Evaluation of pre-rigor proteases injections on cooked beef volatiles at 1 day and 21 days post-mortem

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Abstract

The fact that tenderness plays a major role in consumer acceptance of meat has been known for many years. After appearance and tenderness, flavour is another important component influencing meat palatability. Although proteases are widely used in the meat industry to tenderize meat, they can also contribute to the formation of amino acids that act as precursors for volatile flavour formation in cooked meat. This research was carried out to determine the effects of pre-rigor injection of beef with nine proteases from plant and microbial sources, after 1 day and 21 days post-mortem storage, on the volatile profile of cooked beef using solid phase microextraction (SPME) in combination with gas chromatography (GC) and mass spectrometry (MS) analysis. The topside of beef was injected with papain (PA), bromelain (BA), actinidin (Ac), zingibain (ZI), Fungal 31 protease (F31), Fungal 60 protease (F60), bacterial protease (BA), kiwi fruit juice (KJ), and *Asparagus* protease (ASP). A non-injected control (C) treatment was also included. In this study, a total of 56 key volatile compounds were found in cooked pre-rigor beef meat injected with proteases at 1 day and 21 days post mortem storage. This included 23 aldehydes, 5 ketones, 3 furans, 8 nitrogen and sulphur compounds, 4 alkanes, 7 alcohols and 6 terpenes. Eleven volatile compounds including camphene, 1,8-cineole, terpineol, citronellol, citral, geraniol, geranial, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene, were only detected in meat treated with ZI at 1 day and 21 days post-mortem storage. 3-methylbutanal and benzaldehyde were significantly increased ($p<0.05$) in the KJ 21 days treated sample. Aldehydes were the main chemical compounds that significantly changed with protease treatments and post mortem storage. Benzaldehyde was significantly increased ($p<0.05$) only in F31 and ASP treated samples from 1 day to 21 days post-mortem storage. A significant increase ($p<0.05$) in 3-methylbutanal was observed in KJ, BA, BR and F31 treated samples at 21 days post-mortem storage. Treatments with BR, PA, ASP, AC, and KJ (except KJ 21 days) proteases underwent fewer changes in the volatile compounds leading to a flavour profile closer to that of the control beef sample.

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Abbreviations

AC Actinidin

ASP *asparagus*

ATP adenosine triphosphate

BA bacterial

Br bromelain

C control

DHE dynamic headspace

DVB/C AR/PDMS divinylbenzene-carboxen-polydimethylsiloxane

GRAS generally recognized as safe

FID flame ionization detector

FSIS food safety inspection service

F31 fungal 31

F60 fungal 60

GC gas chromatography

HS-SPME headspace solid phase microextraction

kg kilogram

KI kiwi juice

LD liquid desorption

Min minute

ML millilitre

MS mass spectrophotometer

No number

PAHs polycyclic aromatic hydrocarbons

PA papain

PCA principal component analysis

PM post-mortem

RI retention index

RT retention time

S second

SBSE Stir Bar Sorptive Extraction

SDE simultaneous distillation–extraction

SIFT-MS Selected ion flow tube mass spectrometry

TD thermal desorption

USDA United States Department of Agriculture

µL microliter

V/W volume/weight

WHC water holding capacity

Wt weight

W/W weight/ weight

ZI zingibain

Chapter 1 Introduction and Objectives

1.1 Introduction

The fact that tenderness plays a major role in consumer acceptance of meat has been known for many years. Boleman et al (1997) reported that high quality meat of consistent tenderness can increase consumer confidence and encourage further purchase of red meat products. A beef quality survey carried out by Robbins et al. (2003) reported that tenderness, flavour and juiciness were the most important factors influencing consumer's eating satisfaction of beef. After appearance and tenderness, flavour is another important component influencing meat palatability. A large multiple-city study found flavour to be the most important factor that influenced consumer's meat buying habits and preferences when tenderness was held constant (Sitz, Calkins, Feuz, Umberger, & Eskridge, 2005). Meat flavour is affected by a variety of factors, such as animal species, breed, sex, age, nutrition, and processing conditions. A number of papers have reported the