), one for each method (M2, M3 and M4).The samples were stored at 2 to 4°C, aged to the designated ageing period, blast frozen and stored in the -80°C freezer until further analysis.

4.2.3 Volatile analysis

4.2.3.1 Sample cooking procedure

Samples were removed from the -80°C freezer and thawed at 4°C for 24 hours. Clam grill (S-143, SILEXIA UK. Ltd, York, United Kingdom) was switched on at least 30 minutes prior to cooking. The average grill plate temperature was set to 180°C and checked using a non-contact thermometer (STTMProPlus, Raytek, Thermimport Quality Control, Zevehuizen, Netherland). The samples were grilled on a clam grill for 3 mins 30 secs to achieve an internal temperature of 65°C. The final temperature of all samples was measured using a k-thermocouple thermometer (HI93532, Hanna Instruments Ltd, Bedfordshire, UK).

4.2.3.2 Sample preparation

Beef samples for M1 and M2 were cored with a 1.27cm diameter coring device. Intact beef cores (2±0.1g) were placed into a 20ml glass vial with magnetic metal cap (320018R-2375 and 535050TB-18M, JG Finneran, Vineland, USA). Beef samples for M3 and M4 were cut into 0.5cm^3 cubes. The beef cubes were immersed in liquid nitrogen for 5 mins. After the beef cubes were frozen completely, the beef cubes were homogenised with a grinder (Kenwood compact chopper / grinder AT320A, Havant, UK) by quickly turning on and off for five to eight times. For M3 and M4, the samples were stored in a -80°C freezer until further analysis.

The homogenised beef (2±0.1g) was transferred into a 20ml glass vial. The sample weight was measured and recorded. 5µl of an internal standard (25ng/µl of 1,2-dichlorobenzene in methanol solvent) was added into each vial using a glass syringe (Agilent, Santa Clara, US).

4.2.3.3 Extraction of headspace volatiles

For M1, the glass vial containing beef cores was transferred to a 65°C water bath and 5 minutes allowed for equilibration. The headspace volatiles were extracted on to SPME using a manual 75µm carboxen (CAR)/ polydimethylsiloxane (PDMS) SPME fibre and holder (Supelco, Bellefonte, PA, USA). After equilibration, the fibre was inserted into the glass vial for 10 minutes to collect headspace volatiles. Following collection, the SPME fibre was removed from the glass vial and capped with a septum (LB-2, Supelco, Bellefonte, PA, USA).

For M2, M3 and M4, the glass vials containing beef cores (M2) or homogenised beef (M3 and M4) were placed in an automated sampler (Gerstel Multi Purpose Sampler Robotic Pro, Linthicum, MD), and transferred by this to the agitator (Gerstel, Linthicum, MD) to equilibrate for 5 mins at 65°C. The speed of agitator was set to 250rpm. The automated sampler was equipped with 75µm CAR/PDMS SPME fibre (Supelco, Bellefonte, PA, USA) for M2 and M3 while 50/30µm divinylbenzene (DVB)/ carboxen CAR/ polydimethylsiloxane (PDMS) SPME fibre (Supelco, Bellefonte, PA, USA) was selected for M4. After 5mins of equilibration, the SPME

fibre was inserted into the headspace of glass vial to collect the volatile compounds for 10mins.

4.2.3.4 Desorption of headspace volatiles

Volatile analysis was performed using gas chromatography-mass spectrometry (Agilent 5977B MSD/7890B GC) for all methods. In M1, cryogenic focusing was carried out by placing the front part of the column into a bed of dry ice for 5 mins. Extracted volatiles were desorbed from manual CAR/PDMS SPME fibre by manually inserting the fibre into the GC inlet at 280°C for 5 mins while the column was held in the dry ice. For M2, M3 and M4, the automated sampler desorbed the volatiles by transferring the SPME fibre from the agitator to the GC/MS inlet at 280°C for 5 mins.

Following desorption, the oven temperature program was started for all four methods. For all methods, the GC inlet was operated with splitless mode. The compounds were separated using a HP-5MS column (30m length, 0.25mm diameter, 0.25µm film thickness; Agilent, Santa Clara, US) with helium flow 1ml/min. A solvent delay of 2.5 mins was set for M2, M3 and M4. However, due to loss of early eluted volatiles in M1, the solvent delay was removed for M1. This modification will also be applied to M2 to M4 in future experiments to increase the capture of early eluted compounds. The oven method was programmed from 30°C (2 mins holding time) to 270°C (2 mins holding time) at a rate of 8°C/min; the total run time was 34mins. After each run, the SPME fibre was conditioned by exposing at 280°C for 3 minutes (270°C for M4 as this was the maximum temperature for DVB/CAR/PDMS fibre) at the GC/MS inlet for M1 or SPME condition station (Gerstel, Linthicum, MD) for other methods on the GC/MS instrument.

4.2.3.5 Sample stability

In the previous method developed by AFBI and TTU, samples were analysed immediately after cooking. This procedure was followed for M1 and M2. However, samples for M3 and M4 were kept in a -80°C freezer after cooking and prior to analysis. Samples were taken out from the freezer and placed onto the sample tray of

the GC/MS instrument. To verify the stability of the samples on the sample tray due to the change in temperature (-80°C to room temperature), twelve homogenised samples were taken out from the freezer and put on the GC/MS sample tray at the same time. As the time required for one analysis was approximately 40 minutes, the first sample analysis commenced at 0min, the second sample at 40 minutes, the third sample at 80 minutes and the last sample analysis commenced at 440 minutes (7 hours 20 mins). Seven volatile compounds were selected and the changes in quantities are compared in Table 4.1. The data suggested changes were observed from sample 1 to sample 12 for all seven volatile compounds, probably due to the exposure of the sample to room temperature (Table 4.2). The differences for the peak area responses between 12 samples were all within three standard deviations. There was no trend observed with time. However, the changes within the first six samples were less compared to the last six samples. Therefore, it was decided that only six samples should be taken out from the freezer and transferred to the GC/MS sample tray at one time.

Table 4.2 Effect of the length of time on response peak area for sample exposed under room temperature.

		Peak area						
Sample number	Time (min)	Acetoin	2-Heptanone	Methional	2,5-dimethyl-Pyrazine	Benz-aldehyde	Decanal	Dimethyl disulphide
1	0	875739	279630	26675	361545	569564	21619	2478
2	40	731663	420103	30367	344442	709898	32835	1701
3	80	907944	278061	28579	376475	561961	22052	3179
4	120	789547	310483	31798	441753	620072	24056	3198
5	160	902100	268355	33729	350233	565458	14112	3482
6	200	728093	426502	33228	311483	721334	35128	2971
7	240	791683	349631	45215	519605	688160	22788	7085
8	280	849434	354712	46795	756864	857000	28734	9934
9	320	848402	372345	51611	655714	742833	23997	4209
10	360	1445525	513705	38072	338134	770752	34415	2981
11	400	1068404	365828	44438	526280	749784	25050	4657
12	440	1074958	377794	40419	443880	702838	27563	5538
	Mean	917791	359762	37577	452201	688305	26029	4285
	SD	200106.0	71555.4	8059.9	139225.7	92238.0	6074.3	2293.9

SD: standard deviation. All data shown was within three standard deviations from mean.

4.2.3.6 Identification and quantitation of volatile compounds

The MS detected ions within 30-500 m/z range under the electron impact mode at 70eV with scan mode. A liquid solution containing n-alkanes (20ng/µl, C8-C22; Sigma-Aldrich, Dorset, UK) was analysed each day to allow the determination of the linear retention index (LRI) for eluted compounds of interest with reference to retention time of n-alkanes. The compound of interest was identified using the calculated LRI and ion fragmentation pattern obtained from analysis of the authentic compounds under the same GC-MS conditions (Sigma-Aldrich, Dorset, UK).

One target ion and three qualifying ions were chosen for each compound of interest. Each compound of interest was quantified with target ion by using the calibration equation of the authentic compound.

4.2.4 Statistical analysis

The mean and standard deviation of volatile quantity were calculated. Coefficient of variation (CV) was calculated as a measure of variability between methods (Bueno et al., 2019, Chen et al., 2014, Parsons et al., 2009). Data were analysed using linear mixed model (LMM) methodology with ageing period set as fixed factor using the estimation method of residual maximum likelihood (REML) (Ahrens, 1974, Robinson, 1987). Significant results were determined at $\alpha = 0.05$.

4.2.5 Method selection

General and specific criteria were considered to select the most suitable method. Scores were assigned to each general criterion from a scale of 1 (bad) to 5 (very good), considering the results from both trials. These general criteria included the ease of use, amount of sample required for extraction and method flexibility. For specific criteria, scores were awarded from a scale of 1 (bad) to 5 (very good) for each selected volatile compound category. These specific criteria were the range of volatile compounds identified, quantities of volatile compounds detected and the ability of the method to differentiate samples aged to different ageing periods. In addition, reproducibility was also categorised as a specific criterion and scores were awarded from a scale of 1 (bad)

to 10 (very good). The total scores of all the criteria were calculated and the best method was selected.

4.3 Results and Discussion

Three general criteria and four specific criteria were considered which are presented in this section.

4.3.1 General criteria

4.3.1.1 Comparison of the ease of use for all methods

The easiness of the extraction methods was considered by time consumed and manual labour involved, and the results are presented in Table 4.3. M1 was the most time and labour consuming method compared to other methods as it required manual extraction, desorption and cryogenic focusing. M2 was the easiest method because no extra steps were required for sample preparation. A score of 4 was awarded to M3 and M4 because an extra liquid nitrogen homogenisation step was required for these two methods. The sample can be stored in a -80°C freezer after cooking and preparation.

Table 4.3 Justification for scores for the ease of use.

Method	Score (1= not easy to use to 5 = most easy to use)	Justification of score
M1	1	Labour intensive for absorption and desorption.
M2	5	No extra sample preparation step after cooking.
M3	4	Additional liquid nitrogen homogenisation step required.
M4	4	Additional liquid nitrogen homogenisation step required.

4.3.1.2 Evaluation of amount of beef sample required for analysis

The amount of the sample required was evaluated and the results are showed in Table 4.4. Steak was cut into similar size for all methods, approximately 50mm x 50mm.

However, one steak was sufficient to collect enough cores for only one analysis in M1 and M2. Therefore, if there was any technological issue with the analysis, there would not be any sample remaining to repeat the analysis. In contrast, for M3 and M4, the sample was homogenised after being frozen in liquid nitrogen. Generally, there were more than 2g of sample after the homogenisation. Therefore, one sample was able to be used for multiple analyses in M3 and M4. This allowed repeat analysis if the instrument didn't perform well. This also provides an opportunity to carry out duplicate or triplicate analysis for one sample, although this was not performed in this study.

Although it was possible that the beef cores collected for M1 and M2 might not be representative for the sample as connective tissues and internal fat was carefully avoided, the advantage of homogenised sample was that the samples collected are more representative of the whole steak sample. Homogenisation steps may or may not increase the reproducibility of the samples, and this will be evaluated and discussed in section 4.3.2.3.

After a literature search, it was found that sample preparation steps have been varied between reported studies. For example, Legako et al. (2015) collected three cooked beef cores (1.27-cm diameter, 2.5 cm in length), while Yancey et al. (2006) used 10g of frozen minced cooked sample plus 40ml of distilled water. Watanabe et al. (2015) placed two grams of meat into a 10ml Pyrex test tube and cooked the meat in a 180 °C aluminium block bath for 5 minutes then subsequently cooled in ice while Stetzer et al. (2008) employed the liquid nitrogen homogenisation steps after the steaks were cooked. This has been proposed to be the reason for the variation between studies as cooking method or sample preparation process may have influenced the formation of volatile compounds (Domínguez et al., 2014).

Table 4.4 Justification for scores for amount of sample required.

Method	Score (1= higher amount required to 5 = lower amount required)	Justification of score
M1	3	One steak only capable for one analysis
M2	3	One steak only capable for one analysis
M3	5	Multiple analysis could be done on one steak
M4	5	Multiple analysis could be done on one steak

4.3.1.3 Flexibility of the methods

The flexibility of the methods was considered for the extraction methods. M3 and M4 were more flexible, followed by M2 while M1 was the least flexible method (Table 4.5). These scores were assigned based on the flexibilities of sample preparation steps, volatile extraction step and analysing steps. M1 was the least flexible in all aspects, therefore awarded the lowest score. Higher flexibility in the volatile extraction step was reported for M2, but the samples required immediate analyse. Therefore, M3 and M4 were the most flexible, with benefits of repeated measures due to flexibility in analysis steps. These two methods also provided the opportunity to have repeated measurements on one sample as the sample didn't need to be analysed immediately after cooking.

Table 4.5 Justification for scores for flexibility of the method.

Method	Score (1= higher amount required to 5 = lower amount required)	Justification of score
M1	1	Highly restricted method from sample preparation to extraction method
M2	3	Higher flexibility in volatile extraction phase
M3	5	Higher flexibility in sample preparation and volatile extraction phase
M4	5	Higher flexibility in sample preparation and volatile extraction phase

4.3.2 Specific criteria

4.3.2.1 Identification of a range of volatile compounds

Volatile compounds that are commonly identified in cooked beef samples were selected to evaluate the suitability of the extraction method. The identities of detected compounds were determined by comparison of the linear retention index and mass spectra with those of authentic compounds and published data for the linear retention index and mass spectra. The equation and r-squared value obtained from the calibration curve were used to calculate the quantity of volatile compounds in beef samples. An intensive literature search was conducted to compare the published linear retention index. Table 4.6 and Table 4.7 show the compounds identified in Trial A and Trial B respectively.

Table 4.6 Volatile compounds identified in Trial A.

Compounds	Mean LRI M1	M2	M3	M4	Literature LRI	Authentic compound LRI	Equation	r^2	Quanti-fier ion	ID method
Ketones										
Acetoin	699	707	707	703	711 (Jordán et al., 2002)	<800	y=68395x	0.991	45	LRI+MS
2-Heptanone	ND	889	889	889	895 (Kaseleht et al., 2011)	890	y=13511x	0.992	43	LRI+MS
Butyrolactone	904	912	912	912		910	y=74956x	0.998	42	LRI+MS
2,3-Octanedione	ND	ND	985	985	983 (Beaulieu and Grimm, 2001)	993	y=245907x	0.992	43	LRI+MS
Strecker Aldehydes										
3-Methylbutanal	645	649	649	650	652 (Legako et al., 2015)	<800	y=3900x	0.969	44	LRI+MS
2-Methylbutanal	653	658	658	658	659 (Legako et al., 2015)	<800	y=189885x	0.995	57	LRI+MS
Methional	ND	915	914	914	911 (Legako et al., 2015)	914	y=82370x	0.998	104	LRI+MS
Benzaldehyde	957	961	961	960		965	y=190393x	0.997	106	LRI+MS
Benzeneacetaldehyde	ND	1046	1046	1046	1055 (Cuevas et al., 2016)	1050	y=174198x	0.982	91	LRI+MS
n- Aldehydes										
Pentanal	ND	690	690	689		<800	y=10934 x	0.949	44	LRI+MS
Hexanal	791	794	799	795	801 (Beaulieu and Grimm, 2001)	<800	y=42860x	0.973	56	LRI+MS
Heptanal	889	900	900	900	898 (Legako et al., 2015)	904	y=76840x	0.999	70	LRI+MS
Octanal	999	1003	1004	1003	1003 (Beaulieu and Grimm, 2001)	1009	y=419725x	0.990	84	LRI+MS
Nonanal	1107	1106	1106	1106	1107 (Legako et al., 2015)	1106	y=74560x	0.943	57	LRI+MS
Decanal	ND	ND	1207	1207	1205 (Legako et al., 2015)	1204	y=59047x	0.995	57	LRI+MS
Alkenals										
(E)-2-Octenal	ND	ND	1060	1059	1057 (Beaulieu and Grimm, 2001)	1061	y=42083x	0.993	70	LRI+MS
(E)-2-Decenal	ND	ND	1264	1264		1261	y=69041x	0.995	70	LRI+MS
(E)-2-Nonenal	ND	ND	1162	1162	1162 (Beaulieu and Grimm, 2001)	1161	y=45960x	0.995	70	LRI+MS
2-Undecenal	ND	ND	ND	1366	1362 (Nóbrega et al., 2007)	1359	y=46751x	0.999	70	LRI+MS

Compounds	Mean LRI M1	M2	M3	M4	Literature LRI	Authentic compound LRI	Equation	r^2	Quanti-fier ion	ID method
Alkadienals										
2,4-E,E-Decadienal	ND	ND	ND	1319	1318 (Beaulieu and Grimm, 2001)	1313	y=251236x	0.987	152	LRI+MS
Furans										
2-Ethylfuran	ND	ND	694	ND	705 (Cuevas et al., 2017)	694	y=38384x	0.996	81	LRI+MS
2-Pentylfuran	ND	ND	992	992	994 (Legako et al., 2015)	997	y=248597x	0.991	81	LRI+MS
2(5H)-Furanone	ND	913	ND	913		913	y=17728x	0.989	55	LRI+MS
Indoles										
Indole	ND	ND	ND	1298		1295	y=621792x	0.961	117	LRI+MS
3-Methylindole	ND	ND	ND	1392		1388	y=499395x	0.835	130	LRI+MS
Pyrazines										
Pyrazine	ND	ND	729	729	732 (Kaseleht et al., 2011)	735	y=24143x	0.986	80	LRI+MS
Methylpyrazine	ND	ND	ND	819	826(Jeleń et al., 2019)	824	y=246055x	0.967	94	LRI+MS
2,5-Dimethyl Pyrazine	ND	ND	915	915	911(Jeleń et al., 2019)	919	y=480817x	0.952	108	LRI+MS
S-Compounds										
Dimethyl disulphide	731	ND	735	ND		740	y=28364s	0.989	94	LRI+MS
Dimethyl sulfone	ND	920	920	921	931 (Mebazaa et al., 2009)	922	y=29630x	0.990	79	LRI+MS
Terpenes										
α-Pinene	ND	ND	933	933		937	y=72925 x	0.915	93	LRI+MS
o-Cymene	ND	ND	ND	1026	1027 (Choi, 2003)	1028	y=452199x	0.956	119	LRI+MS
D-Limonene	ND	ND	ND	1031		1035	y=103822x	0.846	68	LRI+MS
Saturated Alcohols										
1-Pentanol	ND	ND	763	761	761 (Beaulieu and Grimm, 2001)	<800	y=74296x	0.981	55	LRI+MS
1-Hexanol	ND	ND	870	868	863 (Cuevas et al., 2017)	872	y=13412x	0.992	56	LRI+MS
1-Heptanol	ND	ND	ND	972	969(Beaulieu and Grimm, 2001)	972	y=19808x	0.986	70	LRI+MS

Compounds	Mean LRI M1	M2	M3	M4	Literature LRI	Authentic compound LRI	Equation	r^2	Quanti-fier ion	ID method
Unsaturated Alcohols										
1-Penten-3-ol	ND	ND	ND	675	683 (Jordán et al., 2002)	<800	y=178868x	0.996	57	LRI+MS
1-Octen-3-ol	ND	ND	ND	981	978 (Cuevas et al., 2017)	987	y=246435x	0.948	57	LRI+MS
Small or medium chain acids										
Hexanoic acid	ND	987	998	ND		994	y=225210x	0.991	60	LRI+MS
Heptanoic acid	ND	1073	1076	ND		1069	y=13988x	0.881	60	LRI+MS
Octanoic acid	ND	1169	1162	1170		1170	y=173662x	0.994	60	LRI+MS
Nonanoic acid	ND	1266	ND	ND	1275 (Plaza et al., 2015)	1274	y=144425x	0.991	60	LRI+MS
Alkanes/ alkenes										
Nonane	892	ND	ND	898	896(Högnadóttir and Rouseff, 2003)	900	y=74379x	0.992	57	LRI+MS
Decane	997	ND	ND	1000	1000 (Timón et al., 2004)	1000	y=110264x	0.990	57	LRI+MS
Dodecane	ND	ND	1200	1200	1200 (Cuevas et al., 2017)	1200	y=32063x	0.995	57	LRI+MS
1-Octene	ND	ND	ND	784	790 (Timón et al., 2004)	787	y=124665x	0.994	43	LRI+MS
Pyrrole										
2-Acetylpyrrole	ND	1062	1064	1062		1059	y=25307x	0.986	94	LRI+MS
Others										
Methyl butanoate	ND	716	716	716		<800	y=43013x	0.992	74	LRI+MS
Toluene	746	758	758	742		754	y=217693x	0.990	92	LRI+MS
Phenol	ND	987	988	984	980(Jeleń et al., 2019)	987	y=430754x	0.981	94	LRI+MS
Benzonitrile	ND	ND	992	989	997(Timón et al., 2004)	997	y=382651x	0.976	135	LRI+MS
Pyridine	ND	ND	739	ND		740	y=37353x	0.980	79	LRI+MS

Compounds	Mean LRI M1	M2	M3	M4	Literature LRI	Authentic compound LRI	Equation	r^2	Quanti-fier ion	ID method
Internal Standard										
1,2-Dichlorobenzene	1027	1036	1037	1036		1037	y=57725x	0.983	146	LRI+MS

ND: not detected, LRI: linear retention index. Identification method: LRI+MS= linear retention index or mass spectrum compared with authentic compound and literature. The lowest LRI detected in samples was 600 because hexane was detected amongst the meat volatiles while the lowest LRI detected in runs of authentic compounds was 800 due to the presence of solvent.

For the authentic compound equation, y represents the ion area response and x represents the quantity (ng/ headspace of 2g of beef sample) of volatile compound.

Table 4.7 Volatile compounds identified in Trial B.

Compounds	Mean LRI M2	M3	M4	Literature LRI	Authentic compound LRI	Equation	R^2	Quanti-fier ion	ID method
Ketones									
Acetoin	710	710	711	711 (Jordán et al., 2002)	<800	y=68395x	0.991	45	LRI+MS
2-Hexanone	791	791	791	788 (Machiels et al., 2003)	<800	y=61598x	0.980	43	LRI+MS
2-Heptanone	896	892	896	895 (Kaseleht et al., 2011)	890	y=13511x	0.992	43	LRI+MS
2-Octanone	999	991	993	998/988 (Jeleń et al., 2019)	999	y=133727x	0.901	58	LRI+MS
2-Nonanone	1101	1092	1101	1090 (Nóbrega et al., 2007)	1093	y=183298x	0.974	58	LRI+MS
2-Decanone	1203	1192	1202	1193 (Nóbrega et al., 2007)	1190	y=206917x	0.962	58	LRI+MS
Butyrolactone	919	914	919		910	y=74956x	0.998	42	LRI+MS
2(5H)-Furanone	920	915	920		913	y=17728x	0.989	55	
2,3-Octanedione	992	985	992	983(Beaulieu and Grimm, 2001)	993	y=245907x	0.992	43	LRI+MS
Strecker aldehyde									
3-Methylbutanal	658	625	657	652 (Legako et al., 2015)	<800	y=3900x	0.969	44	LRI+MS
2-Methylbutanal	667	640	666	659 (Legako et al., 2015)	<800	y=189885x	0.995	57	LRI+MS

Compounds	Mean LRI M2	M3	M4	Literature LRI	Authentic compound LRI	Equation	R^2	Quanti-fier ion	ID method
Methional	911	906	911	911 (Legako et al., 2015)	914	y=82370x	0.998	104	LRI+MS
Benzaldehyde	968	962	967	976 (Cuevas et al., 2016)	975	y=190393x	0.997	106	LRI+MS
Benzeneacetaldehyde	1054	1045	1053	1055 (Cuevas et al., 2016)	1050	y=174198x	0.982	91	LRI+MS
n-aldehydes									
Pentanal	700	684	698	700 (Cuevas et al., 2017)	<800	y=10934x	0.949	44	LRI+MS
Hexanal	801	802	801	801(Beaulieu and Grimm, 2001)	802	y=42860x	0.973	56	LRI+MS
Heptanal	907	902	907	898 (Legako et al., 2015)	904	y=76840x	0.999	70	LRI+MS
Octanal	1011	1003	1010	1003 (Beaulieu and Grimm, 2001)	1009	y=41972x	0.990	84	LRI+MS
Nonanal	1114	1104	1114	1107 (Legako et al., 2015)	1106	y=74560x	0.943	57	LRI+MS
Decanal	1213	1205	1214	1205 (Legako et al., 2015)	1204	y=59047x	0.995	57	LRI+MS
Alkenals									
(E)-2-Octenal	ND	1059	1067	1057(Beaulieu and Grimm, 2001)	1061	y=42083x	0.993	70	LRI+MS
(E)-2-Nonenal	1171	1160	1170	1162(Beaulieu and Grimm, 2001)	1161	y=45960x	0.995	70	LRI+MS
(E)-2-Decenal	1272	1262	ND		1262	y=69041x	0.995	70	LRI+MS
2-Undecenal	1367	1366	1366	1362 (Nóbrega et al., 2007)	1359	y=46751x	0.999	70	LRI+MS
Furans									
2-Ethylfuran	700	699	700	705 (Cuevas et al., 2017)	698	y=38384x	0.996	81	LRI+MS
2-Pentylfuran	999	992	999	994 (Legako et al., 2015)	997	y=248597x	0.991	81	LRI+MS
Indole									
Indole	1308	1296	ND		1295	y=621791x	0.961	117	LRI+MS
Pyrazines									
Pyrazine	732	ND	732	732 (Kaseleht et al., 2011)	735	y=24143x	0.986	80	LRI+MS
Methylpyrazine	824	823	824	826 (Jeleń et al., 2019)	824	y=246055x	0.967	94	LRI+MS
2,5-Dimethylpyrazine	915	910	915	911 (Jeleń et al., 2019)	919	y=4808177 x	0.952	108	LRI+MS
Trimethylpyrazine	1009	1002	1009	1003 (Jeleń et al., 2019)	1011	y=288253x	0.996	122	LRI+MS

Compounds	Mean LRI M2	M3	M4	Literature LRI	Authentic compound LRI	Equation	R^2	Quanti-fier ion	ID method
2-Ethyl-3,5-dimethylpyrazine	1088	1080	1088	1083(Kaseleht et al., 2011)	1082	y=180620x	0.993	135	LRI+MS
S-compounds									
Dimethyl disulphide	743	732	742	746 (Timón et al., 2004)	740	y=28364x	0.989	94	LRI+MS
Dimethyl sulfone	925	920	925	931 (Mebazaa et al., 2009)	922	y=29630x	0.990	79	LRI+MS
Dimethyl trisulphide	977	971	ND	977 (Machiels et al., 2003)	981	y=212525x	0.994	126	LRI+MS
2-Acetylthiazole	1027	1020	ND	1016 (Nóbrega et al., 2007)	1025	y=88441x	0.998	84	LRI+MS
Benzothiazole	1241	1229	1240	1243 (Machiels et al., 2003)	1231	y=105962x	0.959	135	LRI+MS
2-methylthiophene	771	771	770		769	y=52085x	0.953	97	LRI+MS
Terpenes									
α-Pinene	ND	935	940	1010 (Cheong et al., 2011)	949	y=72925x	0.915	93	LRI+MS
Limonene	ND	1030	1038	1190 (Cheong et al., 2011)	1035	y=103822x	0.846	119	LRI+MS
o-Cymene	ND	1026	1034	1027 (Choi, 2003)	1028	y=452199x	0.956	68	LRI+MS
Saturated Alcohols									
1-Pentanol	770	768	767	761 (Beaulieu and Grimm, 2001)	<800	y=74296x	0.981	55	LRI+MS
1-Heptanol	ND	971	978	969 (Beaulieu and Grimm, 2001)	972	y=19808x	0.986	70	LRI+MS
Unsaturated Alcohols									
1-Penten-3-ol	ND	672	684	683 (Jordán et al., 2002)	<800	y=178868x	0.996	57	LRI+MS
1-Octen-3-ol	ND	981	987	978 (Cuevas et al., 2017)	987	Y=246435x	0.948	57	LRI+MS
Short or medium chain acids									
3-Methylbutanoic acid	838	851	844		841	y=235667x	0.999	60	LRI+MS
Hexanoic acid	989	985	987		994	y=225210x	0.991	60	LRI+MS
Heptanoic acid	1080	1072	1079		1070	y=13988x	0.881	60	LRI+MS
Octanoic acid	1177	1177	1177	1225 (Jeleń et al., 2011)	1172	y=173662x	0.994	60	LRI+MS
Nonanoic acid	1275	1263	1273	1275 (Plaza et al., 2015)	1274	y=144425x	0.991	60	LRI+MS
Decanoic acid	ND	ND	1371	1380 (Mahattanatawee et al., 2005)	1384	y=6920x	0.836	60	LRI+MS

Compounds	Mean LRI M2	M3	M4	Literature LRI	Authentic compound LRI	Equation	R^2	Quanti-fier ion	ID method
Long chain acids									
Hexadecanoic acid	1979	ND	1972	1973 (Lasekan et al., 2013)	1964	y=37254x	0.929	73	LRI+MS
Octadecanoic acid	2176	ND	2162	2172 (Lasekan et al., 2013)	2166	y=16184x	0.852	73	LRI+MS
alkane/alkene									
Nonane	905	900	905	896 (Högnadóttir and Rouseff, 2003)	900	y=74379x	0.992	57	LRI+MS
Decane	1007	1000	1007	1000 (Timón et al., 2004)	1000	y=110264x	0.990	57	LRI+MS
Dodecane	ND	ND	1209	1200 (Cuevas et al., 2017)	1200	y=32063x	0.995	57	LRI+MS
4E-Octene	815	819	815	813 (Timón et al., 2004)	812	y=8849x	0.991	55	LRI+MS
Pyrrole									
Pyrrole	756	756	756		760	y=41113x	0.995	67	LRI+MS
2-Acetylpyrrole	1069	1060	1068		1059	y=25306x	0.986	94	LRI+MS
Other volatiles									
Toluene	765	764	765	770 (Jordán et al., 2002)	<800	y=217693x	0.990	92	LRI+MS
Phenol	990	982	989	980 (Jeleń et al., 2019)	987	y=430754x	0.981	94	LRI+MS
Methyl butanoate	724	724	723		<800	y=43013x	0.992	74	
Pyridine	749	747	751	753 (Pino et al., 2005)	<800	y=37353x	0.980	79	LRI+MS
3-Furaldehyde	835	834	835	835 (Timón et al., 2004)	839	y=144197x	0.997	95	LRI+MS
Internal Standard									
1,2-Dichlorobenzene	1035	1035	1036		1037	y=57725x	0.983	146	LRI+MS

ND: not detected, LRI: linear retention index. Identification method: LRI+MS = linear retention index or mass spectrum compared with authentic compound and literature.

The lowest LRI detected in samples was 600 because hexane was detected amongst the meat volatiles while the lowest LRI detected in runs of authentic compounds was 800 due to the presence of solvent.

For the authentic compound equation, y represents the ion area response and x represents the quantity (ng/ headspace of 2g of beef sample) of volatile compound.

A total of 53 volatile compounds were selected in Trial A while 65 volatile compounds were identified in Trial B (Table 4.8). These compounds are classified into ketones, Strecker aldehydes, n-aldehydes, alkenals, alkadienal, furans, indoles, pyrazines, sulphur containing compounds, terpenes, alcohols, acids, alkanes or alkenes and other volatiles, which were common compounds identified in beef samples as reported several studies (Rivas-Cañedo et al., 2011, Shahidi et al., 1986, Mottram, 1998). For the purpose of evaluating the method, only the compounds that positively matched with the authentic compound's linear retention index and mass spectrum were included. These were similar to that reported in other studies. For example, 69 compounds were reported by Watanabe et al. (2015), 66 compounds were identified by Vasta et al. (2011) but only 26 were detected by Legako et al. (2015) and 27 compounds were identified by Gardner and Legako (2018).

The results for Trial A showed that only 13 volatile compounds were detected by M1 (Table 4.6). Therefore, this method was excluded in Trial B. Methods 2, 3 and 4 successfully picked up the common beef volatile compounds, including ketones, alcohols and aldehydes. M4 detected the most volatile compounds, followed by M3 and M2, and this was consistent in both trials. The results of Trial A agreed with Trial B, in which M4 detected more compounds compared to other methods.

One of the reasons that influence the volatile compounds detection range was the SPME fibre type used by the extraction method. Two common SPME fibres that have been used extensively by other studies were selected, including CAR/PDMS SPME fibre (M1, M2, M3) and DVB/CAR/PDMS SPME fibre (M4) (Machiels and Istasse, 2003, Rivas-Cañedo et al., 2011, Watanabe et al., 2015). A previous study reported that CAR/PDMS was suitable for the analysis for low-molecular mass compounds, range from 30 to 225 (Elmore et al., 2001a) while DVB/CAR/PDMS fibre contained a layer of DVB/PDMS coating over CAR/PDMS coating, which is suitable for molecular mass compounds ranging from 40-275 (Shirey, 2000). Our results broadly agreed with this because M4 detected more later eluting compounds, such as 2-undecanal and 2,4-E,E-decadienal, which were undetected by other methods in Trial A. Shirey (2000) studied six different SPME fibres including CAR/PDMS and DVB/CAR/PDMS SPME fibres to analyse ten low molecular mass compounds and

the result showed that the peak area responses were highest using CAR/PDMS SPME fibre for nine out of ten compounds, for which DVB/CAR/PDMS SPME fibre had the highest response.

The main compound categories that are commonly found in cooked beef were selected to identify the most suitable method for volatile selection. Scores were assigned to each method based on the number of volatile compounds detected in each compound groups. The results are presented in Table 4.9. M3 and M4 scored higher compared to M1 and M2.

Table 4.8 Number of compounds detected in each compound category by method.

Compound Category	**Trial A**				**Trial B**		
	M1	M2	M3	M4	M2	M3	M4
Ketones	2	3	4	4	9	9	9
Strecker Aldehydes	2	5	5	5	5	5	5
n- Aldehydes	4	5	6	6	6	6	6
Alkenals	0	0	3	4	3	4	3
Alkadienal	0	0	0	1	0	0	0
Furans	0	1	2	2	2	2	2
Indoles	0	0	0	2	1	1	0
Pyrazines	0	0	2	3	5	4	5
S-Compounds	1	1	2	1	6	6	4
Terpenes	0	0	1	3	0	3	3
Saturated Alcohols	0	0	2	3	1	2	2
Unsaturated Alcohols	0	0	0	2	0	2	2
Short or medium chain acids	0	4	3	1	5	5	6
Long chain acids	0	0	0	0	2	0	2
Alkanes/ alkenes	2	0	1	4	3	3	4
Pyrrole	0	1	1	1	2	2	2
Others	1	3	5	4	5	5	5
Internal Standard	1	1	1	1	1	1	1
Total	**13**	**24**	**38**	**47**	**56**	**60**	**61**

Table 4.9 Score allocation for the range of volatile compounds detected.

	Score* (1= high number of volatile compounds detected to 5 = low number of volatile compounds detected)			
	M1	M2	M3	M4
Strecker aldehydes	1	5	5	5
Aldehyde	2	4	5	5
2-alkenals	0	4	5	5
Pyrazine compounds	0	4	3	5
Acids	0	5	3	4
Furans	0	3	4	4
Sulphur containing compounds	1	4	5	3
Ketones	1	4	5	5
Total (40-points)	**5**	**32**	**35**	**36**

*Scores are assigned based on the number of compounds detected in each category for Trial A and B, which are recorded in Table 4.8.

4.3.2.2 Quantities of volatile compounds for all methods

The mean, standard deviation and CV of the quantities of volatile compounds in Trial A and Trial B are presented in Table 4.10 and Table 4.11a-c.

Table 4.10 Mean, standard deviation and coefficient of variation of volatile compounds quantities (ng/ headspace of 2g of beef sample) based on four methods in Trial A.

Compounds	**M1**			**M2**			**M3**			**M4**		
	Mean	STD	CV	Mean	STD	CV	Mean	STD	CV	Mean	STD	CV
Ketones												
Acetoin	0.97	0.350	36%	66.8	16.26	24%	54.2	15.37	28%	12.2	2.94	24%
2-Heptanone	ND	ND	ND	1.05	0.237	23%	21.9	17.82	81%	10.7	6.83	64%
Butyrolactone	0.12	0.051	44%	6.3	6.49	104%	4.6	0.75	16%	1.4	0.40	28%
2,3-Octanedione	ND	ND	ND	ND	ND	ND	17.7	11.44	65%	14.0	9.50	68%
Strecker Aldehydes												
3-Methylbutanal	13.3	5.44	41%	244.1	88.02	36%	89.5	39.93	45%	8.7	2.06	24%
2-Methylbutanal	0.26	0.073	28%	6.5	2.81	43%	1.7	0.87	50%	0.18	0.071	40%
Methional	ND	ND	ND	0.55	0.261	47%	0.53	0.227	42%	0.23	0.052	23%
Benzaldehyde	0.13	0.024	19%	1.9	0.43	23%	3.5	0.97	28%	2.4	0.64	26%
Benzeneacetaldehyde	ND	ND	ND	0.24	0.09	37%	1.3	1.41	112%	2.7	2.53	95%
n- Aldehydes												
Pentanal	ND	ND	ND	22.8	11.47	50%	913.9	272.77	30%	80.0	49.47	62%
Hexanal	0.44	0.434	99%	26.3	17.32	66%	857.3	209.15	24%	185.6	84.63	46%
Heptanal	0.09	0.104	120%	1.0	0.39	40%	45.2	23.38	52%	20.2	11.69	58%
Octanal	0.14	0.121	88%	0.68	0.303	45%	14.8	7.42	50%	16.6	11.10	67%
Nonanal	0.19	0.204	108%	0.63	0.381	61%	11.1	5.16	46%	25.5	14.87	58%
Decanal	ND	ND	ND	ND	ND	ND	0.08	0.041	53%	0.72	0.465	64%
Alkenals												
(E)-2-Octenal	ND	ND	ND	ND	ND	ND	1.8	1.17	67%	2.4	1.54	63%
(E)-2-Decenal	ND	ND	ND	ND	ND	ND	0.09	0.075	81%	0.85	0.654	77%
(E)-2-Nonenal	ND	ND	ND	ND	ND	ND	0.56	0.428	77%	2.0	1.42	70%
2-Undecenal	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.56	0.479	85%

Compounds	M1			M2			M3			M4		
	Mean	STD	CV	Mean	STD	CV	Mean	STD	CV	Mean	STD	CV
Alkadienals												
2,4-E,E-Decadienal	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.3	0.17	66%
Furans												
2-Ethylfuran	ND	ND	ND	ND	ND	ND	5.7	2.51	44%	ND	ND	ND
2-Pentylfuran	ND	ND	ND	ND	ND	ND	2.8	1.89	66%	2.7	1.93	73%
2(5H)-Furanone	ND	ND	ND	3.8	4.52	118%	ND	ND	ND	0.92	0.254	28%
Indoles												
Indole	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.28	0.16	59%
3-methylindole	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.17	0.18	103%
Pyrazines												
Pyrazine	ND	ND	ND	ND	ND	ND	3.4	0.32	9%	0.57	0.05	9%
Methylpyrazine	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.07	0.03	40%
2,5-Dimethyl Pyrazine	ND	ND	ND	ND	ND	ND	0.30	0.10	35%	0.14	0.06	45%
S-Compounds												
Dimethyl disulphide	0.30	0.356	117%	ND	ND	ND	1.2	1.39	118%	ND	ND	ND
Dimethyl sulfone	ND	ND	ND	0.68	0.317	47%	0.50	0.186	37%	0.51	0.193	38%
Terpenes												
α-Pinene	ND	ND	ND	ND	ND	ND	5.4	2.81	52%	3.8	2.06	54%
o-Cymene	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.02	0.009	44%
D-Limonene	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.11	0.041	38%
Saturated Alcohols												
1-Pentanol	ND	ND	ND	ND	ND	ND	35.9	29.28	82%	6.2	4.02	65%
1-Hexanol	ND	ND	ND	ND	ND	ND	34.2	64.20	188%	19.5	18.76	96%
1-Heptanol	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.2	6.57	92%
Unsaturated Alcohols												

Compounds	M1			M2			M3			M4		
	Mean	STD	CV	Mean	STD	CV	Mean	STD	CV	Mean	STD	CV
1-Penten-3-ol	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.26	0.142	55%
1-Octen-3-ol	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.6	4.84	74%
Small or medium chain acids												
Hexanoic acid	ND	ND	ND	1.0	0.54	54%	17.4	4.73	27%	ND	ND	ND
Heptanoic acid	ND	ND	ND	1.3	1.35	102%	4.6	2.22	49%	ND	ND	ND
Octanoic acid	ND	ND	ND	0.10	1.000	51%	0.73	0.745	91%	0.13	0.010	27%
Nonanoic acid	ND	ND	ND	0.09	0.075	88%	ND	ND	ND	ND	ND	ND
Alkanes/ alkenes												
Nonane	0.17	0.024	14%	ND	ND	ND	ND	ND	ND	0.40	0.141	35%
Decane	0.45	0.079	17%	ND	ND	ND	ND	ND	ND	0.92	0.173	19%
Dodecane	ND	ND	ND	ND	ND	ND	0.20	0.058	29%	1.0	0.26	27%
1-Octene	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.16	0.029	18%
Pyrrole												
2-Acetylpyrrole	ND	ND	ND	1.2	0.43	36%	1.1	0.65	59%	1.4	0.84	61%
Others												
Methyl butanoate	ND	ND	ND	4.7	0.61	13%	4.2	1.29	31%	0.42	0.134	32%
Toluene	0.08	0.052	66%	1.4	0.35	25%	1.3	0.23	18%	0.19	0.031	17%
Phenol	ND	ND	ND	0.22	0.066	30%	0.30	0.034	11%	0.41	0.163	39%
Benzonitrile	ND	ND	ND	ND	ND	ND	0.18	0.015	8%	0.30	0.040	13%
Pyridine	ND	ND	ND	ND	ND	ND	1.0	0.54	53%	0.38	0.117	31%
Internal Standard												
1,2-Dichlorobenzene	13.5	3.51	26%	94.0	14.06	15%	50.3	12.93	26%	67.8	22.56	33%

Each value represents the replicate analysis of 12 samples.

ND: not detected, SED: standard deviation, CV: coefficient of variation.

Table 4.11a Trial B: Mean, standard deviation and correlation variation of volatile compounds quantities (ng/ headspace of 2g of beef sample) for samples with different ageing period (A) using Method 2.

	A4			**A7**			**A21**			**A49**			**A120**			***P*** (Ageing effect)
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV (%)	Mean	SD	CV	Mean	SD	CV	
Low MW ketone																
Acetoin	126^{b}	39.6	31%	112^{b}	13.4	12%	124^{b}	56.8	46%	88^{ab}	37.2	42%	56^{a}	30.9	55%	**0.019**
2-Hexanone	0.63^{a}	0.389	62%	1.37^{b}	1.082	79%	0.38^{a}	0.153	41%	0.73^{ab}	0.334	46%	0.61^{a}	0.135	22%	**0.045**
2-Heptanone	4.4^{a}	2.98	67%	6.1^{a}	2.31	38%	4.3^{a}	1.58	36%	5.5^{a}	1.41	25%	15.1^{b}	4.18	28%	**<0.001**
2-Octanone	0.13^{a}	0.114	87%	0.18^{a}	0.113	62%	0.12^{a}	0.035	28%	0.13^{a}	0.043	33%	0.32^{b}	0.072	23%	**0.002**
2-Nonanone	0.19^{a}	0.234	126%	0.29^{a}	0.324	113%	0.10^{a}	0.027	27%	0.15^{a}	0.056	38%	0.57^{b}	0.186	33%	**0.003**
2-Decanone	0.11	0.134	119%	0.18	0.210	120%	0.06	0.024	42%	0.06	0.029	47%	0.20	0.070	36%	0.156
Butyrolactone	3.1^{a}	0.85	28%	5.2^{ab}	0.88	17%	5.2^{ab}	0.93	18%	8.6^{c}	4.53	53%	6.0^{bc}	1.11	19%	**0.005**
2(5H)-Furanone	3.6	1.16	32%	7.8	3.93	51%	5.1	2.55	50%	6.8	7.98	117%	5.0	1.66	33%	0.485
2,3-Octanedione	1.75	0.604	34%	1.52	1.480	98%	0.77	0.485	63%	0.98	0.314	32%	0.79	0.212	27%	0.119
Strecker aldehyde																
3-Methylbutanal	129^{a}	77	60%	262^{ab}	152	58%	372^{b}	173	46%	586^{c}	300	51%	594^{c}	45	8%	**<0.001**
2-Methylbutanal	3.8^{a}	2.12	56%	8.6^{ab}	4.70	55%	11.4^{b}	4.72	41%	19.9^{c}	11.11	56%	21.2^{c}	1.93	9%	**<0.001**
Methional	0.37^{a}	0.046	12%	0.52^{ab}	0.123	24%	1.20^{b}	0.490	41%	2.18^{c}	1.123	51%	2.26^{c}	0.486	22%	**<0.001**
Benzaldehyde	3.4^{a}	0.59	17%	3.8^{a}	0.94	25%	3.9^{a}	0.84	21%	5.7^{b}	1.66	29%	6.0^{b}	0.78	13%	**<0.001**
Benzeneacetaldehyde	1.1^{a}	0.12	10%	1.5^{a}	0.39	26%	3.7^{b}	1.59	43%	5.8^{c}	2.31	40%	7.2^{c}	2.43	34%	**<0.001**
n-aldehydes																
Pentanal	61.8	26.44	43%	82.1	72.33	88%	30.2	12.38	41%	40.8	9.90	24%	32.3	5.03	16%	0.080
Hexanal	71.8^{b}	33.05	46%	70.9^{b}	49.67	70%	21.7^{a}	11.53	53%	23.2^{a}	13.38	58%	13.8^{a}	5.75	42%	**0.001**
Heptanal	4.7	1.83	39%	12.2	15.64	128%	2.9	1.31	46%	2.5	1.11	44%	2.3	0.87	39%	0.111
Octanal	3.2	1.39	44%	7.7	8.93	117%	2.3	0.99	44%	2.2	1.72	77%	1.8	0.61	35%	0.118
Nonanal	4.5	2.06	45%	9.3	7.87	85%	3.7	1.16	31%	4.6	4.86	106%	3.1	0.69	23%	0.126

	A4			A7			A21			A49			A120			*P*
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV (%)	Mean	SD	CV	Mean	SD	CV	(Ageing effect)
Decanal	0.26	0.123	48%	0.30	0.088	29%	0.23	0.121	54%	0.17	0.088	51%	0.18	0.046	26%	0.159
Tridecanal	1.02	0.815	80%	0.84	1.294	153%	0.89	1.187	133%	1.00	0.959	96%	0.66	0.203	31%	0.968
2-alkenals																
(E)-2-Nonenal	0.21^{ab}	0.163	77%	0.30^{b}	0.264	88%	0.09^{a}	0.049	53%	0.09^{a}	0.049	56%	0.07^{a}	0.006	9%	**0.039**
(E)-2-Decenal	0.28^{b}	0.196	70%	0.21^{ab}	0.111	53%	0.13^{a}	0.071	55%	0.11^{a}	0.078	69%	0.09^{a}	0.015	17%	**0.037**
2-Undecenal	0.14	0.151	112%	0.10	0.060	63%	0.05	0.040	83%	0.03	0.021	82%	0.01	0.002	17%	0.051
Furans																
2-ethylfuran	0.63	0.244	39%	1.02	1.024	100%	0.33	0.092	28%	0.48	0.318	66%	0.78	0.155	20%	0.170
2-pentylfuran	0.34^{ab}	0.230	68%	0.50^{b}	0.313	63%	0.16^{a}	0.055	35%	0.14^{a}	0.050	36%	0.21^{a}	0.068	33%	**0.009**
Indole																
Indole	0.009	0.0034	37%	0.008	0.0028	34%	0.009	0.0024	28%	0.010	0.0036	36%	0.012	0.0024	20%	0.241
Pyrazines																
Pyrazine	0.59^{a}	0.186	32%	0.86^{ab}	0.288	33%	0.92^{ab}	0.484	53%	1.41^{bc}	0.838	59%	1.73^{c}	0.301	17%	**0.002**
Methylpyrazine	0.76^{a}	0.495	65%	1.46^{a}	0.484	33%	1.60^{ab}	1.112	70%	2.92^{bc}	2.319	79%	3.32^{c}	0.699	21%	**0.007**
2,5-Dimethylpyrazine	1.1^{a}	0.81	71%	2.3^{a}	0.71	32%	2.7^{ab}	1.90	70%	4.9^{b}	3.93	81%	5.1^{b}	1.13	22%	**0.011**
Trimethylpyrazine	1.1^{a}	0.85	78%	2.3^{abc}	0.79	34%	2.3^{ab}	1.40	61%	4.0^{bc}	3.29	80%	4.4^{c}	1.18	27%	**0.017**
2-Ethyl-3,5-dimethylpyrazine	0.55^{a}	0.554	73%	1.00^{a}	0.444	30%	1.33^{a}	1.098	56%	2.67^{b}	3.170	81%	3.15^{b}	1.054	27%	**0.002**
S-compounds																
Dimethyl disulphide	0.63^{a}	0.277	44%	3.79^{ab}	5.019	133%	1.45^{a}	0.932	64%	3.83^{ab}	3.060	80%	7.16^{b}	5.472	76%	**0.039**
Dimethyl sulfone	4.8	0.72	15%	5.9	1.75	30%	4.2	1.22	29%	5.2	1.15	22%	4.9	1.15	24%	0.245
Dimethyl trisulphide	0.02	0.005	31%	0.09	0.127	147%	0.03	0.016	60%	0.07	0.108	153%	0.17	0.190	112%	0.170
2-Acetylthiazole	0.11	0.050	48%	0.12	0.047	38%	0.17	0.054	32%	0.20	0.110	57%	0.16	0.049	30%	0.166
Benzothiazole	0.08^{a}	0.014	18%	0.10^{ab}	0.017	18%	0.11^{bc}	0.028	25%	0.13^{c}	0.024	18%	0.17^{d}	0.011	7%	**<0.001**
2-Methylthiophene	0.19^{a}	0.112	58%	0.31^{ab}	0.122	40%	0.19^{a}	0.081	42%	0.19^{a}	0.104	54%	0.35^{b}	0.134	38%	**0.047**
Saturated Alcohols																

	A4			**A7**			**A21**			**A49**			**A120**			***P***
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV (%)	Mean	SD	CV	Mean	SD	CV	(Ageing effect)
1-Pentanol	4.9	0.73	15%	4.7	0.92	20%	4.6	0.39	8%	5.0	0.96	19%	5.2	0.73	14%	0.708
Small chain and medium acids																
3-Methylbutanoic acid	0.07^{a}	0.038	55%	0.10^{a}	0.062	67%	0.11^{a}	0.069	60%	0.84^{a}	1.631	194%	2.85^{b}	2.128	75%	**0.002**
Hexanoic acid	1.0	0.14	14%	1.2	0.61	50%	1.1	0.22	20%	1.1	0.28	25%	1.4	0.23	16%	0.314
Heptanoic acid	2.2	0.38	17%	3.3	2.75	82%	2.0	0.48	24%	1.8	0.49	27%	2.4	0.65	27%	0.334
Octanoic acid	0.22	0.076	35%	0.25	0.133	54%	0.19	0.046	25%	0.18	0.066	37%	0.24	0.061	25%	0.552
Nonanoic acid	0.38	0.214	56%	0.33	0.217	67%	0.19	0.070	37%	0.28	0.214	77%	0.33	0.189	57%	0.477
Long chain acids																
Hexadecanoic acid	2.43	3.030	125%	0.63	1.139	181%	0.55	0.631	115%	0.86	1.437	166%	0.10	0.103	102%	0.152
Octadecanoic acid	1.59	2.176	137%	0.39	0.740	189%	0.37	0.312	84%	0.58	1.014	176%	0.11	0.046	43%	0.220
Toal alkane/alkene																
Nonane	1.2	0.39	33%	1.1	0.35	32%	1.5	0.24	15%	1.2	0.31	25%	1.4	0.43	32%	0.266
Decane	2.1	0.68	33%	2.1	0.69	33%	2.8	0.45	16%	2.2	0.45	21%	2.5	0.84	34%	0.243
4E-Octene	0.13	0.103	77%	0.27	0.496	186%	1.49	2.303	155%	1.50	2.292	152%	1.81	1.231	68%	0.238
Pyrrole																
Pyrrole	0.045^{a}	0.017	38%	0.068^{ab}	0.017	26%	0.053^{a}	0.017	33%	0.080^{b}	0.034	43%	0.081^{b}	0.017	20%	**0.026**
2-Acetylpyrrole	6.9	1.15	17%	7.8	2.86	37%	8.0	3.55	45%	9.1	6.02	66%	5.8	1.62	28%	0.541
Other volatiles																
Toluene	0.77^{a}	0.20	26%	1.11^{ab}	0.21	19%	1.54^{b}	0.13	9%	2.81^{c}	0.96	34%	4.08^{d}	0.59	14%	**<0.001**
Phenol	0.11	0.03	25%	0.11	0.02	15%	0.12	0.03	25%	0.12	0.03	24%	0.14	0.02	11%	0.172
Methyl butanoate	2.9	1.14	40%	2.9	0.70	24%	3.9	0.97	25%	3.2	0.93	29%	5.3	5.62	105%	0.473
Pyridine	0.82	0.35	43%	1.12	0.39	35%	0.91	0.16	17%	1.36	0.81	60%	0.64	0.58	92%	0.165
3-Furaldehyde	0.07^{a}	0.017	23%	0.13^{ab}	0.030	24%	0.18^{b}	0.061	35%	0.16^{b}	0.044	27%	0.18^{b}	0.077	42%	**0.006**
Internal Standard																

	A4			**A7**			**A21**			**A49**			**A120**			***P***
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV (%)	Mean	SD	CV	Mean	SD	CV	(Ageing effect)
1,2-Dichlorobenzene	79.6	15.89	20%	85.4	27.38	32%	116.0	23.08	20%	92.9	25.49	27%	91.4	27.83	30%	0.133

Each value represents the replicate analysis of 6 samples. a, b, c: Letters in the same row which do not share a common superscript are significantly different. SD: standard deviation, CV: coefficient variation, ***P***: probability.

Table 4.11b Trial B: Mean standard deviation and correlation variation (CV) of volatile compounds (ng/ headspace of 2g of beef sample) for samples with different ageing period (A) using Method 3.

	A4			**A7**			**A21**			**A49**			**A120**			***P*** (Ageing effect)
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
Ketones																
Acetoin	82	28.3	35%	89	30.3	34%	91	42.3	47%	102	38.6	38%	56	38.4	68%	0.267
2-Hexanone	0.92	0.338	37%	0.83	0.232	28%	1.00	0.248	25%	1.20	0.469	39%	1.22	0.199	16%	0.168
2-Heptanone	4.9	1.73	35%	13.6	8.77	65%	8.9	7.62	86%	9.4	10.70	114%	11.5	3.27	28%	0.333
2-Octanone	0.07^{a}	0.019	28%	0.10^{a}	0.050	50%	0.07^{a}	0.034	49%	0.11^{a}	0.080	72%	0.26^{b}	0.156	60%	**0.003**
2-Nonanone	0.06^{a}	0.046	81%	0.06^{a}	0.026	46%	0.05^{a}	0.008	17%	0.07^{a}	0.023	34%	0.52^{b}	0.511	99%	**0.006**
2-Decanone	0.051	0.0613	121%	0.039	0.030	77%	0.028	0.012	42%	0.030	0.011	36%	0.082	0.049	60%	0.126
Butyrolactone	2.2^{a}	1.07	50%	2.0^{a}	0.71	36%	2.7^{ab}	1.48	56%	4.0^{b}	2.05	53%	4.0^{b}	0.40	10%	**0.026**
2(5H)-Furanone	2.2	1.33	61%	1.0	0.49	50%	1.5	1.29	88%	1.3	0.75	57%	2.6	0.76	29%	0.055
2,3-Octanedione	1.9	1.72	90%	6.0	4.66	78%	4.8	6.51	134%	4.4	9.36	212%	1.2	0.81	69%	0.539
Strecker aldehyde																
3-Methylbutanal	136^{a}	120.8	89%	112^{a}	97.7	87%	187^{ab}	122.3	66%	375^{c}	231.6	62%	320^{bc}	80.7	25%	**0.011**
2-Methylbutanal	3.2^{a}	3.15	98%	2.6^{a}	2.62	103%	4.6^{ab}	3.59	78%	10.6^{c}	6.83	64%	9.0^{bc}	2.73	31%	**0.006**
Methional	0.25^{a}	0.137	56%	0.25^{a}	0.197	80%	0.35^{a}	0.218	62%	0.48^{a}	0.354	73%	1.44^{b}	0.682	47%	**<0.001**
Benzaldehyde	2.7^{a}	1.49	55%	2.2^{a}	0.86	39%	2.7^{a}	1.34	50%	2.7^{a}	0.85	31%	6.3^{b}	1.93	31%	**<0.001**
Benzeneacetaldehyde	0.49^{a}	0.306	62%	0.74^{a}	0.843	114%	0.79^{a}	0.567	72%	1.19^{a}	1.199	101%	5.22^{b}	2.104	40%	**<0.001**
n-aldehydes																
Pentanal	80	75.2	94%	224	154.6	69%	156	136.0	87%	116	171.9	148%	64	25.5	40%	0.209
Hexanal	88	57.9	66%	228	146.7	64%	161	106.2	66%	128	203.7	159%	36	12.0	34%	0.118
Heptanal	7.5	4.55	60%	29.1	19.81	68%	16.4	11.61	71%	14.0	21.62	154%	7.2	2.24	31%	0.082
Octanal	4.1	1.34	33%	12.6	8.33	66%	7.1	4.41	62%	7.4	9.31	127%	7.2	3.13	43%	0.229
Nonanal	5.8	1.92	33%	12.4	6.30	51%	7.9	3.64	46%	8.3	7.93	95%	14.8	7.04	48%	0.079
Decanal	0.15^{a}	0.073	49%	0.24^{ab}	0.081	34%	0.16^{a}	0.065	42%	0.18^{a}	0.115	64%	0.30^{b}	0.109	37%	**0.044**

	A4			A7			A21			A49			A120			*P* (Ageing effect)
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
2-alkenals																
(E)-2-Octenal	0.22	0.120	54%	0.81	0.550	68%	0.46	0.377	82%	0.48	0.808	168%	0.08	0.033	40%	0.109
(E)-2-Nonenal	0.10	0.051	54%	0.48	0.339	70%	0.21	0.151	74%	0.26	0.490	191%	0.13	0.038	30%	0.150
(E)-2-Decenal	0.043	0.0094	22%	0.135	0.0920	68%	0.062	0.0296	48%	0.074	0.1080	147%	0.074	0.0331	44%	0.208
2-Undecenal	0.021	0.0083	40%	0.047	0.0315	67%	0.023	0.0110	47%	0.033	0.0355	107%	0.017	0.0227	135%	0.234
Furans																
2-Ethylfuran	1.49	0.975	65%	5.25	3.950	75%	3.06	2.473	81%	2.59	4.252	164%	0.95	0.313	33%	0.118
2-Pentylfuran	0.42	0.198	47%	1.62	1.168	72%	1.05	0.919	88%	0.96	1.671	174%	0.36	0.104	29%	0.212
Indole																
Indole	0.054	0.0679	127%	0.032	0.0367	117%	0.030	0.0216	72%	0.027	0.0193	71%	0.005	0.0010	21%	0.292
Pyrazines																
Methylpyrazine	0.42^{a}	0.248	59%	0.29^{a}	0.126	43%	0.46^{a}	0.356	78%	0.81^{a}	0.692	85%	1.62^{b}	0.812	50%	**0.001**
2,5-Dimethylpyrazine	4.9^{a}	2.72	55%	3.6^{a}	1.59	44%	5.1^{a}	3.29	65%	8.2^{a}	6.54	79%	16.0^{b}	8.61	54%	**0.003**
Trimethylpyrazine	0.67^{a}	0.478	72%	0.42^{a}	0.180	44%	0.64^{a}	0.500	78%	1.13^{a}	1.083	96%	2.19^{b}	1.424	65%	**0.011**
2-Ethyl-3,5-dimethylpyrazine	0.32^{a}	0.330	68%	0.27^{a}	0.237	63%	0.38^{a}	0.354	67%	0.79^{a}	0.856	90%	1.59^{b}	1.211	61%	**0.009**
3,5-Dimethyl-2-isobutylpyrazine	0.010^{a}	0.0097	96%	0.014^{a}	0.0198	143%	0.018^{a}	0.0136	75%	0.065^{a}	0.0605	93%	0.207^{b}	0.1538	74%	**<0.001**
S-compounds																
Dimethyl disulfide	3.33	3.551	107%	0.40	0.163	41%	1.74	1.994	115%	2.03	1.763	87%	1.52	0.590	39%	0.191
Dimethyl trisulfide	0.023	0.0280	121%	0.006	0.0050	80%	0.027	0.0378	140%	0.015	0.0078	53%	0.038	0.0199	53%	0.209
2-Acetylthiazole	0.100	0.0531	53%	0.073	0.0208	29%	0.084	0.0464	55%	0.093	0.0819	89%	0.174	0.0843	48%	0.065
Benzothiazole	0.54	0.710	132%	0.10	0.013	13%	0.11	0.043	41%	0.17	0.127	77%	0.37	0.123	34%	0.114
2-Methylthiophene	0.53^{ab}	0.114	21%	0.34^{a}	0.071	21%	0.51^{ab}	0.101	20%	0.67^{bc}	0.453	68%	0.94^{c}	0.212	23%	**0.003**
Dimethyl sulfone	6.25	2.276	36%	4.26	2.850	67%	3.96	1.277	32%	3.53	1.485	42%	4.65	0.606	13%	0.145
Terpenes																
α-Pinene	6.6	4.57	69%	5.3	2.37	45%	4.2	0.81	19%	4.4	1.76	40%	2.7	0.71	27%	0.122

	A4			A7			A21			A49			A120			*P* (Ageing effect)
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
o-Cymene	0.085	0.0218	26%	0.077	0.0203	26%	0.069	0.0228	33%	0.066	0.0119	18%	0.067	0.0164	24%	0.386
D-Limonene	0.15	0.117	78%	0.15	0.114	77%	0.08	0.062	74%	0.13	0.115	87%	0.04	0.012	31%	0.206
Saturated Alcohols																
1-Pentanol	3.2	2.50	79%	14.1	9.89	70%	7.3	3.83	53%	7.5	12.21	162%	3.1	1.65	53%	0.093
1-Heptanol	0.58	0.439	75%	6.78	5.681	84%	1.82	1.612	89%	2.85	6.008	211%	1.08	0.440	41%	0.062
Unsaturated Alcohols																
1-Penten-3-ol	0.55	0.356	65%	2.21	1.615	73%	1.16	0.648	56%	1.08	1.532	142%	0.46	0.102	22%	0.056
1-Octen-3-ol	0.49	0.441	91%	2.42	1.919	79%	1.36	1.040	76%	1.25	2.466	197%	1.36	0.454	33%	0.309
Small or medium chain acids																
3-Methylbutanoic acid	0.08^{ab}	0.054	64%	0.06^{a}	0.032	58%	0.09^{ab}	0.040	44%	0.15^{b}	0.087	60%	0.15^{b}	0.060	41%	**0.045**
Hexanoic acid	1.8	0.78	43%	3.8	2.26	60%	2.7	1.45	53%	2.2	2.30	105%	1.4	0.21	16%	0.129
Heptanoic acid	2.6	0.84	33%	5.8	3.48	60%	3.5	1.80	51%	3.2	3.22	102%	2.1	0.48	24%	0.084
Octanoic acid	0.12	0.020	16%	0.16	0.062	39%	0.12	0.044	36%	0.12	0.068	56%	0.19	0.046	24%	0.094
Nonanoic acid	4.5	0.02	0%	4.5	0.03	1%	4.5	0.03	1%	4.5	0.05	1%	4.5	0.04	1%	0.207
Total alkane/alkene																
Nonane	1.10^{a}	0.289	26%	1.05^{a}	0.262	25%	1.00^{a}	0.174	17%	1.04^{a}	0.168	16%	1.59^{b}	0.453	28%	**0.009**
Decane	2.6^{a}	1.12	43%	2.0^{a}	0.98	48%	1.9^{a}	0.75	39%	2.41^{a}	0.88	37%	5.2^{b}	1.95	37%	**<0.001**
4E-Octene	0.53	0.437	82%	0.97	1.073	110%	0.86	0.808	94%	1.06	0.632	60%	1.68	1.319	79%	0.305
Pyrrole																
Pyrrole	0.43^{a}	0.112	26%	0.26^{a}	0.035	13%	0.39^{a}	0.143	37%	0.45^{ab}	0.304	67%	0.64^{b}	0.137	21%	**0.013**
2-Acetylpyrrole	3.1	1.19	39%	2	0.66	33%	2.3	1.65	71%	2.2	2.02	92%	4.1	1.89	46%	0.158
Other volatiles																
Toluene	3.2	3.07	96%	1.1	0.53	47%	1.6	0.25	15%	2.3	1.28	57%	2.4	0.69	28%	0.206
Phenol	0.114^{a}	0.0136	12%	0.105^{a}	0.0203	19%	0.100^{a}	0.0162	16%	0.095^{a}	0.0264	28%	0.157^{b}	0.0098	6%	**<0.001**
Methyl butanoate	2.9	2.41	82%	4.0	3.47	86%	4.3	3.12	72%	6.0	6.85	114%	12.3	10.81	88%	0.103

	A4			**A7**			**A21**			**A49**			**A120**			***P*** (Ageing effect)
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
Pyridine	0.53	0.32	61%	0.37	0.20	55%	0.77	0.78	101%	0.43	0.11	25%	0.31	0.12	38%	0.301
3-Furaldehyde	0.13	0.06	44%	0.13	0.08	61%	0.13	0.09	68%	0.18	0.21	118%	0.29	0.13	45%	0.131
Internal Standard																
1,2-Dichlorobenzene	50.5^{bc}	20.54	41%	34.1^{a}	12.33	36%	35.4^{ab}	8.29	23%	37.5^{ab}	8.16	22%	58.7^{c}	10.49	18%	**0.009**

Each value represents the replicate analysis of 6 samples.

a, b, c: Letters in the same row which do not share a common superscript are significantly difference. SD: standard deviation, CV: coefficient variation, ***P***: probability

Table 4.11c Trial B: Mean, standard deviation and coefficient variation (CV) of volatile compounds quantities (ng/ headspace of 2g of beef sample) for samples with different ageing period (A) using Method 4.

	A4			**A7**			**A21**			**A49**			**A120**			***P*** (Ageing effect)
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
Ketones																
Acetoin	43^{a}	22.2	52%	48^{a}	13.2	28%	35^{a}	21.1	60%	70^{b}	10.9	16%	39^{a}	16.3	41%	**0.016**
2-Hexanone	0.65^{a}	0.154	24%	0.70^{a}	0.133	19%	0.67^{a}	0.350	53%	1.28^{b}	0.393	31%	0.84^{a}	0.269	32%	**0.003**
2-Heptanone	10.2	10.07	99%	10.7	7.35	69%	5.4	2.59	48%	4.4	1.23	28%	7.4	1.89	25%	0.262
2-Octanone	15.7^{b}	18.09	116%	15.0^{b}	14.97	100%	5.0^{ab}	5.07	101%	1.2^{a}	1.03	86%	0.3^{a}	0.13	51%	**0.044**
2-Nonanone	0.06^{a}	0.015	23%	0.06^{a}	0.024	42%	0.05^{a}	0.010	20%	0.11^{a}	0.053	49%	0.38^{b}	0.416	110%	**0.025**
2-Decanone	0.043^{a}	0.0134	31%	0.037^{a}	0.0159	43%	0.027^{a}	0.0112	41%	0.046^{a}	0.0256	55%	0.080^{b}	0.0347	44%	**0.005**
Butyrolactone	3.3	3.29	100%	2.1	1.12	54%	2.8	1.22	43%	3.9	0.84	22%	3.5	0.43	12%	0.417
2(5H)-Furanone	3.0	2.59	86%	1.2	0.85	72%	1.9	1.39	74%	2.1	0.59	28%	2.4	0.79	33%	0.285
2,3-Octanedione	8.6	10.04	116%	7.8	7.59	97%	2.6	2.54	98%	1.9	2.88	151%	0.4	0.25	60%	0.079
Strecker aldehydes																
3-Methylbutanal	35^{a}	28.0	81%	24^{a}	14.2	59%	51^{a}	22.1	44%	126^{b}	53.6	42%	136^{b}	22.6	17%	**<0.001**
2-Methylbutanal	0.88^{a}	0.703	80%	0.54^{a}	0.368	68%	1.70^{a}	0.885	52%	4.05^{b}	1.815	45%	4.35^{b}	0.852	20%	**<0.001**
Methional	0.23^{a}	0.174	76%	0.21^{a}	0.064	31%	0.33^{a}	0.114	34%	0.61^{b}	0.123	20%	1.04^{c}	0.349	33%	**<0.001**
Benzaldehyde	2.9	0.87	30%	2.3	0.71	31%	2.5	0.85	34%	3.3	1.02	31%	3.2	1.15	36%	0.230
Benzeneacetaldehyde	1.22^{a}	1.020	84%	0.84^{a}	0.444	53%	1.45^{a}	0.719	50%	3.03^{b}	0.738	24%	4.36^{c}	1.111	26%	**<0.001**
n-aldehydes																
Pentanal	81^{bc}	81.8	101%	97^{c}	73.6	76%	44^{abc}	37.3	85%	19^{ab}	9.3	50%	17^{a}	7.7	46%	**0.044**
Hexanal	119^{b}	101.7	86%	146^{b}	91.6	63%	67^{ab}	67.3	100%	23^{a}	21.2	91%	9^{a}	3.7	40%	**0.008**
Heptanal	23.9^{b}	27.34	115%	26.3^{b}	19.03	72%	9.2^{ab}	8.02	87%	4.3^{a}	1.82	42%	4.2^{a}	1.56	37%	**0.041**
Octanal	16.8^{bc}	15.47	92%	19.9^{c}	11.71	59%	8.5^{ab}	5.54	65%	5.8^{a}	2.42	42%	7.2^{ab}	3.39	47%	**0.048**
Nonanal	23	17.4	75%	26	12.0	46%	13	6.5	52%	10	5.7	57%	15	7.8	52%	0.072

	A4			A7			A21			A49			A120			*P* (Ageing effect)
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
Decanal	0.80^{bc}	0.490	62%	0.87^{c}	0.343	40%	0.47^{ab}	0.098	21%	0.49^{ab}	0.301	62%	0.40^{a}	0.113	28%	**0.043**
2-alkenals																
(E)-2-Octenal	1.31^{b}	1.467	112%	1.28^{b}	1.103	86%	0.51^{ab}	0.414	81%	0.15^{a}	0.118	77%	0.11^{a}	0.044	39%	**0.042**
(E)-2-Nonenal	1.50	1.997	133%	1.30	1.248	96%	0.37	0.386	106%	0.13	0.072	57%	0.12	0.007	6%	0.082
2-Undecenal	0.26	0.343	130%	0.24	0.212	88%	0.07	0.073	103%	0.03	0.021	79%	0.03	0.007	25%	0.072
Furans																
2-Ethylfuran	1.873^{b}	2.0423	109%	1.986^{b}	1.4429	73%	0.808^{ab}	0.8005	99%	0.305^{a}	0.1990	65%	0.302^{a}	0.0871	29%	**0.040**
2-Pentylfuran	1.630^{b}	1.7336	106%	1.972^{b}	1.6018	81%	0.732^{ab}	0.7036	96%	0.312^{a}	0.1835	59%	0.288^{a}	0.0882	31%	**0.040**
Pyrazines																
Pyrazine	0.21^{ab}	0.074	36%	0.19^{a}	0.051	28%	0.25^{abc}	0.113	44%	0.40^{c}	0.216	53%	0.36^{bc}	0.133	38%	**0.030**
Methylpyrazine	0.29^{ab}	0.183	63%	0.16^{a}	0.070	44%	0.38^{abc}	0.231	61%	0.59^{bc}	0.438	74%	0.62^{c}	0.193	31%	**0.018**
2,5-Dimethylpyrazine	0.64^{ab}	0.481	75%	0.32^{a}	0.145	46%	0.72^{ab}	0.430	60%	1.20^{b}	0.888	74%	1.16^{b}	0.404	35%	**0.039**
Trimethylpyrazine	0.82	0.672	81%	0.38	0.210	56%	0.84	0.552	66%	1.43	1.199	84%	1.30	0.605	47%	0.121
3,5-Dimethyl-2-isobutylpyrazine	0.48^{ab}	0.653	87%	0.25^{a}	0.158	43%	0.52^{ab}	0.520	61%	1.02^{b}	1.043	69%	0.95^{b}	0.547	41%	**0.036**
S-compounds																
Dimethyl disulfide	0.42^{ab}	0.431	103%	0.12^{a}	0.115	96%	0.25^{a}	0.213	84%	0.48^{ab}	0.540	111%	0.76^{b}	0.162	22%	**0.033**
Benzothiazole	1.25	1.720	138%	0.19	0.061	33%	0.22	0.051	24%	0.40	0.211	52%	0.43	0.110	26%	0.149
2-Methylthiophene	0.42	0.200	47%	0.38	0.074	19%	0.45	0.153	34%	0.47	0.173	37%	0.55	0.142	26%	0.459
Dimethyl sulfone	4.8	2.24	47%	4.9	2.36	48%	4.8	0.96	20%	4.7	2.33	50%	6.1	1.87	31%	0.717
Terpenes																
α-Pinene	4.4^{a}	0.75	17%	4.0^{a}	1.50	38%	3.7^{a}	1.05	28%	4.5^{a}	0.90	20%	6.9^{b}	1.84	27%	**0.002**
Limonene	0.13	0.020	16%	0.12	0.036	31%	0.12	0.013	11%	0.12	0.025	21%	0.10	0.045	44%	0.682
o-Cymene	0.037	0.0072	19%	0.029	0.0091	31%	0.028	0.0074	26%	0.033	0.0071	21%	0.043	0.0138	32%	0.063
Saturated Alcohols																

	A4			A7			A21			A49			A120			*P* (Ageing effect)
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
1-Pentanol	5.9^{b}	6.14	103%	5.7^{b}	4.27	75%	2.1^{ab}	1.50	72%	1.2^{a}	0.29	24%	0.6^{a}	0.38	63%	**0.027**
1-Heptanol	6.66	9.618	144%	4.75	5.206	110%	0.75	1.109	148%	0.11	0.044	39%	0.96	0.521	54%	0.115
Unsaturated Alcohols																
1-Penten-3-ol	0.66	0.752	115%	0.68	0.525	77%	0.37	0.329	90%	0.12	0.076	64%	0.20	0.088	44%	0.115
1-Octen-3-ol	4.50	5.908	131%	4.28	4.422	103%	0.49	0.523	107%	0.17	0.193	113%	1.26	0.385	31%	0.078
Small and medium chain acids																
3-Methylbutanoic acid	0.034^{a}	0.0139	41%	0.028^{a}	0.0158	57%	0.033^{a}	0.0188	56%	0.044^{a}	0.0105	24%	0.127^{b}	0.0612	48%	**<0.001**
Hexanoic acid	1.19	1.031	87%	0.84	0.301	36%	0.76	0.314	42%	0.73	0.218	30%	1.46	0.515	35%	0.138
Heptanoic acid	1.8^{ab}	1.22	68%	1.3^{a}	0.38	29%	1.2^{a}	0.40	34%	1.1^{a}	0.30	27%	2.6^{b}	1.03	40%	**0.015**
Octanoic acid	0.15^{a}	0.047	32%	0.11^{a}	0.023	21%	0.13^{a}	0.088	66%	0.15^{a}	0.052	34%	0.29^{b}	0.121	41%	**0.002**
Nonanoic acid	0.12^{b}	0.069	55%	0.10^{ab}	0.026	27%	0.07^{a}	0.015	23%	0.07^{a}	0.014	21%	0.15^{b}	0.069	46%	**0.015**
Decanoic acid	0.54	0.125	23%	0.46	0.176	38%	0.47	0.133	28%	0.53	0.178	33%	0.66	0.075	11%	0.159
Long chain acids																
Hexadecanoic acid	0.13	0.092	71%	0.12	0.102	84%	0.12	0.056	46%	0.13	0.054	41%	0.15	0.048	32%	0.963
Octadecanoic acid	0.52	0.203	39%	0.41	0.030	7%	0.64	0.412	64%	0.43	0.101	24%	1.48	2.022	136%	0.258
Total alkane/alkene																
Nonane	1.4	0.42	31%	1.2	0.40	33%	1.3	0.50	37%	1.6	0.22	14%	1.7	0.52	31%	0.336
Decane	8	4.3	54%	6	2.4	38%	8	7.0	83%	11	5.8	54%	5	1.8	33%	0.385
Dodecane	2.1	1.15	56%	2.3	1.24	54%	2.4	1.06	44%	3.6	1.57	43%	2.0	0.75	38%	0.143
4E-Octene	0.16^{a}	0.088	56%	0.37^{a}	0.163	44%	0.40^{a}	0.285	72%	1.6^{b}	1.449	90%	1.5^{b}	0.834	55%	**0.004**
Pyrrole																
Pyrrole	0.27	0.139	51%	0.23	0.029	13%	0.22	0.083	38%	0.31	0.119	38%	0.26	0.042	16%	0.393
2-Acetylpyrrole	4.38^{c}	1.141	26%	2.22^{a}	0.804	36%	3.20^{ab}	1.253	39%	3.26^{abc}	0.697	21%	3.99^{bc}	0.941	24%	**0.010**
Other volatiles																

	A4			**A7**			**A21**			**A49**			**A120**			***P*** (Ageing effect)
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
Toluene	2.6	1.92	73%	1.1	0.49	44%	1.5	0.30	20%	2.3	0.56	24%	1.6	0.36	23%	0.057
Phenol	0.16	0.034	21%	0.15	0.033	22%	0.16	0.039	24%	0.15	0.028	19%	0.12	0.015	12%	0.228
Methyl butanoate	1.0	0.77	76%	1.0	0.51	49%	1.9	1.24	67%	2.5	1.53	60%	2.4	2.17	92%	0.201
Pyridine	0.33	0.136	42%	0.28	0.065	23%	0.75	0.746	100%	0.34	0.144	42%	0.35	0.077	22%	0.163
3-Furaldehyde	0.09	0.041	44%	0.06	0.012	21%	0.09	0.040	44%	0.10	0.017	17%	0.10	0.019	19%	0.103
Internal Standard																
1,2-DDichlorobenzene	52.3	13.11	25%	44.1	12.23	28%	47.9	14.46	30%	58.3	23.56	40%	62.8	15.78	25%	0.300

Each value represents the replicate analysis of 6 samples.

a, b, c: Letters in the same row which do not share a common superscript are significantly difference. SD: standard deviation, CV: coefficient variation, ***P***: probability.

The total quantity of each compound category was calculated and is presented in Table 4.12. Strecker aldehyde was detected in highest quantity in M2. Aldehydes, acids and furans were detected in highest quantity by M3. For M4, 2-alkenals were detected in the highest quantity compared to other extraction methods.

Table 4.12 Total quantities (ng/ headspace of 2g of beef sample) of volatile compounds in each compound category.

	Trial 1				Trial 2*		
	M1	M2	M3	M4	M2	M3	M4
Strecker aldehydes	14	253	97	14	411	238	82
Aldehyde	0.8	51	1842	329	104	289	167
2-Alkenals	0	0	2.5	5.8	0.4	0.8	1.5
Pyrazine compounds	0	0	3.7	0.8	11.8	10.2	3.5
Acids	0	2.5	22.1	0.2	6.3	10.6	4.3
Furans	0	3.8	8.5	3.6	0.9	3.5	2.0
Sulphur containing compounds	0.3	0.7	1.7	0.5	9.0	7.3	6.4
Ketones	1.1	74	98	38	122	103	73

*Total quantity of each compound category was calculated using the average quantity of volatile compounds across five ageing periods.

Another factor that can influence the detection quantity was the form of the sample. As discussed above, although liquid-nitrogen homogenised sample possessed extra advantages such as extra flexibility in conducting analysis (samples can be stored in a -80°C freezer prior to analysis), it was also important that the liquid-nitrogen homogenised step did not disrupt the quantity of volatile compounds. This was proven as the quantity of total volatile compound in M2 and M3 was similar in Trial B (Table 4.12). Higher differences in Trial A compared to Trial B were due to a smaller number of volatile compounds detected in M2 compared to M3. Detection range and quantity can also be influenced by absorption time, desorption time, amount of sample used and temperature of absorption. However, all these factors were kept constant for both trials.

Table 4.13 Score allocation for the quantity of volatile compounds detected.

	Score* (1= higher quantity of volatile compounds detected to 5 = lower quantity of volatile compounds detected)			
	M1	M2	M3	M4
Strecker aldehydes	1	5	2	1
Aldehyde	0	1	5	2
2-Alkenals	0	1	2	5
Pyrazine compounds	0	3	4	2
Acids	0	2	4	1
Furans	0	3	5	3
Sulphur containing compounds	1	4	4	3
Ketones	0	5	4	3
Total (40-points)	2	19	26	17

*Scores are assigned based on the quantity of volatile compounds detected in Trial A and B, which was recorded in Table 4.12.

4.3.2.3 Reproducibility of the methods

Coefficients of variation (CV) were calculated for each method in Trial A and Trial B and recorded in Table 4.10 and Table 4.11. The CV were categorised into high, medium high, medium low and low reproducibility for CV less than 35%, between 35% to 50%, between 50% to 85% and over 85%, respectively (Table 4.14). Due to the low number of compounds detected by M1, it was difficult to analyse the reproducibility of this method. The results in Table 4.14 show that the reproducibility of M2 and M4 were better compared to M3. More compounds were grouped into medium low and low reproducibility categories in Trial B, probably due to the lower number of samples (n=6) analysed per treatment group compared to Trial A. Scores were awarded to each extraction method on the basis of the number of volatile compounds in the reproducibility category and the results are recorded in Table 4.15.

Approximately half of the volatile compounds had CVs lower than 50% (Table 4.14). These CVs were improved compared to the previous study on pork (Elmore et al., 2001a), which in all cases had variability of over 50% for DVB/CAR/PDMS SPME fibre and over 67% for CAR/PDMS fibre. Mallia et al. (2005) reported the CVs for SPME method ranged from 2.9% to 10.7% while the purge and trap extraction method (dynamic headspace extraction) showed higher CV ranging from 8.2% to 26.9% for cheese sample; lower CVs were observed compared to our study as the cheese sample was more homogeneous and only 10 volatile compounds were considered for the CVs.

Correction to internal standard, 1,2-dichlorobenzene, did not improve the CV (data not shown). This was probably because the variation of the response of the internal standard was found to be significantly different between samples groups in M3 for Trial B. It was interesting to note that M3 had lower reproducibility compared to M2, and the only difference between these two methods was the liquid nitrogen homogenisation steps. It was thought that the homogenisation step would reduce the variation and increase the reproducibility because it will increase the representativeness of the sample. However, the overall variation between samples did not decrease. The causes were unclear and a future experiment is recommended to explore the effect of liquid-nitrogen freezing and homogenisation step separately to explain this finding. The reproducibility of M3 and M4 can also be improved by repeated measurements for every steak sample and this was impossible for M2 due to the limitation on the amount of sample required. On the other hand, the reproducibility of M4 was just slightly lower than M2, by using DVB/CAR/PDMS SPME fibre. It might be of interest to investigate the reproducibility of this fibre with cooked beef without liquid-nitrogen homogenisation.

Table 4.14 Number of compounds in each reproducibility classes in Trial A and Trial B.

		Trial A			Trial B*			
Reproducibility	CV	M1	M2	M3	M4	M2	M3	M4
High	<35%	5	7	13	16	16	9	14
Medium high	35%-50%	3	8	8	9	17	8	17
Medium low	50%-85%	1	4	14	18	19	36	26
Low	≥85%	5	5	3	5	6	8	5

*Mean CVs of 5 ageing periods were used in Trial B.
Refers to Table 4.11a-c for volatile compounds individual CV.

Table 4.15 Scores allocation for method reproducibility.

Method	**Score*** (1= lower reproducibility to 10 = higher reproducibility)
M1	3
M2	8
M3	5
M4	8

*Scores are assigned based on the number of compounds in each reproducibility class in Trial A and B, which are recorded in Table14.

4.3.2.4 Volatiles from cooked beef subjected to extended ageing periods using different extraction methods

Previous studies showed that ageing of beef increased flavour characteristics and intensity of aftertaste (Gorraiz et al., 2002), and improved overall flavour (Jeremiah et al., 1991). These effects were also extended to beef volatile compounds, which have been well documented (Gorraiz et al., 2002, Resconi et al., 2018, Stetzer et al., 2008, Watanabe et al., 2015). Longer ageing periods will lead to a higher degree of proteolysis which increases the concentration of amino acid beef flavour precursors (Koutsidis et al., 2008). It was not the primary aim for this trial to investigate the effect of extended ageing period. Rather the trial aimed to identify which method was best to demonstrate the effect of extended ageing period on volatile compounds in beef from one animal using M2, M3 and M4 only. Nevertheless, the results showed that extended ageing had significant effects on 28, 20 and 31 volatile compounds using M2, M3 and M4, respectively. These compounds included Strecker aldehydes, aldehydes, alkenals, pyrazines and acids.

4.3.2.4.1 Strecker aldehydes

Strecker aldehydes selected were 3-methylbutanal, 2-methylbutanal, methional, benzaldehyde and benzeneacetylaldehyde (Table 4.7). These compounds were formed through Strecker degradation of amino acids (Kosowska et al., 2017). Benzeneacetylaldehyde is derived from Strecker degradation of phenylalanine while benzaldehyde is a degradation product of tyrosine (Huang and Ho, 2012). Strecker aldehydes play important roles in beef flavour or aroma. For example, 2-methylbutanal has been reported to contribute to fruity, floral or sweet aroma while 3-

methylbutanal is related to caramel, malty, fatty or green aroma (Burdock, 2010, MacLeod and Ames, 1986).

Strecker aldehydes generally increased with extended ageing (Table 4.11a-c, Figure 4.2). 3-Methylbutanal and 2-methylbutanal increased with extended ageing, and the effects were significant for all methods (Table 4.11a-c, Figure 4.2). In agreement with the results from this study, Ma et al. (2012) observed a significant increase in 3-methylbutanal from beef aged for 1 day to 21 days, although the sample was treated with kiwifruit crude juice, papain, bromelain and fungal protease. Benzaldehyde increased gradually with ageing in M2 and M3 but no significant change was observed in M4 (Figure 4.2). The quantity of benzeneacetylaldehyde increased gradually with ageing, and the effect was consistent for all methods. Watanabe et al. (2015) reported a gradual increase in benzeneacetylaldehyde for beef aged from day 2 to day 30, but no change was observed for benzaldehyde.

All of the five compounds in Figure 4.2 were significantly affected using M2 at significance level of $\boldsymbol{P}<0.001$; the same trend was observed for M4 except no significant changes were observed in benzaldehyde while the compounds were significantly affected using M3 at $\boldsymbol{P}<0.001$ or $\boldsymbol{P}<0.01$ significance levels. Thus, M2 differentiated the beef samples aged to different periods the best.

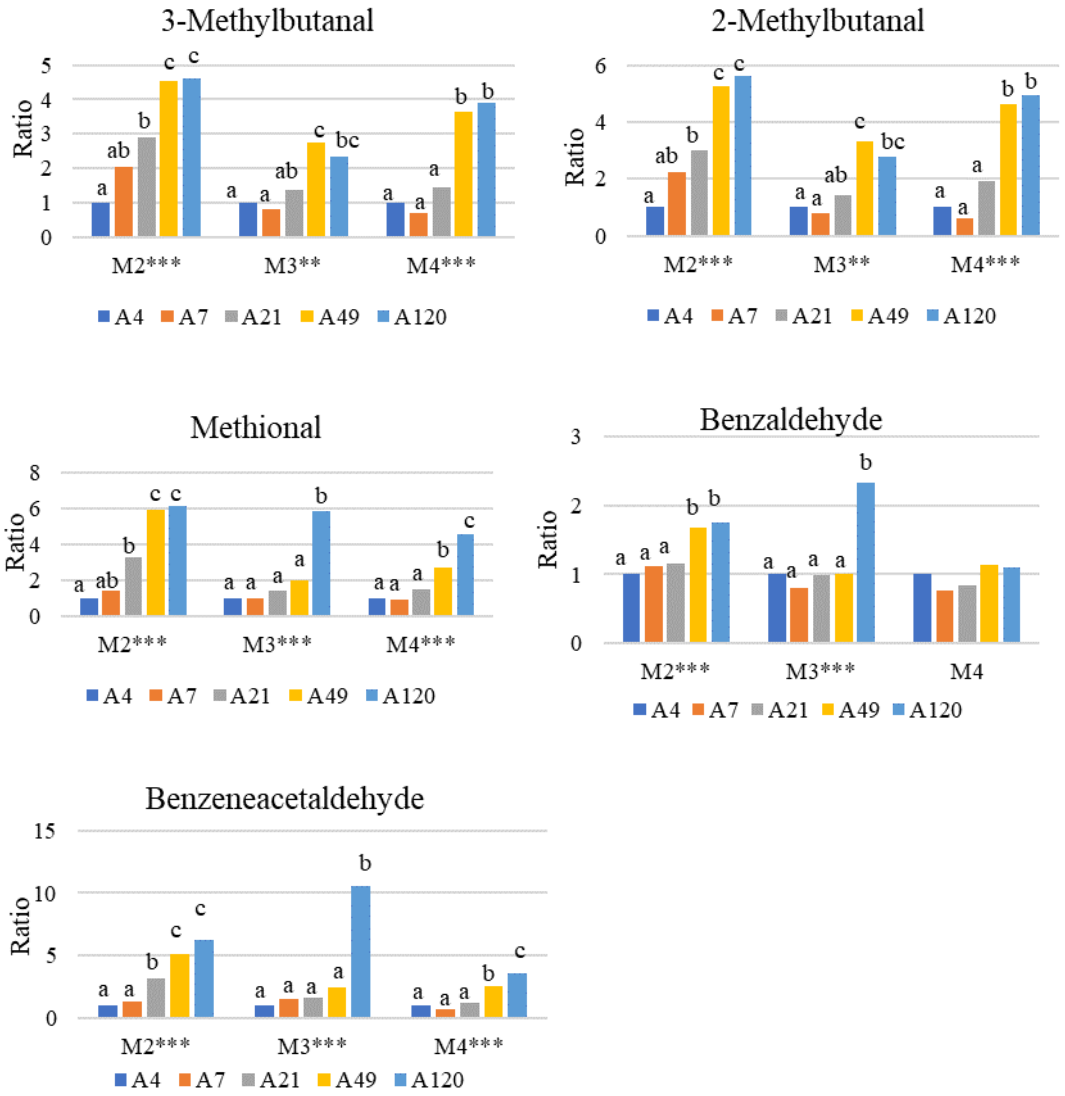


Figure 4.2 Changes in the quantities of Strecker aldehydes in relation to the quantity of volatile compound from cooked beef sample aged for 4 days.
Significance levels: ****P*<0.01 and *****P*<0.001. Different superscripts on the graph represent significant differences.

4.3.2.4.2 Aldehydes

Aldehydes, including pentanal, hexanal, heptanal, octanal and decanal have been reported in cooked beef (Machiels et al., 2004) which are produced through lipid oxidation and thermal oxidation (Domínguez et al., 2014). Several significant differences were observed for aldehydes for beef subjected to extended ageing periods. Low molecular weight n-aldehydes such as pentanal (***P***<0.05), hexanal (***P***<0.01) and heptanal (***P***<0.05), decreased with extended ageing period, although the differences

were not always significant (Table 4.11a-c, Figure 4.3). This result concurred with the study conducted by Ba et al. (2014), where the concentration of nonanal and octanal reduced significantly from 7 days to 28 days aged beef. In lamb samples, significant reduction in n-aldehydes, such as pentanal, hexanal, octanal, nonanal and decanal, were also reported by Rivas-Cañedo et al. (2013) after 6-days of post-mortem storage. Different trends were observed by Watanabe et al. (2015), where heptanal increased significantly ($\boldsymbol{P}<0.05$) from day 2 to day 30. Another study showed minimal change from 0 to 2 weeks and substantial change for beef aged from 2 to 4 weeks (Coppock and MacLeod, 1977). It has also been reported that low molecular weight aldehydes occurred after 21 days ageing; this is because lipid oxidation would be relatively low during the first 3 weeks of vacuum packed ageing (Ma et al., 2012). High molecular weight aldehydes such as octanal decreased significantly ($\boldsymbol{P}<0.05$) from day 7 to day 120, although it was only significant in M4 (Figure 4.3).

The results in Figure 4.3 shows that M4 was the best method to differentiate the quantities of all the n-aldehydes for beef aged for different periods. Although the trend was similar in M2 and M3, these two methods only significantly differentiated one n-aldehyde.

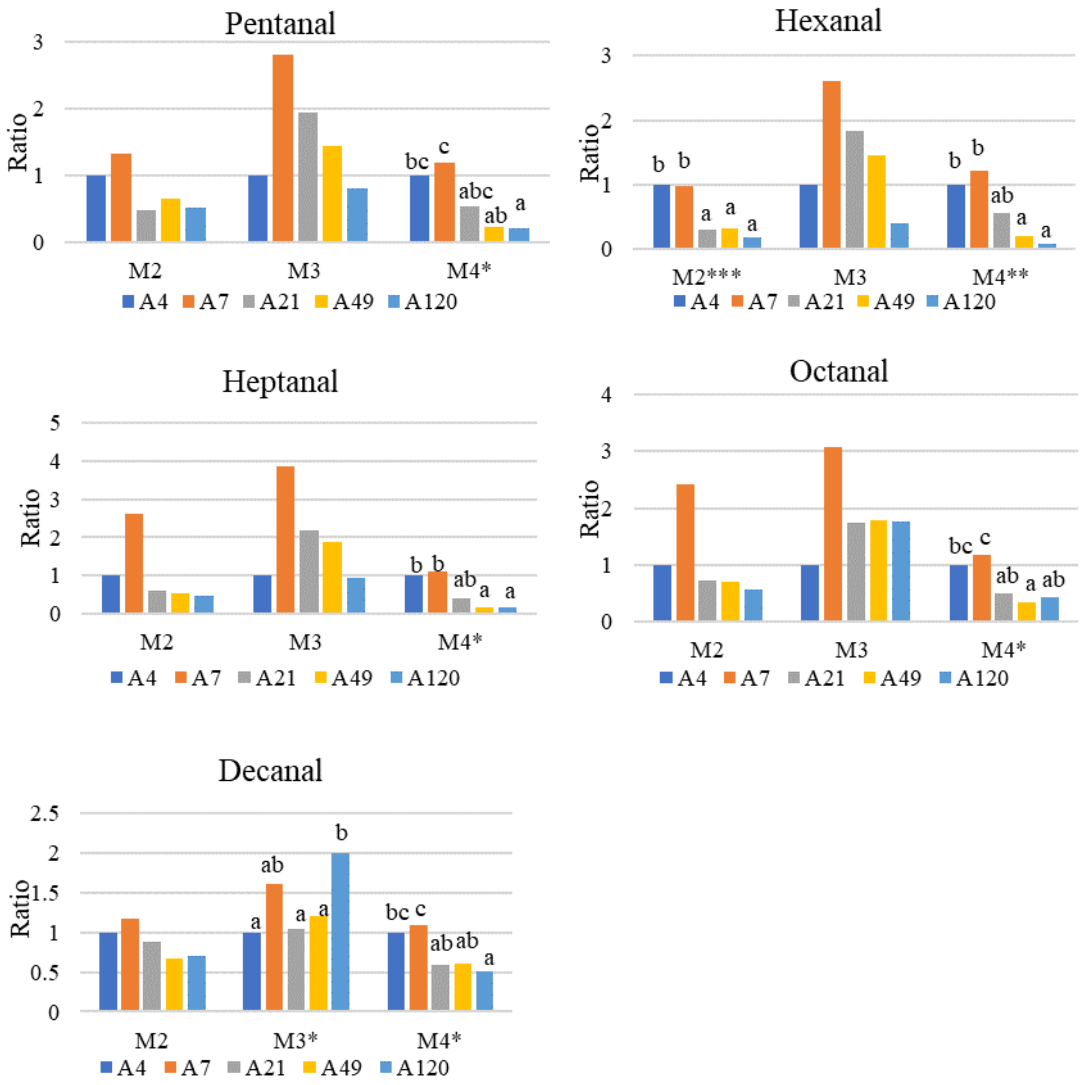


Figure 4.3 Changes in the quantities of n-aldehydes in relation to the quantity of volatile compound from cooked beef sample aged for 4 days.
Significance levels: *P<0.05, **P<0.01 and ***P<0.001. Different superscripts on the graph represent significant differences.

4.3.2.4.3 2-Alkenals

Several 2-alkenals were quantified in beef samples, including (E)-2-octenal, (E)-2-nonenal and (E)-2-decenal. However, (E)-2-nonenal and (E)-2-decenal were undetected by M2 and M4, respectively. This might be caused by the low concentration of these volatile compounds in the beef sample, which was 0.001ppm to 0.011ppm for (E)-2-octenal, 0.001ppm to 0.015ppm for (E)-2-nonenal and 0.0000094ppm to 0.0028ppm for (E)-2-decenal. The detection limit of (E)-2-octenal,

(E)-2-nonenal and (E)-2-decenal were previously reported to be 0.003ppm, 0.00008ppm and 0.004ppm, respectively (Kerth and Miller, 2015).

There were trends showing that 2-alkenals reduced gradually with extended ageing after 7 days, with some effects more significant than the others (Figure 4.4). M2 was the best method to differentiate the samples although it did not detect (E)-2-octenal. M4 significantly differentiated the quantity of (E)-2-nonenal (Table 4.11a-c, Figure 4.4).

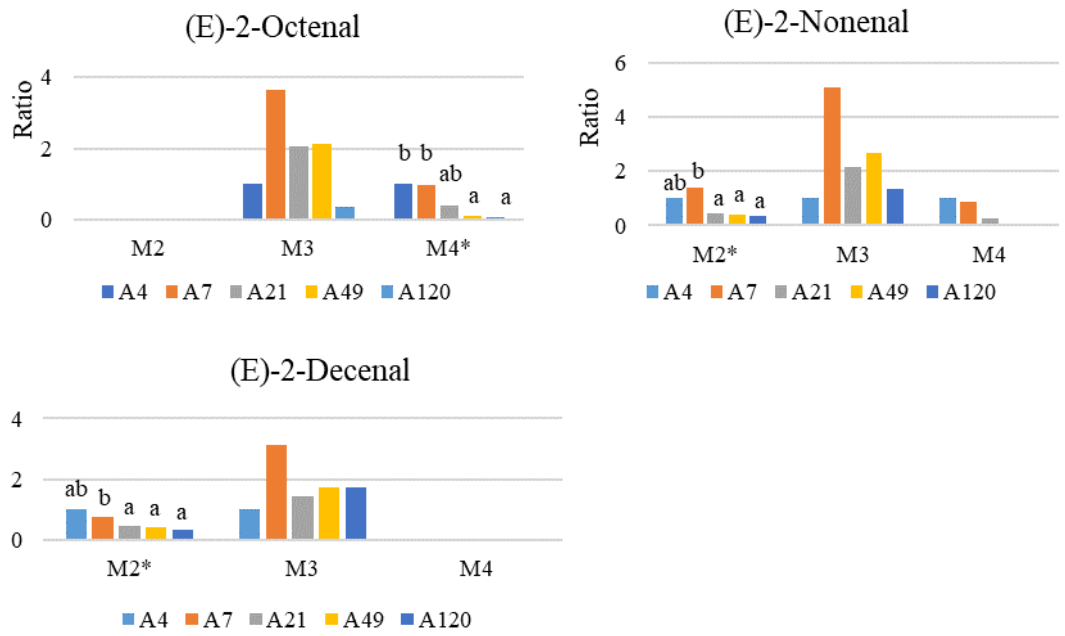


Figure 4.4 Changes in the quantities of alkenals in relation to the quantity of volatile compound from cooked beef sample aged for 4 days.
Significance levels: *P<0.05, **P<0.01 and ***P<0.001. Different superscripts on the graph represent significant differences.

4.3.2.4.4 Pyrazines

Numerous pyrazines were identified in the beef samples and most of these compounds were significantly affected by ageing (Figure 4.5). Ageing significantly (P<0.05) increased the concentration of pyrazine compounds (Table 4.11a-c, Figure 4.5). This agreed with the study conducted by Watanabe et al. (2015). The compound, 2-ethyl-3,5-dimethyl pyrazine was detected by M4, which was previously described as one of the most important nitrogenous compounds for roast beef aroma (Specht and Baltes, 1994). Notably, amino acids increased with ageing (Mullen et al., 2000) and sugar

(Koutsidis et al., 2008) also increased with storage, which probably explains the increased of pyrazine compounds.

M2 was the best extraction method to differentiate all pyrazine compounds in Figure 4.5. M3 successfully differentiated all the pyrazine compounds detected, except the method did not pick up pyrazine and this method only detected differences between sample aged for 120 days compared to other samples. M4 also differentiated all pyrazine compounds, except for trimethylpyrazine.

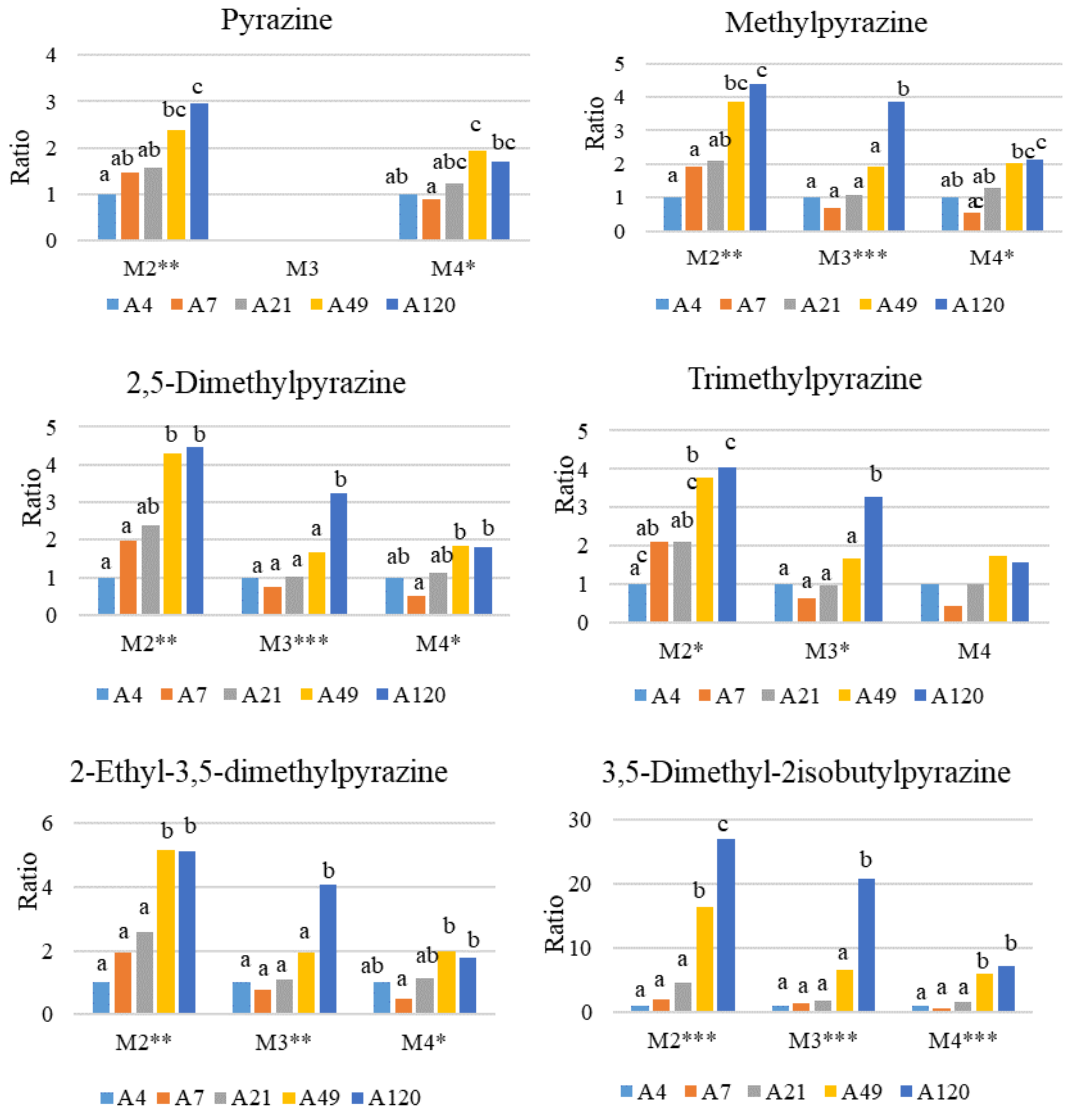


Figure 4.5 Changes in the quantities of pyrazine compounds in relation to the quantity of volatile compound from cooked beef sample aged for 4 days. Significance levels: **P*<0.05, ***P*<0.01 and ****P*<0.001. Different superscripts on the graph represent significant differences.

4.3.2.4.5 Acid compounds

Figure 4.6 shows that 3-methylbutanoic acid (***P***<0.001) increased significantly with extended ageing (Table 4.11a-c, Figure 4.6). The effects extended to medium chain acids, including heptanoic acid (***P***<0.05), octanoic acid (***P***<0.01) and nonanoic acid (***P***<0.05). Stetzer et al. (2008) reported increases in acid compounds due to ageing (7 days and 14 days), although some of the samples were enhanced with phosphate and salt. These compounds increased significantly probably as the result of rise in the fatty acid levels with ageing because of the lipolytic enzyme activity (Hood and Allen, 1971).

M4 significantly differentiated all the acid compounds in Figure 4.6. Although very highly significance level (***P***<0.001) changes in the quantity of 3-methylbutanoic acid was reported in M4 due to post-mortem ageing, higher change in 3-methylbutanoic acid was observed in M2 compared to M3 and M4, therefore these graphs were plotted separately in Figure 4.6. M2 and M3 only significantly differentiated 3-methylbutanoic acid but not other acid compounds.

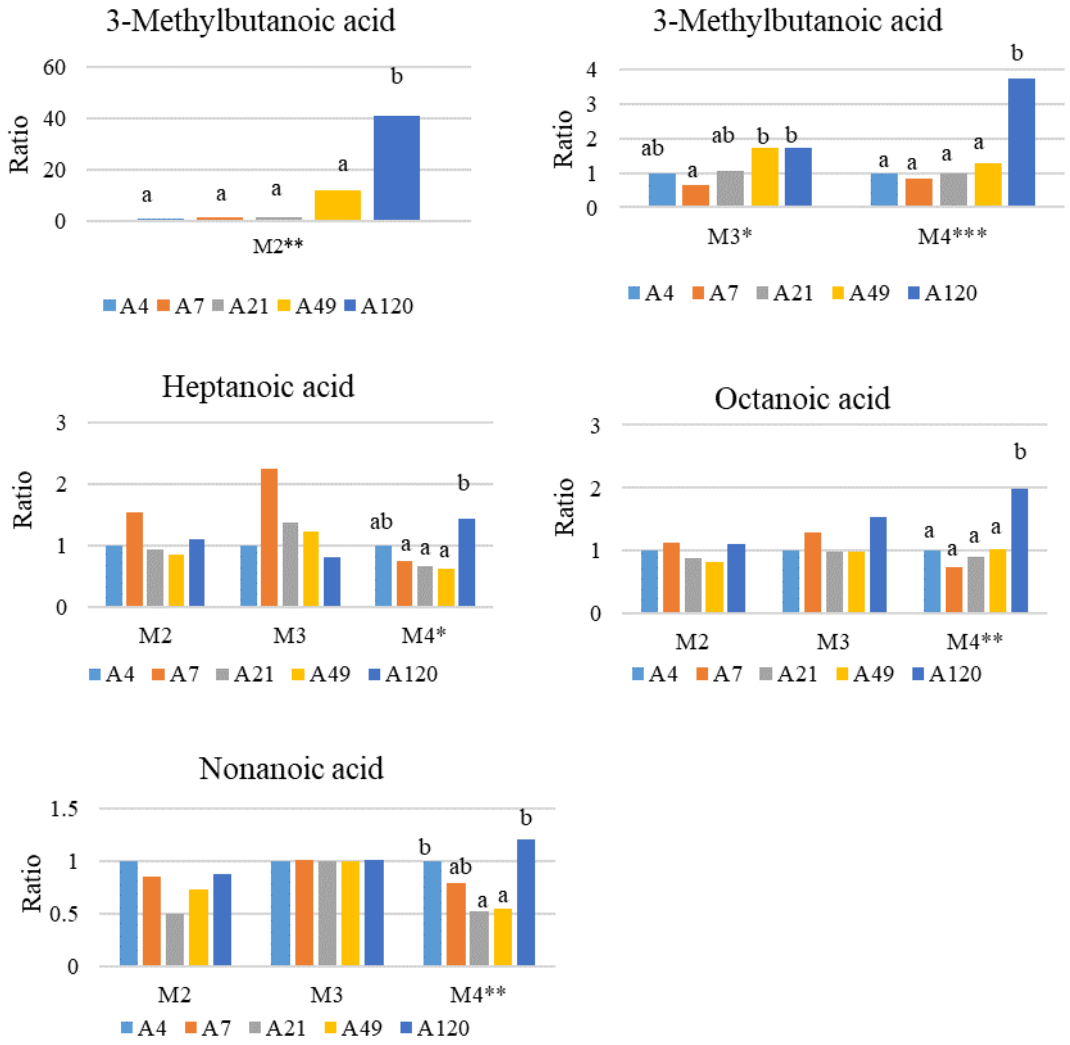


Figure 4.6 Changes in the quantities of acid compounds in relation to the quantity of volatile compound from cooked beef sample aged for 4 days.
Significance levels: *P<0.05, **P<0.01 and ***P<0.001. Different superscripts on the graph represent significant differences.

4.3.2.4.6 Scores achieved by the extraction method for the ability to differentiate cooked beef subjected to extended ageing periods

The effects of extended ageing on volatile groups were generally in agreement between methods (Table 4.11a-c). Results showed that extended ageing increased the quantities of Strecker aldehydes and M2 was the best in differentiating this compound category (Figure 4.2). Significant decreases in the quantities of aldehydes were observed using M4, although the trend was mostly not significant using M2 or M3 (Figure 4.3). Some 2-alkenals decreased with extended ageing, although the trend was not consistently significant in all methods (Figure 4.4). Pyrazine compounds increased

with extended ageing with M2 best differentiating the compounds in this category with beef subjected to extended ageing period (Figure 4.6). M4 also identified increases in the quantities of acids due to extended ageing (Figure 4.6).

Table 4.16 Scores allocation for the ability to differentiate beef samples aged to different periods by each volatile compound category.

Ability to differentiate cooked beef subjected to extended ageing periods	Score*		
	M2	M3	M4
Strecker aldehydes	5	2	3
Aldehyde	2	1	5
2-alkenals	5	0	3
Pyrazine compounds	5	2	2
Acids	1	1	5
Total (25 points)	**18**	**6**	**18**

*Scores were assigned based on the ability of extraction method to differentiate cooked beef subjected to extended ageing periods reported in Section 4.3.2.4.1 to 4.3.2.4.5.

4.3.3 The advantages and disadvantages of the different extraction methods

After careful consideration of all criteria, specific scores were assigned for each method, and the results are presented in Table 4.17. Overall, M1 achieved the lowest score compared to other methods. Therefore, M1 was excluded from Trial B. This method was hard to use, labour-intensive, least flexible and did not detect many volatile compounds. M2 and M4 achieved higher scores compared to M3, and both of these methods had their advantages and disadvantages. For example, M2 requires less preparation as the sample is analysed immediately after cooking. However, this means that the method is less flexible. One might wish to conduct volatile analysis on the same sample as was being tested in consumer or sensory panels. In this scenario, it might be difficult to analyse all the samples immediately after cooking, so M4 might be more suitable. In addition, sample size might be one of the restrictions in designing an experiment. Thus, M4 might be more suitable in this scenario.

It was also important to note that M2 had slightly better reproducibility compared to M4 (Table 4.17). The reproducibility of M4 might increase if repeated measurements are taken per sample, considering it is possible to replicate or triplicate the same steak sample due to the lower amount of sample required for the analysis.

Table 4.17 General and specific criteria for selection of extraction method.

Criteria	Weighting	M1	M2	M3	M4
General criteria					
Ease of use	5	1	5	4	4
Amount of beef samples required	5	3	3	5	5
Flexibility of the methods	5	1	3	5	5
	Subtotal	**5**	**11**	**14**	**14**
Specific criteria					
i) Detection range of compounds					
Strecker aldehydes	5 for each category	1	5	5	5
Aldehyde		2	4	5	5
2-alkenals		0	4	5	4
Pyrazine compounds		0	4	4	5
Acids		0	5	3	4
Furans		0	3	4	4
Sulphur containing compounds		1	4	5	3
Ketones		1	4	5	5
	Subtotal	**5**	**33**	**36**	**35**
ii) Detection quantities of the compounds					
Strecker aldehydes	5 for each category	1	5	2	1
Aldehyde		0	1	5	2
2-alkenals		0	1	2	5
Pyrazine compounds		0	3	4	2
Acids		0	2	4	1
Furans		0	3	5	3
Sulphur containing compounds		1	4	4	3
Ketones		0	5	4	3
	Subtotal	**2**	**19**	**26**	**17**
iii) Reproducibility of the method	10	3	8	5	7
	Total*	**15**	**71**	**81**	**73**
iv) Ability to differentiate cooked beef subjected to extended ageing periods					
Strecker aldehydes	5 for each category	NA	5	2	3
Aldehyde		NA	2	1	5
2-alkenals		NA	5	0	3
Pyrazine compounds		NA	5	2	2
Acids		NA	1	1	5
	Subtotal	**NA**	**18**	**6**	**18**
	Final Total	**15**	**90**	**87**	**91**

*A total is calculated excluding one of the specific criterion (ability to differentiate cooked beef subjected to extended ageing periods) as M1 was not included for this criterion.

4.4 Conclusions

This study investigated the effectiveness of alternative SPME extraction methods. Seven criteria were considered to select the more suitable methods for cooked beef volatile analysis, including the ease of use, amount of beef sample required, method flexibility, detection range of volatile compounds, quantities of volatile compounds, ability to differentiate samples subjected to different ageing period and reproducibility of the extraction methods. In conclusion, the comparison of three SPME methods showed that the M2 and M4 had strengths for different compound groups. M2 was easier to use compared to all other methods. However, this method was less flexible compared to M4 as the sample should be analysed as soon as possible after cooking. An extra liquid nitrogen homogenisation preparation step was required for M4. M4 had extra flexibility as the sample can be stored in a -80ºC freezer and could be analysed afterwards. Due to the quantity of sample required, it was possible for repeated analysis for one sample. On the other hand, M2 had slightly higher reproducibility compared to M4, although the reproducibility of M4 is possible to improve using repeated analysis for every sample.

4.4.1 Future Direction

The impact of a few specific elements of the methods were considered for further improvement. A 2.5 minute solvent delay was applied to M2 to M4. This solvent delay can be removed in future experiments to capture early eluted volatile compounds such as methanethiol. For the analysis, 5µl of 25ng/µl of internal standard (1,2-dichlorobenzene in methanol) was added into all samples. The concentration of internal standard should be adjusted according to the extraction method to ensure that it was not too concentrated and that the peak did not override other volatile peaks in the analysis. Other frozen methods could be considered because it was difficult to ensure the samples were completely frozen after 5 minutes immersion in liquid nitrogen. It is also recommended that beef samples be cut into smaller cubes to ensure the inside of the sample is completely frozen. Thus, further improvements to the method may be possible with future experimentation.

4.5 References

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Chapter 6 General Discussion and Future Directions for Research

In this chapter, the significant findings of the studies reported in this Thesis will be discussed in Section 6.1. In addition, the important implications of the studies for the meat industry and meat science will be outlined in Section 6.2, while Section 6.3 will suggest future developments and recommendations for further research.

6.1 Significance of the results

The responsibility of producing high eating quality beef products rests with farmers, beef producers, processors and retailers. As demonstrated in previous trials, eating quality of beef is vital to ensure consumer satisfaction which guarantees consumers repurchase intent (Harrington, 1994). Consumers are the end-users of the product and it's an expensive product in the customers' shopping basket. Consumers demand beef products that are produced with good farming practices, and are safe, nutritious and most importantly, of high eating quality (Verbeke et al., 2010). Thus, understanding consumer perception or liking of the product is important for the beef industry to deal with such a perishable product. Grunert (2006) proposed that the attributes of meat are normally unknown by the consumers when they purchase the product, and thus, are affected by the information available, which are known as the quality cues. However, the consumer liking of beef, intrinsic quality cues and eating quality of the meat are fundamentally linked to consumer satisfaction. Many studies have reported that pre- and post-slaughter factors of the meat affected eating quality, and thus, consumer liking of beef (Lawrie, 1998). It is expected that these factors can affect beef eating quality in different ways and that consumers can perceive these factors in different ways, depending on socio-economic status and cultural origin (Oliver et al., 2006, Prescott et al., 2001, Sañudo et al., 1998). Pre- and post- slaughter factors such as animal breed, sex and hanging method were included in Chapter 2 with the aim to create a wide range of eating quality attributes.

While many papers have evaluated the average consumers' palatability trait scores, it is evident that consumers may not be regarded as "average" (Lyford et al., 2010, Oliver et al., 2006, Sánchez et al., 2012). Historically, Northern Ireland and the Republic of Ireland have exported high proportion of their beef products to the Great Britain. For this reason, the response or perception of beef amongst consumers in Great Britain relative to those in Northern Ireland and the Republic of Ireland is of commercial relevance to the beef industry. This has prompted the study of similarities and differences between consumers from different regions in Chapter 2. Three consumer panels were conducted, involving 120 consumers from each region (Belfast, Cork and Reading). Few differences were found between regions on which beef samples consumers preferred. For each region, a modified MSA model was created and the results showed little differences on the weightings for tenderness, juiciness, flavour liking and overall liking. Willingness to pay (WTP) for "unsatisfactory", "satisfactory everyday", "better than everyday" and "premium" beef product were transferable across different region, with slightly higher WTP observed for Reading and Cork consumers compared to Belfast consumers, indicating the suitability to establish the MSA star system in Northern Ireland, the Republic of Ireland and Great Britain to maximise profit.

The consumers' socioeconomic background, behavioural factors, consumption frequency of beef muscles and motivation of beef choice were also investigated in these three regions. Consumers with different consumption habits or motivations of beef choice had significant effects on the consumers' palatability traits and WTP. The higher score for all palatability traits observed among Reading consumers compared to Belfast and Cork consumers may be due to the observed differences in motivation for beef choice and consumption frequency of beef muscles. Consumers from Reading paid less attention to the origin of beef product and the healthiness of beef product. In addition, consumers from Reading also routinely consumed lower quality beef such as rump and topside, which might explain why these consumers scored higher on the same striploin beef (higher quality meat) compared to consumers from Cork and Belfast.

Four cluster groups were identified in Chapter 2 and these clusters showed distinct differences in scoring pattern and/or liking of beef. These cluster groups were described as "easy-pleased", "bull-beef liker", "tender-beef liker" and "fastidious"

consumers. The variability of consumer liking of meat has been frequently linked to the sensory preferences (Oliver et al., 2006, Oltra et al., 2015, Prescott et al., 2001).

A study was conducted in Chapter 3 to evaluate the effect of value-added processes on the beef quality of different muscles with a series of chemical and instrumental analyses. Beef samples that were treated with extract solutions enhancement and underwent tenderisation treatment were compared with untreated samples. In this study, analyses were conducted to try and understand the consumer results conducted in Australia which showed that consumers preferred treated samples over the untreated samples. Sugar analysis showed clear effects, with increasing sugar concentration and decreased sugar phosphates concentration for value-added beef injected with extract solution. The clear differences in sugar and sugar phosphate concentrations might be one of the reasons that impacted the flavour liking of beef. Volatile analysis showed clear muscle effects but the effects of value-added process were generally small. Value-added beef also had higher moisture content due to the addition of extract solution. Sucrose and dimethyl disulphide were correlated to consumer flavour liking score. Principal component analysis showed that consumers' palatability traits were highly associated with moisture content, sucrose, glucose, ribose and fructose. It was also possible that salt was added in the preparation of extract solution; however, such analysis was not conducted.

The lack of significant results for volatile analysis in Chapter 3 might be due to the variation in the amount of extract solution contained in the core collected for volatile analysis. Manual SPME was a labour-intensive method which may have introduced additional variability. This prompted the development of a more robust SPME extraction method for cooked beef samples in Chapter 4. General criteria such as ease of use, amount of beef sample and flexibility of the methods were considered. Specific criteria with higher weightings were also considered, including the reproducibility of the method, detection range of compound and detection quantity of compound. Post-mortem ageing was introduced to identify the ability of the extraction methods to differentiate samples aged for different ageing periods. Two methods achieved high scores for most of the criteria; automatic SPME-cored beef (CAR/PDMS fibre) and automatic SPME- liquid nitrogen homogenised beef (DVB/CAR/PDMS fibre). These two methods had higher reproducibility and were less labour intensive compared to the manual SPME method and can be employed in different situations. For example,

the high reproducibility automatic SPME-cored beef (CAR/PDMS fibre) method can be used whenever it's possible to conduct the volatile analysis straight after cooking and this method requires less preparation. Automatic SPME- liquid nitrogen homogenised beef (DVB/CAR/PDMS fibre) is suitable to use if the researcher is planning to collect the beef samples during consumer/ trained sensory panels to analyse in the future. It's also possible to collect repeated measurements for liquid nitrogen homogenised samples.

In Chapter 5, packaging methods (modified atmosphere packaging, overwrapped packaging and vacuum skin packaging) significantly affected several lipid degradation volatile compounds and a Maillard compound, which was 3-methylbutanal. These results showed that high oxygen concentration in modified atmosphere packaging induced higher generation of lipid degradation compounds which might explain the lower consumer liking scores for these samples compared to vacuum skin packaging or overwrapped packaging. Higher concentration of n-aldehydes, Strecker aldehydes, sulphur containing compounds, acids, esters and ketones were reported in rump samples compared to striploin samples. These two muscles were separated in the principal component analysis, which might be due to the differences in the lipid content. Post-mortem ageing had a significant impact on the quantities of 20 volatile compounds, in which the samples that aged for an extended period (49 days) had higher concentrations of Strecker aldehydes, ketones, pyrazines, alcohols and acids compared to beef aged for 14 days and 21 days. Therefore, the results from this chapter indicated that the packaging method, beef muscles and post-mortem ageing affected the volatile profile of cooked beef and provide the beef industry the opportunity to develop beef products with favourable flavour profiles that target consumer preferences and thus improve the overall beef experience.

The relationships between sensory attributes and consumer liking with some instrumental measurements such as Warner Bratzler Shear Force, volatile compounds and sugar content were analysed using principal component analysis and preference mapping. These analyses provide indications of possible pre- or post-slaughter processes and identify the sensory attributes that could be related to consumer liking of beef. This could be valuable for the commercial markets and those developing products to be introduced to different markets.

6.2 Implications for the beef industry

The investigation and identification of pre-slaughter factors such as animal breed, sex and post-slaughter factors such as hanging method, which have important effects on the consumer liking of beef, could allow the beef industry to target animals before slaughter and produce meat with the high and consistent eating quality that consumers demand for a particular market. The results showed that beef from steers generally had higher quality compared to bulls and cows, although consumers from one cluster group rated beef from steers and bulls similarly. In terms of post-slaughter processing, tenderstretch hanging method significantly affected the eating quality of the product, as has been reported by many before (Ahnström et al., 2012, Sørheim and Hildrum, 2002). On the other hand, beef from dairy breeds had higher eating quality compared to continental breeds. However, dairy breed animals tend to be viewed less favourably by the beef industry due to smaller size and poor muscle shape. The results from this study showed the possibility for marketing beef from dairy breeds as premium product to maximise profit despite the economic loss due to animal size or muscle shape.

Considering the results reported in the literature and in this Thesis, it seems possible that the beef industry could target consumers according to their region or preferences. Generally, the consumers from Great Britain had similar preferences towards the same beef compared to consumers from the Republic of Ireland and Northern Ireland, which showed that beef consumer panels conducted in the Republic of Ireland or Northern Ireland were representative to those in Great Britain. However, consumers in Great Britain had different consumption frequencies for rump and topside compared those from Northern Ireland and the Republic of Ireland. This finding is interesting as the results from Chapter 3 and Chapter 5 showed that beef from striploin (STR045) had higher eating quality compared to beef from rump muscles (RMP131 and RMP231). Consumers from the four cluster groups in Chapter 2 also perceived beef differently. This information provides insights for beef industries in the Republic of Ireland and Northern Ireland that are marketing beef products to other regions or targeting certain consumer groups, indicating that the differences in consumption habit or liking should be taken into account.

When different value-added processes were evaluated, extract or phosphate solutions enhancement and tenderising processes had positive impacts on eating quality of beef

according to consumer panel results. This is interesting as these value-added processes were applied after 22 days of post-mortem ageing. Future research should investigate if earlier introduction of value-added processes could enhance the eating quality to reduce the cost associated with storage of meat. Although there was some variation between trials, value-added processes had significant impacts on several flavour volatile compounds. Packaging methods also had some impacts on the volatile profile of grilled beef and results from consumer studies in Australia showed modified atmosphere packaging had a negative impact on beef eating quality.

The relationships observed between sensory panels and chemical analyses are observed in principal component analysis and preference mapping. For example, attributes such as "tenderness", "salty" flavour and "roast beef" flavour were associated with consumer liking. Principal component analysis also showed that sugars such as ribose, sucrose and fructose were closely associated with consumer liking. These relationships should be properly investigated and examined through control experiments which will allow further understanding in these areas.

6.3 Recommendations for future work

Considering the results of the studies conducted, the following considerations for future study and research are recommended.

- There are some analyses that the author would like to conduct, however, it was impossible to address every aspect of these studies in the time available. For example, in Chapter 2, inclusion of more consumers from different regions could increase the representation of consumers from each country. Fatty acid analysis could help give us a better understanding of the fat composition in different beef samples and how this affects consumer liking of beef in Chapter 3 and Chapter 5. In Chapter 3, analysis of salt content in the enzyme treatments may have explained why consumers preferred beef treated with enzyme treatments.

- The study in Chapter 2 showed that the consumers from different regions had differences in the consumers' sensory score with some differences in the weighting of tenderness, juiciness, flavour liking and overall liking on the MQ4 equation using beef sourced from the Republic of Ireland and Northern Ireland. Interestingly, other studies also found differences in consumer sensory scores between consumers from Australia, France, Republic of Ireland, Northern Ireland and Poland using beef sourced from each country. However, no study has investigated the consumer liking of Irish or Northern Irish beef for consumers from other European or Asian countries. Comparison studies should be conducted between Irish/Northern Irish consumers and consumers from international markets using beef sourced in Ireland and following industry practises on the island of Ireland. Such study would help the beef industry in the Republic of Ireland and Northern Ireland gain a better understanding of how consumers from international markets perceive beef products from Ireland and Northern Ireland.

- Enhancement treatments improved the overall eating quality of beef and changed the volatile compound profile when the samples were treated after 22 days of post-mortem ageing. However, no study has investigated the effect on the volatile profile of beef if enhancement treatment is introduced at an earlier stage of post-mortem ageing (e.g. 4 days or 7 days). Studies have suggested that injection of extract solution during early post-mortem ageing improve tenderness of meat (Han, 2008, Toohey et al., 2011). Therefore, it would be interesting to study the effect of early post-mortem enhancement on the volatile profile of beef and investigate at which stage of post-mortem ageing enhancement gives optimal tenderness and flavour profile for beef.

- Addition of sugars into uncooked beef samples increased the eating quality of beef and appeared to affect the concentrations of other sugars and sugar phosphates (Chapter 3). However, it was unclear why addition of one sugar will affect the concentration of other sugars and sugar phosphate. Sucrose was added into the value-added beef and the results showed increased in glucose, fructose and ribose. The increase in fructose and glucose were expected due to

hydrolysation of sucrose. However, the increase in ribose was unexpected and this should be further investigated because ribose has flavour generating potential (Koutsidis et al., 2008, Meinert et al., 2009).

- The advancement in analytical instruments provides opportunities to develop new methods for volatile analysis. Two samples formed were used, cooked cored beef and liquid nitrogen-homogenisation beef. The reproducibility of the liquid-nitrogen-homogenisation beef was slightly affected compared to the cooked cored beef. However, it was unclear that whether the reproducibility of the extraction method was affected by liquid nitrogen freezing or the homogenisation process. Future research should investigate which process will introduce variation and reduce the reproducibility of the method. In addition, cored beef should be analysed with DVB/CAR/PDMS fibre to investigate if the reproducibility is reduced by liquid nitrogen homogenisation step with this fibre. Other extraction methods such as dynamic headspace should be further explored.

- Muscle had significant impacts on many volatile compounds. This reflects that individual beef muscles have different volatile profiles using the grill cooking method. However, it is possible that the volatile profile of various beef muscles is affected differently when cooked by other cooking methods. In fact, a study conducted by Vierck et al. (2019) showed that cooking method directly affected the flavour profile of striploin steak while Farmer et al., (2009) reported significant interaction for cooking method with muscle and position within muscles for all consumer sensory scores (tenderness, juiciness, flavour liking, overall liking, MQ4 and satisfaction category). Future studies should investigate the effects of beef muscles with different cooking methods because cooking methods might affect beef muscles differently according to the composition of the meat (e.g. intramuscular fat, connective tissue content).

The results of this Thesis revealed that there were differences between regions even though these regions are very close geographically. The increasing importance of flavour liking among consumers highlights the necessity to understand the effects of

pre- and post- slaughter processes on volatile profile of cooked beef. The results reported in this Thesis will help inform future research on the role of volatile compounds in relation to consumer preferences.

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Annex

Annex 2.1 Quantitative Descriptive Analysis- Appearance significance interactions.

	Pale EXAP	Chestnut EXAP	Juicy EXAP	Charred EXAP	Bloody EXAP	RedJui EXAP	BrownJui EXAP	Greasy EXAP	Tight INAP	Lean INAP
Hang.Breed										
AT Cont	28.2	49.9	17.5ab	29.8	5.2	7.5b	15.4	10.6	57.3	75.8
AT Dairy	15.8	61.5	19.5b	40.5	5.2	6.9b	21.8	11.9	59.1	74.7
TS Cont	28.3	47.5	14.5a	27.9	3.3	3.6a	16.4	9.4	58.2	76.5
TS Dairy	16.3	61.5	23.1c	40.1	6.7	9.1b	20.5	11.7	58.8	74.6
avSED	2.56	2.53	1.65	2.42	1.38	1.64	1.82	0.87	2.20	1.51
P	0.951	0.42	0.005	0.502	0.067	0.007	0.381	0.344	0.733	0.681
Breed.Sex										
Cont Bulls	31.1c	44.9	18.4c	27.8	4.7ab	7.2bc	16.8a	9.5a	51.8a	76.1abc
Cont Cows	22.5b	53.6	17.9bc	29.2	6.1bc	6.5abc	16.4a	11.3ab	59.6b	73.8ab
Cont Steers	31.2c	47.6	11.7a	29.4	2.0a	3.0a	14.6a	9.3a	61.8b	78.6c
Dairy Bulls	20.0b	56.5	23.7d	37.8	8.9c	10.7c	19.3a	11.6ab	59.7b	72.7a
Dairy Cows	16.7ab	62.9	14.0ab	37.5	2.6a	3.5ab	17.9a	10.5a	60.6b	76.7bc
Dairy Steers	11.6a	65.0	26.2d	45.5	6.4bc	9.8c	26.2b	13.3b	56.7ab	74.4ab
avSED	3.56	3.46	2.02	3.22	1.80	2.13	2.34	1.13	2.78	1.87
P	0.02	0.236	<0.001	0.191	0.001	0.003	0.004	0.009	0.005	0.014
Sex.Position										
Bulls M	25.4	52.1	19.9	32.3	6.4	7.5	19.9	11.2	55.3	71.9a
Bulls A	26.9	49.0	20.3	30.5	4.7	8.6	17.2	10.5	56.7	77.8bc
Bulls P	24.4	51.1	22.9	35.7	9.3	10.8	17.0	9.9	55.3	73.5ab
Cows M	17.9	60.4	15.8	31.2	3.1	3.3	19.2	10.7	58.0	73.5ab
Cows A	21.1	53.5	15.2	32.6	4.2	4.2	15.3	10.6	61.6	74.6abc
Cows P	19.8	60.8	16.9	36.3	5.6	7.4	17.0	11.3	60.7	77.5bc
Steers M	22.7	54.4	21.8	36.3	5.2	6.8	20.4	11.5	60.7	77.9c
Steers A	22.2	54.7	16.2	36.3	4.0	6.6	18.4	12.3	59.9	75.2abc
Steers P	19.2	59.8	18.7	39.8	3.5	5.8	22.5	10.3	57.1	76.3bc
avSED	3.08	3.20	2.49	3.24	1.91	2.27	2.57	1.19	3.16	2.25
P	0.652	0.24	0.37	0.966	0.161	0.468	0.53	0.455	0.693	0.026
Hang.Breed.Sex										
AT Cont Bulls	29.1	48.0abc	22.7ef	28.2	6.7	12.0	17.6	10.3	52.8	77.2
AT Cont Cows	21.1	57.3def	19.3cde	33.7	5.9	7.8	14.9	11.7	58.4	71.9
AT Cont Steers	34.5	44.4ab	10.3a	27.4	3.0	2.7	13.7	9.8	60.8	78.3

	Pale EXAP	Chestnut EXAP	Juicy EXAP	Charred EXAP	Bloody EXAP	RedJui EXAP	BrownJui EXAP	Greasy EXAP	Tight INAP	Lean INAP
AT Dairy Bulls	20.6	54.3cde	20.6de	35.3	9.1	11.1	18.2	11.4	62.2	72.1
AT Dairy Cows	16.3	63.3fg	13.1ab	39.6	2.2	2.1	19.7	11.3	61.5	77.0
AT Dairy Steers	10.5	66.8g	24.9ef	46.5	4.3	7.5	27.6	13.0	53.7	74.8
TS Cont Bulls	33.2	41.9a	13.9abc	27.4	2.7	2.4	15.9	8.7	50.9	75.0
TS Cont Cows	23.9	49.9abcd	16.5bcd	24.8	6.2	5.2	17.8	10.8	60.9	75.7
TS Cont Steers	27.9	50.8bcd	13.0ab	31.4	1.0	3.3	15.6	8.9	62.9	78.8
TS Dairy Bulls	19.3	58.7defg	26.9f	40.3	8.6	10.2	20.4	11.8	57.2	73.3
TS Dairy Cows	17.1	62.6efg	14.9abc	35.4	2.9	4.9	16.2	9.7	59.7	76.3
TS Dairy Steers	12.6	63.2fg	27.4f	44.6	8.6	12.1	24.8	13.6	59.6	74.0
avSED	4.55	4.49	2.85	4.26	2.42	2.87	3.18	1.52	3.83	2.62
P	0.149	0.019	0.024	0.237	0.464	0.682	0.201	0.64	0.498	0.313
Hang.Breed.Position										
AT Cont M	26.2	52.0ab	17.2	30.5	5.2	6.6	15.8	10.8	57.1	75.0
AT Cont A	31.5	45.9a	17.5	27.3	4.5	6.2	14.5	10.6	59.0	77.0
AT Cont P	27.0	51.8ab	17.7	31.5	5.9	9.7	16.0	10.4	55.9	75.2
AT Dairy M	16.3	60.8cd	21.4	36.6	4.4	5.4	26.0	11.3	58.8	73.3
AT Dairy A	17.3	60.1cd	18.3	42.0	5.5	8.2	20.0	13.1	60.4	76.0
AT Dairy P	13.8	63.5d	18.8	42.8	5.7	7.1	19.5	11.4	58.2	74.6
TS Cont M	30.3	45.6a	14.6	26.7	2.7	1.9	17.4	9.7	56.1	76.3
TS Cont A	26.6	47.8a	10.4	28.2	2.2	2.0	15.0	9.6	60.2	74.5
TS Cont P	28.1	49.3ab	18.5	28.7	5.1	6.9	16.8	9.0	58.3	78.8
TS Dairy M	15.3	64.2d	23.5	39.3	7.2	9.5	20.1	12.7	60.1	73.2
TS Dairy A	18.1	56.0bc	22.8	35.0	5.1	9.4	18.4	11.3	58.2	75.9
TS Dairy P	15.6	64.4d	22.9	46.0	7.8	8.2	22.9	11.1	58.3	74.5
avSED	3.40	3.57	2.88	3.66	2.17	2.58	2.94	1.36	3.63	2.59
P	0.193	0.047	0.317	0.07	0.659	0.619	0.241	0.424	0.679	0.483
Hang.Sex.Position										
AT Bulls M	21.2a	54.7	20.8	33.0	7.1	10.4	20.6	12.0	58.4	71.2
AT Bulls A	30.7e	48.0	21.3	28.8	5.8	10.5	18.5	10.6	59.2	79.0
AT Bulls P	22.7abcd	50.8	23.0	33.4	10.8	13.8	14.6	9.9	54.8	73.8
AT Cows M	18.0a	62.7	13.6	35.1	2.8	3.2	18.8	9.9	56.2	72.9
AT Cows A	20.0a	54.9	17.3	35.5	5.2	4.7	14.6	12.2	62.2	75.7
AT Cows P	18.2a	63.2	17.8	39.4	4.3	6.9	18.6	12.3	61.4	74.7
AT Steers M	24.7abcde	51.9	23.6	32.5	4.6	4.5	23.3	11.3	59.2	78.5
AT Steers A	22.5abc	56.0	15.0	39.6	4.0	6.3	18.7	12.6	57.7	74.9
AT Steers P	20.3a	59.0	14.1	38.8	2.3	4.5	20.0	10.4	54.9	76.3
TS Bulls M	29.7bcde	49.5	19.1	31.5	5.7	4.6	19.3	10.3	52.1	72.7

	Pale EXAP	Chestnut EXAP	Juicy EXAP	Charred EXAP	Bloody EXAP	RedJui EXAP	BrownJui EXAP	Greasy EXAP	Tight INAP	Lean INAP
TS Bulls A	23.0abcde	50.0	19.3	32.1	3.6	6.6	15.8	10.5	54.3	76.6
TS Bulls P	26.1abcde	51.4	22.8	37.9	7.7	7.7	19.3	9.8	55.7	73.3
TS Cows M	17.9a	58.2	18.0	27.3	3.3	3.4	19.5	11.6	59.8	74.2
TS Cows A	22.2abc	52.1	13.0	29.8	3.3	3.7	16.1	9.0	61.1	73.5
TS Cows P	21.4ab	58.4	16.1	33.3	7.0	8.0	15.3	10.3	60.0	80.3
TS Steers M	20.7a	57.0	20.1	40.2	5.8	9.2	17.4	11.7	62.3	77.4
TS Steers A	21.9ab	53.4	17.5	33.0	4.0	6.8	18.2	11.9	62.2	75.5
TS Steers P	18.1a	60.7	23.2	40.8	4.6	7.1	25.0	10.1	59.3	76.3
avSED	4.24	4.42	3.52	4.51	2.67	3.17	3.61	1.67	4.45	3.17
P	0.044	0.393	0.093	0.18	0.857	0.873	0.18	0.323	0.691	0.637
Breed.Sex.Position										
Cont BullsM	29.8	45.9	18.2bcdef	29.8	4.0abcd	5.6	19.76c	10.5	50.8	74.4
Cont Bulls A	33.3	44.1	19.0cdef	23.7	5.1abcde	7.0	16.1abc	9.2	54.6	77.9
Cont Bulls P	30.2	44.8	17.9bcdef	29.9	5.0abcde	9.0	14.5abc	8.7	50.1	76.0
Cont Cows M	21.7	55.8	16.4bcde	26.2	4.0abcd	2.3	17.9abc	10.7	57.4	73.7
Cont Cows A	22.8	49.4	15.0bcd	29.1	4.1abcd	5.2	11.7a	11.5	61.0	72.0
Cont Cows P	23.0	55.6	22.4efg	32.5	10.0ef	12.0	19.4c	11.6	60.4	75.7
Cont Steers M	33.2	44.7	13.1abc	29.8	3.8abcd	4.9	12.1ab	9.6	61.5	78.9
Cont Steers A	31.0	47.0	7.8a	30.5	0.9a	0.1	16.5abc	9.6	63.1	77.4
Cont Steers P	29.4	51.3	14.1abc	27.9	1.4ab	3.9	15.3abc	8.8	60.9	79.4
Dairy Bulls M	21.0	58.3	21.6defg	34.7	8.7def	9.4	20.1c	11.8	59.7	69.5
Dairy Bulls A	20.4	53.9	21.6defg	37.3	4.3abcd	10.2	18.2abc	11.9	58.8	77.7
Dairy Bulls P	18.6	57.3	28.0gh	41.4	13.5f	12.5	19.4c	11.1	60.4	71.1
Dairy Cows M	14.1	65.1	15.2bcd	36.2	2.1abc	4.2	20.4c	10.8	58.6	73.4
Dairy Cows A	19.4	57.6	15.3bcd	36.2	4.4abcd	3.3	19.0bc	9.7	62.3	77.3
Dairy Cows P	16.6	66.0	11.5ab	40.1	1.3ab	2.9	15.0abc	11.0	61.0	79.4
Dairy Steers M	12.3	64.2	30.7h	42.8	6.5bcde	8.7	28.6d	13.4	59.9	77.0
Dairy Steers A	13.4	62.4	24.7fgh	42.1	7.2cde	13.0	20.4c	14.9	56.7	73.0
Dairy Steers P	9.0	68.4	23.2efg	51.7	5.5abcde	7.7	29.7d	11.7	53.3	73.2
avSED	4.48	4.62	3.52	4.64	2.72	3.23	3.66	1.70	4.49	3.18
P	0.813	0.991	0.041	0.253	0.005	0.092	0.006	0.71	0.839	0.619

Abbreviation: AT- straight hung, TS- aitch hung, Cont- continental, A- anterior, M- middle, P- posterior, avSED: average standard error, ***P***-probablility

Annex 2.2 Quantitative Descriptive Analysis- texture on cutting and mouthfeel

	Texture on cutting			Mouthfeel						
	TenderTXC	CrumblyTXC	FibrousTXC	TenderMOU	SpongyMOU	SucculeMOU	StickyMOU	BallsMOU	CrumblyMOU	GreasyMOU
Hang.Breed										
AT Cont	47	20.1	24.4	43.4	31	24.06ab	18.8	24.7	22.7	13.3
AT Dairy	47.9	18.2	22	43.9	30.9	27.2b	17	23.7	17.4	15.1
TS Cont	48.7	16.1	23.1	46.4	26.2	22.1a	18.1	25.9	22.6	12.5
TS Dairy	53.6	12.8	21.1	48.4	26.8	31.9c	16.9	23.3	17.9	15.2
avSED	2.547	1.912	1.599	2.8	1.907	2.442	1.091	1.692	2.471	0.867
P	0.213	0.656	0.906	0.585	0.833	0.022	0.697	0.47	0.941	0.426
Breed.Sex										
Cont Bulls	51.2	19.9	23.4	48.4	29.3	23.9ab	18.9	25	23.2	13
Cont Cows	38.4	13.6	28.8	34.5	34.2	23.1ab	16.4	28.7	15.1	13.1
Cont Steers	53.9	20.9	19.1	51.8	22.2	22.2ab	20.2	22.2	29.7	12.6
Dairy Bulls	50	15.9	20.3	45.3	30	29.0b	17	24.4	16.1	14.6
Dairy Cows	41.6	12.4	25	35.4	34	21.5a	15.4	26.1	15.1	14
Dairy Steers	60.5	18.2	19.4	57.6	22.6	38.2c	18.5	20	21.8	16.9
avSED	3.507	2.536	2.016	3.895	2.479	3.357	1.336	2.133	3.45	1.144
P	0.314	0.721	0.311	0.303	0.971	<0.001	0.898	0.784	0.181	0.081
Hang.Position										
AT M	48.1	20	24.5	43	31	25.8	18.9	24.9	19.8ab	14.3
AT A	48	18	22.6	44.4	31.9	25	17.5	24.3	19.3ab	14.3
AT P	46.2	19.5	22.4	43.4	29.8	26	17.4	23.4	21.2ab	14
TS M	51.4	14.8	23.5	47.7	26	27.6	17	24.5	19.2a	13.3
TS A	51.3	14.7	20.8	47.7	26.6	25.1	17.2	23.4	23.2b	14.1
TS P	50.7	13.9	22.1	46.8	26.9	28.3	18.5	26	18.3a	14
avSED	2.35	1.984	1.866	2.484	2.084	2.265	1.348	1.985	2.133	0.91
P	0.892	0.68	0.871	0.922	0.655	0.776	0.256	0.399	0.042	0.673

	Texture on cutting			Mouthfeel						
	TenderTXC	CrumblyTXC	FibrousTXC	TenderMOU	SpongyMOU	SucculeMOU	StickyMOU	BallsMOU	CrumblyMOU	GreasyMOU
Hang.Breed.Sex.Position										
AT Cont Bulls M	52.3	22.9	24.7	44.4	32.3	30.8cdefghi	17.7	23.8	23.6	11.8abcd
AT Cont Bulls A	53.4	18.4	28	50	32.3	26.0abcdef	20.3	25.9	25.6	14.4abcdefgh
AT Cont Bulls P	50.7	26.6	18.9	48.4	27.5	23.4abcdef	17.8	24.6	27	12.7abcdefg
AT Cont Cows M	38.3	15.8	33.3	31.5	35.3	17.3a	16.3	29.1	13.5	15.2abcdefghi
AT Cont Cows A	35.1	14.6	27.3	30.7	38.3	26.0abcdef	17.2	28.6	10.4	15.8cdefghi
AT Cont Cows P	37.2	10.8	28.5	35.7	32.2	25.2abcdef	17.3	24.8	13.9	12.1abcdefg
AT Cont Steers M	55.4	26.9	20.5	53.6	26.7	23.4abcdef	24.7	20.8	28.5	13.2abcdefgh
AT Cont Steers A	51.5	22.4	18.4	50.5	30.4	20.8abc	20.9	25.3	31.5	11.1ab
AT Cont Steers P	49.2	22.6	19.8	45.8	23.6	22.8abcde	17.4	19.9	30.6	13.1abcdefg
AT Dairy Bulls M	43.8	17.5	19.8	39.6	36.8	19.5abc	20.3	25.8	16.3	14.7abcdefghi
AT Dairy Bulls A	45.1	19.1	19	37.4	33.2	21.7abc	15.4	27.1	16	14.3abcdefgh
AT Dairy Bulls P	46.2	23.3	21.7	45.6	30	29.5bcdefgh	19.9	26.5	17.5	15.2abcdefghi
AT Dairy Cows M	36.8	20.9	26	33.7	31.6	21.3abc	16.4	29.8	13.1	14.6abcdefgh
AT Dairy Cows A	38.2	14	23.5	33.3	33.8	17.0a	14.2	20.7	13.1	12.7abcdefg
AT Dairy Cows P	41.6	13.8	23.3	35.4	39.8	24.2abcdef	15	22.8	16.5	15.4abcdefghi
AT Dairy Steers M	61.9	15.8	22.7	55.3	23.6	42.4ik	18.2	20.3	23.8	16.2defghi
AT Dairy Steers A	64.7	19.5	19.5	64.7	23.4	38.6ghijk	17.1	18.1	19.2	17.8hi
AT Dairy Steers P	52.3	20.1	22.2	49.7	26	31.0cdefghij	16.8	21.9	21.4	15.2abcdefghi
TS Cont Bulls M	54.1	17.7	21.9	49.6	28	17.0a	19.6	26.1	21.4	13.3abcdefgh
TS Cont Bulls A	49.3	17.8	20.9	51.5	25	22.0abc	21.1	25.5	24.4	13.2abcdefgh
TS Cont Bulls P	47.5	16.2	25.7	46.5	30.7	23.9abcdef	16.6	24.4	17	12.5abcdefg
TS Cont Cows M	37.6	12.8	33.6	36.9	33.2	26.9abcdefg	15.4	28.8	16.6	10.8a
TS Cont Cows A	39.1	15.5	23.8	31.2	35.5	18.2ab	15.7	29.7	19.7	11.7abcd
TS Cont Cows P	43.3	11.8	26.4	41.4	30.3	25.2abcdef	16.6	31.5	16.6	12.7abcdefg
TS Cont Steers M	59.1	17	20	55.5	18.8	22.4abcd	16.4	23.9	27.4	12.1abcde

	Texture on cutting			Mouthfeel						
	TenderTXC	CrumblyTXC	FibrousTXC	TenderMOU	SpongyMOU	SucculeMOU	StickyMOU	BallsMOU	CrumblyMOU	GreasyMOU
TS Cont Steers A	50.8	20.1	15.1	52.1	17.6	19.1abc	19.2	19.7	30.9	13.5abcdefgh
TL Cont Steers P	57.3	16.4	20.7	53.1	16.4	24.6abcdef	22.7	23.4	29.3	12.4abcdefg
TS Dairy Bulls M	52	10.9	17.5	48.2	27.5	34.0defghijk	14.6	20.1	16.3	11.6abc
TS Dairy Bulls A	58.2	13.8	24.1	53.2	27.1	35.0fghijk	14.3	23	19.6	16.7efghi
TS Dairy Bulls P	54.7	11	19.7	47.9	25.6	34.4efghijk	17.9	23.8	10.6	15.0abcdefghi
TS Dairy Cows M	42.7	7	30	35.3	29.5	22.9abcde	18.5	29.5	14.9	16.6eghi
TS Dairy Cows A	45.8	6.5	25.3	37.7	34.5	21.9abc	11.6	22.4	16.2	12.1abcdef
TS Dairy Cows P	44.5	11.9	21.8	37	35.1	21.6abc	16.7	31.3	16.8	12.5abcdefg
TS Dairy Steers M	62.6	23.4	17.8	60.8	19.2	42.3ik	17.4	18.4	18.3	15.5bcdefghi
TS Dairy Steers A	64.6	14.3	15.8	60.3	19.6	34.6efghijk	21.2	20	28.5	17.7hi
TS Dairy Steers P	56.9	16	18.3	54.9	23.5	40.0hijk	20.3	21.2	19.7	19.2i
avSED	6.237	5.072	4.62	6.687	5.249	6.002	3.294	4.908	5.805	2.32
P	0.871	0.357	0.692	0.731	0.997	0.049	0.43	0.836	0.691	0.02

Abbreviation: AT- straight hung, TS- aitch hung, Cont- continental, A- anterior, M- middle, P- posterior, avSED: average standard error, ***P***- probablility

Annex 2.3 Quantitative Descriptive Analysis- aroma significance interactions.

	RstBfAR	GrilStkAR	BeefyAR	CharAR	FattyAR	BloodyAR	MealyAR	HerbyAR	AcridAR	FarmyardAR	SpiceAR
Hang.Breed											
AT Cont	26.5	33.6	28.5	22.9	6.4	7.51ab	11.3	5.4	5.4	5.1	5.42ab
AT Dairy	28.4	36.2	30.3	32.8	7.2	6.37ab	11.9	4.8	5.6	6.2	4.73ab
TS Cont	32.2	32.4	29.7	21.4	5.8	5.56a	12.1	4.5	5.6	4.7	4.44a
TS Dairy	30.4	36.9	34.6	30.8	7.8	8.26b	11.6	4.6	5.5	4.6	5.56b
avSED	1.83	1.84	1.63	2.11	0.75	1.14	1.08	0.71	0.91	0.87	0.51
Prob	0.193	0.399	0.172	0.995	0.211	0.017	0.454	0.493	0.816	0.214	0.012
Breed.Sex											
Cont Bulls	29.3	31.5	30.1	22.33a	6.5	7.43b	11.9	5.0	5.6	5.0	5.0
Cont Cows	29.5	32.7	30.2	22.12a	6.1	7.90bc	11.4	5.0	5.8	5.5	5.2
Cont Steers	29.3	34.7	27.2	22.10a	5.8	4.28a	11.9	4.9	5.1	4.2	4.5
Dairy Bulls	29.2	34.2	33.7	26.50ab	8.4	10.43c	13.0	4.4	5.5	6.8	5.1
Dairy Cows	29.5	35.6	31.0	31.83bc	5.8	4.56a	11.5	4.9	4.5	4.4	5.3
Dairy Steers	29.5	39.8	32.5	37.08c	8.2	6.96ab	10.8	4.7	6.7	5.0	5.0
avSED	2.35	2.30	2.09	2.75	0.97	1.42	1.41	0.88	1.15	1.12	0.63
P	0.995	0.718	0.305	0.023	0.099	0.001	0.558	0.937	0.189	0.15	0.911
Hang.Position											
AT M	28.0	36.1	30.5	26.8	6.8	6.2	12.1	4.6	6.1	6.0	6.01b
AT A	25.9	32.2	27.0	27.4	6.8	6.6	12.0	5.7	5.4	5.6	4.45a
AT P	28.5	36.4	30.7	29.4	6.8	8.0	10.8	4.9	5.0	5.4	4.77a
TS M	31.0	35.8	31.8	25.5	6.5	7.8	11.3	4.6	5.2	4.8	4.44a
TS A	32.6	34.7	33.2	25.3	7.2	5.8	12.1	4.6	5.1	4.4	4.91ab
TS P	30.3	33.4	31.4	27.6	6.7	7.1	12.2	4.4	6.5	4.7	5.65ab
avSED	2.04	2.19	1.82	2.30	0.80	1.37	1.16	0.86	1.04	0.99	0.64
P	0.187	0.24	0.062	0.958	0.789	0.353	0.403	0.673	0.205	0.931	0.014
Sex.Position											
Bulls M	30.0	36.7	32.0	24.8	6.7	9.7	12.8	4.9	4.54ab	4.96abc	5.2
Bulls A	29.2	30.7	29.8	23.4	7.9	7.1	12.7	5.0	6.64bc	7.03c	4.7
Bulls P	28.6	31.2	33.9	25.0	7.8	10.0	11.8	4.1	5.40abc	5.73bc	5.4
Cows M	29.0	34.3	32.0	27.0	6.2	5.3	12.3	4.4	5.79abc	5.04abc	5.9
Cows A	28.8	32.4	30.1	25.3	6.3	5.9	11.2	5.5	5.08abc	4.70abc	5.1
Cows P	30.7	35.8	29.8	28.7	5.3	7.5	10.8	4.9	4.69abc	5.11abc	4.9
Steers M	29.5	36.8	29.6	26.6	7.1	6.1	10.0	4.4	6.57bc	6.20bc	4.6
Steers A	29.7	37.3	30.3	30.3	6.8	5.6	12.1	5.0	4.03a	3.17a	4.3
Steers P	29.0	37.8	29.6	31.9	7.1	5.2	11.8	5.0	7.16c	4.41ab	5.3

	RstBfAR	GrilStkAR	BeefyAR	CharAR	FattyAR	BloodyAR	MealyAR	HerbyAR	AcridAR	FarmyardAR	SpiceAR
avSED	2.56	2.71	2.29	2.92	1.02	1.69	1.48	1.06	1.30	1.24	0.78
P	0.892	0.226	0.427	0.578	0.51	0.444	0.409	0.839	0.038	0.05	0.638
Hang.Breed.Position											
AT Cont M	26.7	35.2	30.2	25.18abc	6.5	6.0	12.6	4.6	6.0	5.5	6.5
AT Cont A	25.3	30.1	25.4	20.97ab	6.3	6.4	11.1	5.8	5.7	4.9	4.3
AT Cont P	27.6	35.6	30.1	22.66abc	6.5	10.2	10.3	5.8	4.4	4.9	5.5
AT Dairy M	29.3	37.1	30.9	28.38cde	7.1	6.5	11.6	4.6	6.2	6.5	5.5
AT Dairy A	26.4	34.2	28.6	33.73ef	7.3	6.8	12.8	5.7	5.1	6.2	4.7
AT Dairy P	29.4	37.2	31.4	36.21f	7.1	5.9	11.3	4.0	5.5	5.9	4.0
TS Cont M	30.8	33.4	30.6	18.90a	5.8	6.5	11.8	4.6	5.3	5.7	3.3
TS Cont A	33.7	32.0	29.9	23.96abc	7.2	4.4	12.4	5.0	5.4	3.9	4.5
TS Cont P	32.1	31.7	28.7	21.43ab	4.5	5.8	12.0	3.9	6.3	4.6	5.5
TS Dairy M	31.1	38.1	33.1	32.05def	7.3	9.1	10.8	4.6	5.1	4.0	5.6
TS Dairy A	31.6	37.4	36.4	26.60bcd	7.3	7.3	11.7	4.2	4.8	4.9	5.3
TS Dairy P	28.6	35.2	34.2	33.84ef	8.8	8.4	12.3	4.9	6.8	4.9	5.8
avSED	2.92	3.12	2.62	3.32	1.16	1.95	1.68	1.22	1.48	1.42	0.90
P	0.845	0.945	0.901	0.01	0.119	0.351	0.735	0.312	0.974	0.672	0.279
Hang.Breed.Sex.Position											
AT Cont Bulls M	28.6	38.9	33.9	28.8abcdefgh	6.8	5.4	11.2	3.7	4.2	3.6	5.9
AT Cont Bulls A	23.0	25.1	27.4	19.7abcde	6.6	8.8	12.0	5.9	6.9	6.5	4.6
AT Cont Bulls P	28.2	32.0	29.7	23.2abcdef	7.8	12.3	12.4	6.2	4.3	5.5	6.8
AT Cont Cows M	26.4	32.9	28.1	27.1abcdefgh	6.7	4.7	13.8	4.9	7.7	6.8	7.4
AT Cont Cows A	25.3	31.9	24.2	24.2abcdefg	7.5	8.2	8.1	4.9	8.4	5.7	4.3
AT Cont Cows P	22.7	34.8	31.7	22.1abcdef	5.9	13.1	8.7	6.5	3.8	5.3	4.0
AT Cont Steers M	25.2	33.7	28.5	19.7abcd	5.9	7.7	12.7	5.3	6.2	6.0	6.3
AT Cont Steers A	27.5	33.3	24.5	18.9abc	4.8	2.2	13.3	6.6	1.9	2.5	3.9
AT Cont Steers P	31.8	39.9	28.8	22.7abcdef	5.8	5.2	9.8	4.7	5.2	4.1	5.7
AT Dairy Bulls M	27.3	34.7	31.4	23.3abcdef	7.7	10.6	12.4	5.1	2.9	9.0	4.2
AT Dairy Bulls A	27.1	29.7	28.4	28.3abcdefgh	8.0	5.9	12.7	4.4	6.0	8.9	4.9
AT Dairy Bulls P	26.0	29.8	37.4	28.5abcdefgh	8.4	10.2	11.4	3.4	5.9	9.1	4.4
AT Dairy Cows M	29.8	36.2	30.4	30.5defgh	5.9	2.9	12.2	4.1	6.5	3.2	6.3
AT Dairy Cows A	26.0	34.5	26.8	30.5defgh	6.3	4.9	12.5	5.7	4.6	4.1	4.7
AT Dairy Cows P	35.3	38.7	28.8	42.7ijk	4.7	3.5	9.3	3.8	3.6	4.1	3.1
AT Dairy Steers M	30.7	40.4	30.9	31.3efghi	7.8	6.0	10.1	4.5	9.2	7.3	6.0
AT Dairy Steers A	26.1	38.6	30.7	42.5jk	7.4	9.6	13.3	7.0	4.8	5.6	4.3
AT Dairy Steers P	27.0	43.0	28.0	37.4hijk	8.2	3.9	13.2	4.9	7.1	4.6	4.6
TS Cont Bulls M	30.6	35.1	28.3	18.4ab	5.8	9.9	14.2	6.0	6.8	4.4	4.9

	RstBfAR	GrilStkAR	BeefyAR	CharAR	FattyAR	BloodyAR	MealyAR	HerbyAR	AcridAR	FarmyardAR	SpiceAR
TS Cont Bulls A	33.5	31.7	30.9	22.7abcdef	8.0	4.1	11.9	4.6	6.2	6.5	3.9
TS Cont Bulls P	32.0	26.1	30.1	21.1abcdef	4.1	4.1	9.6	3.4	5.2	3.8	4.2
TS Cont Cows M	29.3	32.0	34.7	18.1a	5.4	6.4	11.2	3.1	4.2	6.1	2.4
TS Cont Cows A	36.8	28.2	30.3	19.5abcd	6.2	5.3	12.7	7.2	5.2	2.6	6.2
TS Cont Cows P	36.4	36.5	32.0	21.8abcdef	4.8	9.8	13.6	3.3	5.8	6.8	7.1
TS Cont Steers M	32.5	33.0	28.9	20.2abcde	6.0	3.3	10.1	4.7	4.8	6.5	2.6
TS Cont Steers A	30.9	36.1	28.3	29.7bcdefgh	7.3	3.8	12.6	3.3	4.7	2.6	3.5
TS Cont Steers P	28.0	32.5	24.0	21.4abcdef	4.7	3.5	12.9	5.0	7.8	3.2	5.3
TS Dairy Bulls M	33.4	38.2	34.4	28.7abcdefgh	6.3	12.7	13.3	5.0	4.3	2.9	5.7
TS Dairy Bulls A	33.0	36.1	32.6	22.9abcdef	9.0	9.8	14.2	5.4	7.5	6.2	5.3
TS Dairy Bulls P	28.3	36.7	38.4	27.3abcdefgh	10.9	13.4	13.9	3.3	6.2	4.6	6.3
TS Dairy Cows M	30.3	36.2	34.6	32.2fghij	7.0	7.3	12.0	5.6	4.8	4.2	7.4
TS Dairy Cows A	27.3	34.9	39.0	27.0abcdefgh	5.1	5.2	11.6	4.2	2.2	6.3	5.1
TS Dairy Cows P	28.3	33.2	26.5	28.1abcdefgh	5.7	3.6	11.6	6.0	5.5	4.3	5.4
TS Dairy Steers M	29.6	40.0	30.2	35.2ghij	8.6	7.3	7.3	3.1	6.1	4.9	3.7
TS Dairy Steers A	34.4	41.1	37.7	29.9cdefgh	7.7	6.8	9.3	3.0	4.7	2.0	5.5
TS Dairy Steers P	29.0	35.7	37.6	46.12k	9.8	8.2	11.4	5.5	8.6	5.8	5.7
avSED	5.11	5.40	4.56	5.80	2.03	3.37	2.94	2.12	2.58	2.48	1.55
P	0.158	0.63	0.239	0.033	0.76	0.199	0.402	0.31	0.943	0.438	0.217

Abbreviation: AT- straight hung, TS- aitch hung, Cont- continental, A- anterior, M- middle, P- posterior, avSED: average standard error, ***P***-probablility

Annex 2.4 Quantitative Descriptive Analysis- flavour significance interactions.

	IntensityFL	GrilStkFL	RstBfFL	BeefyFL	CharGrillFL	MetallicFL	SaltyFL	SourFL	BitterFL	SweetFL	EarthyFL	RancidFL
Breed.Position												
Cont M	46.3	29.9	26.0	32.8	20.2a	11.5	11.1	10.6	4.2	10.8	10.3	3.8
Cont A	45.5	28.5	27.5	31.1	21.2a	10.7	10.4	10.9	3.6	11.3	11.0	2.9
Cont P	45.3	28.6	26.1	33.0	21.4a	11.6	11.1	11.2	4.1	10.6	12.7	2.7
Dairy M	52.1	32.9	29.6	34.6	25.8b	13.2	10.8	10.0	4.0	12.7	11.2	3.1
Dairy A	50.5	33.5	29.1	36.4	28.7b	11.5	10.3	9.5	3.1	13.1	10.7	3.1
Dairy P	53.5	36.1	29.1	35.9	35.4c	11.1	12.2	9.6	4.3	12.7	10.5	2.4
avSED	2.27	2.18	1.80	1.89	2.24	0.98	0.79	0.93	0.86	1.05	1.05	0.75
P	0.459	0.326	0.7	0.39	0.017	0.305	0.381	0.744	0.815	0.968	0.1	0.66
Hang.Breed.Position												
AT Cont M	45.4	30.7	24.3	30.0a	20.6	12.4	12.0	11.2	4.9	11.0	10.8	4.4
AT Cont A	44.3	28.2	24.5	30.6ab	19.5	11.4	10.1	11.3	3.7	12.4	10.3	3.4
AT Cont P	46.4	30.2	27.1	34.7abcd	22.1	12.4	10.9	11.0	3.1	10.8	13.2	3.0
AT Dairy M	51.7	32.9	29.0	34.3abc	25.6	12.8	10.2	10.7	3.5	13.1	11.8	4.1
AT Dairy A	48.8	33.0	27.8	33.0abc	29.0	11.3	9.4	9.2	3.6	13.0	10.2	2.5
AT Dairy P	51.5	37.7	28.7	34.3abc	36.1	11.7	12.6	9.6	4.4	11.7	10.8	2.5
TS Cont M	47.2	29.1	27.7	35.6bcd	19.9	10.6	10.3	9.9	3.6	10.5	9.7	3.1
TS Cont A	46.7	28.7	30.5	31.5ab	23.0	9.9	10.6	10.5	3.6	10.2	11.7	2.4
TS Cont P	44.3	26.9	25.1	31.3ab	20.6	10.8	11.4	11.3	5.0	10.4	12.1	2.4
TS Dairy M	52.5	32.9	30.2	35.0abcd	26.0	13.5	11.5	9.4	4.5	12.3	10.7	2.1
TS Dairy A	52.2	34.0	30.4	39.7d	28.5	11.7	11.2	9.9	2.7	13.1	11.3	3.8
TS Dairy P	55.5	34.5	29.5	37.5cd	34.8	10.6	11.9	9.6	4.3	13.8	10.3	2.3
avSED	3.20	3.09	2.55	2.66	3.16	1.38	1.12	1.32	1.21	1.48	1.48	1.07
P	0.467	0.97	0.445	0.049	0.739	0.78	0.189	0.729	0.196	0.572	0.954	0.358

Abbreviation: AT- straight hung, TS- aitch hung, Cont- continental, A- anterior, M- middle, P- posterior, avSED: average standard error, ***P***- probablility

Annex 2.5 Quantitative Descriptive Analysis- aftertaste significance interactions.

	IntensityAF	RstBfAF	AcidicAF	BitterAF	SaltyAF
Breed.Position					
Cont M	25.3a	15.6	8.0	2.4	7.0
Cont A	25.5a	16.1	7.9	1.9	6.8
Cont P	27.1ab	16.2	7.8	2.4	5.7
Dairy M	33.2c	19.2	8.8	2.8	7.1
Dairy A	29.4b	18.9	6.8	2.7	6.7
Dairy P	29.8b	17.1	7.0	2.4	7.4
avSED	1.67	1.37	0.89	0.55	0.67
P	0.045	0.31	0.251	0.57	0.107

Abbreviation: Cont- continental, A- anterior, M- middle, P- posterior, avSED: average standard error, ***P***- probablility.

Annex 2.6 Consumer Panel- REML Analysis (*Significance Interaction).

	Aroma Liking	Tenderness	Juiciness	Flavour Liking	Overall Liking	CMQ4
Hang.Breed						
AT Cont	57.25a	50.43	49.17	52.85	52.47	51.64
AT Dairy	57.78a	51.88	54.66	56.97	56.11	54.95
TS Cont	57.09a	54.30	50.02	56.06	56.00	54.91
TS Dairy	62.24b	58.98	60.10	62.15	61.96	60.94
avSED	1.579	2.466	2.162	1.724	1.951	1.911
Prob	0.034	0.388	0.129	0.401	0.399	0.310
Breed.Sex						
Cont Bulls	59.92	61.99d	56.83c	59.40c	61.30c	60.49d
Cont Cows	53.63	36.06a	41.82a	46.93a	43.85a	42.23a
Cont Steers	57.95	59.05cd	50.14b	57.03c	57.56c	57.11cd
Dairy Bulls	59.02	52.74c	52.51bc	56.61bc	55.74bc	54.78c
Dairy Cows	56.36	43.86b	50.49b	52.21b	50.76b	49.10b
Dairy Steers	64.64	69.70e	69.14d	69.86d	70.61d	69.96e
avSED	2.071	3.413	2.954	2.200	2.640	2.593
Prob	0.058	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Hang.Position						
AT M	55.91	49.08a	51.16	53.13	52.54	51.54
AT A	58.39	52.16ab	52.41	56.05	55.17	54.25
AT P	58.24	52.23ab	52.17	55.55	55.17	54.10
TS M	58.36	55.48b	55.90	58.16	58.04	57.09
TS A	61.64	60.72c	56.70	61.25	61.71	60.77
TS P	58.99	53.73b	52.59	57.91	57.19	55.91
avSED	1.675	2.209	2.029	1.954	1.894	1.840
Prob	0.545	0.048	0.215	0.507	0.182	0.136
Location.Breed.Sex						
Belfast Cont Bulls	55.96	62.29	57.79fg	60.05	61.59gij	60.96
Belfast Cont Cows	49.40	33.18	40.46a	42.97	40.09a	38.92
Belfast Cont Steers	58.96	62.01	57.77fg	59.59	60.73fgij	60.48
Belfast Dairy Bulls	54.82	49.38	51.14cdef	54.14	51.90cde	51.74
Belfast Dairy Cows	55.00	45.65	51.35def	50.73	50.91bcd	49.32
Belfast Dairy Steers	61.45	66.74	67.00hi	66.42	66.11jk	66.48
Cork Cont Bulls	57.86	59.23	52.65def	56.94	58.91efghij	57.79
Cork Cont Cows	53.96	35.44	42.13ab	47.83	43.87ab	42.36
Cork Cont Steers	55.27	54.51	44.87abcd	54.13	54.02cdefgh	53.29
Cork Dairy Bulls	58.29	49.30	50.07bcdef	54.89	53.96cdefg	52.45
Cork Dairy Cows	55.18	42.35	46.65abcd	49.96	47.63bc	46.65
Cork Dairy Steers	64.29	68.87	68.90i	70.52	71.31kl	70.10
Reading Cont Bulls	65.94	64.44	60.05gh	61.21	63.40ij	62.72
Reading Cont Cows	57.54	39.56	42.88abc	50.00	47.59bc	45.43
Reading Cont Steers	59.63	60.62	47.77abcde	57.38	57.93defghi	57.55

Reading Dairy Bulls	63.96	59.53	56.31fg	60.81	61.37fhij	60.14
Reading Dairy Cows	58.91	43.58	53.47efg	55.95	53.73cdef	51.32
Reading Dairy Steers	68.17	73.49	71.51i	72.64	74.41l	73.32
avSED	3.061	4.357	3.913	3.480	3.596	3.508
Prob	0.299	0.073	0.027	0.244	0.029	0.054
Location.Breed.Position						
Belfast Cont M	49.87a	48.91	50.00	50.85ab	49.95ab	49.91ab
Belfast Cont A	58.48bcd	54.80	53.32	55.79abcde	56.02bcde	55.32bcd
Belfast Cont P	55.97abc	53.76	52.70	55.95abcde	56.44bcdef	55.12bcd
Belfast Dairy M	55.72abc	49.59	56.91	54.67abcd	53.11abc	52.90abc
Belfast Dairy A	60.47cde	62.43	60.35	63.61f	63.22efg	62.81e
Belfast Dairy P	55.09abc	49.76	52.23	53.01abc	52.59abc	51.83abc
Cork Cont M	55.33abc	51.68	48.58	52.02abc	52.47abc	51.71abc
Cork Cont A	59.16cd	53.48	51.06	57.47bcdef	55.86bcd	55.15bcd
Cork Cont P	52.59ab	44.03	40.01	49.42a	48.47a	46.57a
Cork Dairy M	60.85cde	52.38	57.65	58.67cdef	58.57cdefg	56.65bcde
Cork Dairy A	56.20abc	51.64	53.58	56.18abcde	55.43abcd	54.33bcd
Cork Dairy P	60.71cde	56.50	54.38	60.51def	58.90cdefg	58.21cde
Reading Cont M	60.00cde	55.23	50.73	55.70abcde	56.51bcdef	55.31bcd
Reading Cont A	59.74cd	55.45	47.98	55.29abcde	55.36bcd	54.63bcd
Reading Cont P	63.38de	53.94	52.00	57.60bcdef	57.04cdef	55.78bcde
Reading Dairy M	61.04cde	55.87	57.31	61.97ef	61.13defg	59.42de
Reading Dairy A	66.04e	60.84	61.01	63.56f	64.75g	62.85e
Reading Dairy P	63.96de	59.89	62.96	63.87f	63.63fg	62.51e
avSED	3.014	4.210	3.801	3.452	3.506	3.417
P	0.027	0.053	0.068	0.025	0.032	0.020

(Abbreviation: AT- straight hung, TL- aitch hung, A- anterior, M- middle, P- posterior, avSED: average standard error, ***P*** - probability)

Annex 2.7 Chi-square analysis on socio-demographic and consumers' habit.

		Cluster Group (CG)			
		CG1 n= 121	**CG2 n= 85**	**CG3 n= 96**	**CG3 n= 58**
Age Group	18- 24	25	16	16	6
	25- 34	13	24	15	11
	35- 44	16	13	14	7
	45- 54	21	10	19	12
	55- 64	27	15	21	10
	65+	19	7	11	12
	χ^2	19.68			
	P	0.184			
Gender	Female	62	38	59	24
	Male	59	47	37	34
	χ^2	7.7			
	P	0.053			
Income	Below £25000	27	23	24	16
	£25000-£50000	59	35	42	24
	£50000-£75000	23	18	17	13
	Above £75000	12	9	13	5
	χ^2	3.04			
	P	0.963			
	Blue/Rare	7	7	10	9

		Cluster Group (CG)			
		CG1 n= 121	**CG2 n= 85**	**CG3 n= 96**	**CG3 n= 58**
Doneness	Medium rare	25	19	27	16
	Medium	35	28	19	15
	Medium well	30	18	22	10
	Well Done	23	13	17	8
	χ^2	10.97			
	P	0.532			
Number of children	Yes	37	24	26	17
	No	84	60	69	41
	χ2	0.28			
	P	0.964			
Number of adults	Less than 2	79	64	60	37
	More than 2	42	21	33	21
	χ2	3.34			
	P	0.342			
Important attributes	Tenderness	48	34	37	27
	Juiciness	7	7	3	4
	Flavour	66	43	56	27
	χ2	3.9			
	P	0.691			
Frequency of consumption	I enjoy red meat, it's an important part of my diet.	50	31	39	29
	I like red meat well enough, it's a regular part of my diet.	54	40	41	21
	I do eat some red meat although truthfully it wouldn't worry me if I didn't or I rarely/ never eat red meat	17	14	16	8
	χ2	2.96			
	P	0.814			
Purchase habit	Supermarket	75	55	62	34
	Other	46	29	34	24
	χ2	0.85			
	P	0.836			
Importance level on motivation of beef choice					
It is good value.	Not/ Little important	8	5	8	4
	Moderately important	56	41	42	33
	Very Important	56	39	45	20
	χ2	3.38			
	P	0.759			
It has a good flavour.	Not/ Little important	3	0	3	0
	Moderately important	21	17	20	9
	Very Important	96	67	72	49
	χ2	5.24			
	P	0.513			
It has good tenderness.	Not/ Little important	5	2	5	0
	Moderately important	25	25	26	11
	Very Important	89	57	64	46
	χ2	7.11			
	P	0.311			
It looks good.	Not/ Little important	12	9	10	5
	Moderately important	43	31	29	26
	Very Important	63	45	56	26
	χ2	3.56			
	P	0.735			

		Cluster Group (CG)			
		CG1 n= 121	CG2 n= 85	CG3 n= 96	CG3 n= 58
I know how to cook it.	Not/ Little important	23	21	12	12
	Moderately important	47	37	39	23
	Very Important	49	26	44	22
	$\chi 2$	6.73			
	P	0.374			
It is easy to prepare.	Not/ Little important	26	22	24	17
	Moderately important	59	43	43	26
	Very Important	34	19	28	14
	$\chi 2$	2.55			
	P	0.863			
I enjoy cooking it.	Not/ Little important	20	25	25	12
	Moderately important	65	34	41	23
	Very Important	34	26	29	21
	$\chi 2$	8.06			
	P	0.243			
It is a healthy choice.	Not/ Little important	18	23	20	17
	Moderately important	55	40	44	20
	Very Important	46	21	29	20
	$\chi 2$	9.72			
	P	0.137			
I enjoyed it last time.	Not/ Little important	11	9	11	7
	Moderately important	48	33	30	27
	Very Important	60	41	53	23
	$\chi 2$	4.58			
	P	0.599			
Animal well cared for.	Not/ Little important	19	20	25	12
	Moderately important	47	32	36	29
	Very Important	53	32	33	16
	$\chi 2$	7.85			
	P	0.249			
Environmentally friendly.	Not/ Little important	25	25	31	17
	Moderately important	54	36	39	22
	Very Important	40	22	24	17
	$\chi 2$	5.01			
	P	0.542			
I know where it comes from.	Not/ Little important	15	13	17	9
	Moderately important	53	41	38	23
	Very Important	51	31	40	25
	$\chi 2$	2.52			
	P	0.867			
Consumption frequency for different muscles					
Brisket	Never	75	43	68	34
	<2/ month	42	35	23	21
	≥2/ month	3	5	3	0
	$\chi 2$	11.48			
	P	0.075			
Casserole steak	Never	30	26	23	23
	<2/ month	60	41	49	22
	≥2/ month	28	16	23	12
	$\chi 2$	6.09			
	P	0.413			
Fillet	Never	21	9	15	12
	<2/ month	80	55	58	33
	≥2/ month	19	19	23	13
	$\chi 2$	5.33			

		Cluster Group (CG)			
		CG1 n= 121	**CG2 n= 85**	**CG3 n= 96**	**CG3 n= 58**
	P	0.503			
Frying steak	Never	31	14	26	19
	<2/ month	58	47	44	26
	≥2/ month	30	22	24	12
	$\chi 2$	5.6			
	P	0.469			
Mince	Never	9	7	15	15
	<2/ month	28	16	24	8
	≥2/ month	82	60	57	34
	$\chi 2$	16.45			
	P	0.012			
Lean mince	Never	12	10	14	7
	<2/ month	27	22	25	17
	≥2/ month	80	51	57	33
	$\chi 2$	2.5			
	P	0.869			
Rib eye	Never	44	21	40	20
	<2/ month	63	52	40	31
	≥2/ month	10	10	16	6
	$\chi 2$	10.49			
	P	0.106			
Rump	Never	39	26	38	29
	<2/ month	67	50	47	22
	≥2/ month	12	8	10	5
	$\chi 2$	8.13			
	P	0.229			
Silverside	Never	53	27	53	27
	<2/ month	51	45	36	20
	≥2/ month	14	11	7	10
	$\chi 2$	12.45			
	P	0.053			
Sirloin	Never	13	7	10	3
	<2/ month	81	59	51	38
	≥2/ month	25	18	29	16
	$\chi 2$	6.46			
	P	0.374			
Topside	Never	50	34	48	26
	<2/ month	59	43	41	27
	≥2/ month	9	6	6	4
	$\chi 2$	2.08			
	P	0.913			

P: Probability

Annex 3.1 Interaction of muscle by treatment for weight analysis.

	Weight Added (kg)			
Muscle/ Treatment	RMP005	RMP131	RMP231	STR045
F+T	0.098cdef	0.064abc	0.090abcde	0.053a
F10	0.132efg	0.099def	0.129efg	0.057ab
K+T	0.102def	0.084abcd	0.097bcdef	0.058ab
K10	0.135fg	0.117ef	0.170gh	0.074abcd
P10	0.234i	0.209i	0.211hi	0.073abcd
avSED	0.0195			
P	<0.001			
	Weight Added (%)			
Muscle/ Treatment	RMP005	RMP131	RMP231	STR045
F+T	0.102abcd	0.083a	0.108abcde	0.082a
F10	0.136bcdefg	0.133cdefg	0.146defgh	0.090abc
K+T	0.122abcdefg	0.135defg	0.120abcdefg	0.085ab
K10	0.161efgh	0.156egh	0.200hi	0.117abcdefg
P10	0.273jk	0.277k	0.227ij	0.111abcdef
avSED	0.0261			
P	<0.001			

a,b..g: Different superscripts within a factor represent significant differences. Beef muscles, STR045, RMP005, RMP131 and RMP231 are prepared according to section 3.2.1 while treatment information can be referred to Table 3.1. Abbreviation: avSED: average standard error, ***P***: probability.

Annex 3.2 Interaction of muscle by treatment for consumers' flavour liking score.

	RMP005	RMP131	RMP231	STR045
CON	58.93b	44.56a	45.79a	48.80a
F+T	79.99hj	67.31cde	71.42cdefghij	69.53cdef
F10	68.68cdef	72.78efghij	70.93cdefgh	71.06defghi
K+T	77.08ghij	64.90bc	75.83fghij	66.80cd
K10	75.16fghij	71.22defghij	69.97cdefg	69.53cdef
P10	76.40fghij	72.06defghij	72.72defghij	72.04efghij
avSED	3.772			
P	0.043			

a,b..j: Different superscripts within a factor represent significant differences. Beef muscles, STR045, RMP005, RMP131 and RMP231 are prepared according to section 3.2.1 while treatment information can be referred to Table 3.1. Abbreviation: avSED: average standard error, ***P***: probability.

Annex 3.3 Interaction of muscle by sex for consumers' sensory scores.

	TE	JU	FL	OL	Satisfactory
RMP005 F	75.35cd	77.88e	72.82c	74.33e	4.02c
RMP005 M	78.28d	77.43e	72.59c	74.08de	4.06c
RMP131 F	61.82a	64.46ab	63.94a	62.85a	3.47a
RMP131 M	66.29ab	66.86abcd	67.01ab	66.49abc	3.64ab
RMP231 F	68.72bc	72.91de	69.97bc	70.49cde	3.79bc
RMP231 M	65.94ab	65.63abc	65.59ab	65.67abc	3.66ab
STR045 F	69.92bc	69.06acd	67.98abc	68.26bcd	3.72b
STR045 M	64.53ab	64.17a	64.61ab	64.26ab	3.56ab
avSED	3.195	2.655	2.467	2.650	0.117
P	0.002	0.003	0.040	0.012	0.016

a,b..e: Different superscripts within a factor represent significant differences. Beef muscles, STR045, RMP005, RMP131 and RMP231 are prepared according to section 3.2.1 while treatment information can be referred to Table 3.1. Abbreviation: avSED: average standard error, ***P***: probability.

Annex 3.4 Interaction of treatment by muscle by sex for consumers' juiciness score.

	CON	F+T	F10	K+T	K10	P10
RMP005 F	62.52def	82.94ijklmnopqrstuvwxyz AB	73.00fghijklmnopqrstuvw	85.40ntwyB	77.02hijklmnopqrstuvwxyz AB	86.40ntuwyzAB
RMP005 M	69.48efghijkl	83.43ntuwxyAB	77.06jklmnopqrstuvwxyz AB	72.48fghijklmnopqrst	82.16nqstuvwxyzAB	79.98lnpqrstuvwxyzAB
RMP131 F	36.36a	64.67defgh	71.43fghijklmnopq	66.48defghij	74.05hijklmnopqrstuvwxyz A	73.81fghijklmnopqrstuvwxy
RMP131 M	48.67bc	69.67fghijklm	68.98efghijk	67.56efghij	72.23fhijklmnopqr	74.02hjklmnopqrstuvwxyz
RMP231 F	58.08bcde	70.37defghijklmn	79.0jklmnopqrstuvwxyz AB	81.07klmnopqrstuvwxyz AB	72.50fghijklmnopqrstu	76.44ijklmnopqrstuvwxyz AB
RMP231 M	35.32a	70.83fghijklmnop	68.60efghijk	70.73fghijklmnop	76.59hjklmnopqrstuvwxyz AB	71.69fghijklmnopq
STR045 F	57.23cd	72.78fghijklmnopqrstuv	72.32fghijklmnopqrs	67.65efghij	73.53hijklmnopqrstuvwx	70.85fghijklmnop
STR045 M	47.40b	66.03efghi	70.37fghijklmno	64.37defg	68.89efghijk	67.98efghij
avSED	5.5926					
P	0.035					

a,b..B: Different superscripts within a factor represent significant differences. Beef muscles, STR045, RMP005, RMP131 and RMP231 are prepared according to section 3.2.1 while treatment information can be referred to Table 3.1. Abbreviation: avSED: average standard error, ***P***: probability.

Annex 3.5 Interaction of treatment by muscle for volatile analysis in Trial 2.

	2-Methyl butanal	Pentanal	Heptane	Hexanal	Heptanal	Octanal
CON RMP005	116.2abcdefghi	0.80a	1.41ab	9.0ab	6.02ab	0.185a
CON RMP131	113.4abcdefghi	3.61abcd	3.09ab	7.8ab	6.60abc	0.053a
CON RMP231	118.3abcdefghi	1.28a	6.05abcd	4.8a	5.03ab	0.293a
CON STR045	80.3abcde	1.92a	2.45ab	2.5a	2.42a	0.016a
F+T RMP005	188.9cegi	10.89bdefghi	19.56fi	60.5abcdefgh	30.92bcfhijk	0.439a
F+T RMP131	88.3abcdefgh	3.46abc	6.14abcde	17.2abcd	9.44abcdefg	0.051a
F+T RMP231	82.6abcdefg	16.99ghi	16.84dfghi	94.0fghij	73.14l	4.754b
F+T STR045	115.8abcdefghi	2.46a	5.35abc	14.7abcd	6.88abcd	0.375a
F10 RMP005	75.2abcd	13.07fghi	13.53cdefghi	76.9efgh	30.41cghijk	0.311a
F10 RMP131	117.2abcdefghi	5.33abcde	8.69abcdefgh	42.9abcdef	13.18abcdefghi	0.190a
F10 RMP231	98.5abcdefghi	12.16fghi	18.80fi	96.4ghij	34.04hjk	0.564a
F10 STR045	155.9bcdefghi	3.61abcd	3.67ab	24.2abcde	3.94a	0.090a
K+T RMP005	47.1a	5.82abcdef	9.89abcdefghi	53.3abcdefgh	23.54abcdefghijk	0.588a
K+T RMP131	166.8bcegi	9.03cdefgh	15.42fhi	72.3fgh	24.99bcdfghijk	0.417a
K+T RMP231	80.8abcdef	6.80abcdef	16.83fhi	35.5abcdef	18.27abcdefghijk	0.435a
K+T STR045	132.8abcdefghi	2.30a	6.00abc	12.4abc	7.58abcde	0.144a
K10 RMP005	72.9abc	11.55cdefghi	14.76cdefghi	70.1bdefgh	32.26cghijk	0.260a
K10 RMP131	160.8bcefghi	10.71efgh	8.28abcdefg	87.8gh	18.24abcdefghij	0.376a
K10 RMP231	168.3abcdefghi	20.24i	30.93j	175.9ik	43.88kl	0.716a
K10 STR045	126.8abcdefghi	3.12ab	4.22ab	18.1abcd	7.76abcdef	0.183a
P10 RMP005	117.6abcdefghi	15.97fhi	10.15bcdefghi	89.9defghi	20.91abcdefghijk	0.056a
P10 RMP131	200.2cefghi	14.46efhi	8.19bcdef	102.2fhijk	29.16abcdefghijk	0.386a
P10 RMP231	130.1abcdefghi	6.44abcdef	13.65cdefghi	57.0abcdefgh	9.86abcdefgh	0.000a
P10 STR045	55.8ab	7.42abcdefg	-0.90a	48.3abcdefg	9.81abcdefgh	0.026a
avSED	51.97	4.209	4.866	30.47	12.63	0.767
P	0.035	0.045	0.021	0.029	0.011	0.004

a,b..k: Different superscripts within a factor represent significant differences. Beef muscles, STR045, RMP005, RMP131 and RMP231 are prepared according to section 3.2.1 while treatment information can be referred to Table 3.1. Abbreviation: avSED: average standard error, ***P***: probability.

Annex 3.6 Interaction of treatment by sex for α-pinene in Trial 2.

Treatment/Sex	F	M
CON	4.656e	0.435abc
F+T	0.008ab	1.015bcd
F10	0.578abcd	0.652bcd
K+T	0.806bcd	0.589bcd
K10	1.100bcd	0.983bcd
P10	-1.443a	1.168bd
avSED	0.573	
P	P<0.001	

a,b..k: Different superscripts within a factor represent significant differences. Treatment information can be referred to Table 3.1. Abbreviation: avSED: average standard error, ***P***: probability.

Annex 5.1 Interactions of muscle by packaging by ageing on consumers' sensory scores.

	TE	OL	MQ4	Satisfactory
RMP131 A14	45.94b	48.92b	48.55b	3.014b
RMP131 A21	38.49a	41.59a	42.12a	2.754a
RMP131 A49	48.91b	48.33b	48.78b	2.974b
STR045 A14	50.27b	54.43c	53.54c	3.142bc
STR045 A21	59.50c	57.98c	57.75c	3.321c
STR045 A49	58.19c	56.30c	56.63c	3.247c
avSED	2.961	2.613	2.522	0.0971
P	P<0.001	0.014	0.014	0.006

a-c: Mean scores with different superscripts within each column for each factor are significantly different. Abbreviation: TE: tenderness, OL: overall liking, STR045: *longissimus lumborum*, RMP131: *gluteus medius*, avSED: average standard error, ***P***: probability.

Annex 5.2 Interactions of muscle by packaging by ageing on volatile compounds.

	3-Methylbutanal	Nonane	2-Ethyl-1-hexanol	1-Octanol
RMP131 MAP A14	18.89d	0.738ab	0.90a	0.286abcde
RMP131 MAP A21	5.17abc	0.958c	7.93abc	0.256abcde
RMP131 MAP A49	4.33abc	0.651a	0.94a	1.139f
RMP131 OWP A14	2.58a	0.665a	16.64de	0.236abcde
RMP131 OWP A21	2.48a	0.778abc	6.62abc	0.463bcde
RMP131 OWP A49	5.17abc	0.734ab	10.81cde	0.360abcde
RMP131 VSP A14	2.08a	0.749abc	0.90a	0.235abcd
RMP131 VSP A21	2.83a	0.880bc	1.10a	0.312abcde
RMP131 VSP A49	4.01abc	0.816abc	3.79abc	0.488cde
STR045 MAP A14	7.17bc	0.691ab	5.41abc	0.229abcd
STR045 MAP A21	7.33c	0.745abc	3.02abc	0.203abc
STR045 MAP A49	4.79abc	0.889bc	4.14abc	0.533de
STR045 OWP A14	2.32a	0.726ab	17.97e	0.182abc
STR045 OWP A21	3.29ab	0.794abc	31.22f	0.179ab
STR045 OWP A49	4.71abc	0.746abc	10.01bcd	0.544e
STR045 VSP A14	2.29a	0.879bc	4.51abc	0.134a
STR045 VSP A21	3.67abc	0.772abc	2.70ab	0.361abcde
STR045 VSP A49	3.91abc	0.664a	0.80a	0.165ab
avSED	1.98	0.105	3.943	0.156
P	0.002	0.049	P<0.001	0.012

a-e: Mean scores with different superscripts within each column for each factor are significantly different. Abbreviation: MAP: Modified air packaging, OWP: overwrapped packaging, VSP: vacuum skin packaging, STR045: *longissimus lumborum*, RMP131: *gluteus medius* avSED: average standard error, ***P***: probability.

Annex 5.3 Interactions of muscle by packaging on volatile compounds.

	Dimethyl sulfide	2-Butyl furan	2,3-Octanedione	2-Ethyl-1-hexanol
RMP131 MAP	1.000b	0.0367ab	4.582d	3.26a
RMP131 OWP	1.161b	0.0187a	0.902ab	11.36b
RMP131 VSP	1.581c	0.0329a	2.223bc	1.93a
STR045 MAP	0.459a	0.0286a	2.117abc	4.19a
STR045 OWP	0.365a	0.0594b	0.484a	19.73c
STR045 VSP	0.523a	0.0348a	2.969cd	2.67a
avSED	0.132	0.0115	0.844	2.277
P	0.024	0.008	0.036	0.032

a-d: Mean scores with different superscripts within each column for each factor are significantly different. Abbreviation: MAP: Modified air packaging, OWP: overwrapped packaging, VSP: vacuum skin packaging, STR045: *longissimus lumborum*, RMP131: *gluteus medius*, avSED: average standard error, ***P***: probability.

Annex 5.4 Interactions of muscle by ageing on volatile compounds.

	3-Methylbutanal	3-Hydroxy-2-butanone	2,4-Pentadione	Methyl hexanoate	Methyl octanoate
RMP131 A14	7.848b	32.82c	23.35c	2.464a	0.534a
RMP131 A21	3.493a	28.73c	16.12b	6.545b	1.290b
RMP131 A49	4.504a	16.94b	6.56a	5.787b	0.836ab
STR045 A14	3.925a	13.29ab	5.51a	1.699a	0.744ab
STR045 A21	4.763a	13.16ab	6.99a	0.796a	0.255a
STR045 A49	4.472a	9.57a	2.45a	2.951a	0.821ab
avSED	1.143	2.456	3.397	1.314	0.325
P	0.006	0.002	0.018	0.025	0.014

a-c: Mean scores with different superscripts within each column for each factor are significantly different. Abbreviation: STR045: *longissimus lumborum*, RMP131: *gluteus medius*, avSED: average standard error, ***P***: probability.

Annex 5.5 Interactions of packaging by ageing on volatile compounds.

	2,3-Butanedione	3-Methylbutanal	1-Octanol
MAP A14	6.079c	13.03c	0.258ab
MAP A21	5.214bc	6.25b	0.229ab
MAP A49	5.061bc	4.56ab	0.836c
OWP A14	4.698b	2.45a	0.209a
OWP A21	4.964bc	2.88a	0.321ab
OWP A49	1.928a	4.94ab	0.452b
VSP A14	4.645b	2.18a	0.185a
VSP A21	4.493b	3.25a	0.336ab
VSP A49	3.052a	3.96ab	0.327ab
avSED	0.638	1.4	0.11
P	0.047	P<0.001	0.003

a-c: Mean scores with different superscripts within each column for each factor are significantly different. Abbreviation: MAP: Modified air packaging, OWP: overwrapped packaging, VSP: vacuum skin packaging, avSED: average standard error, ***P***: probability.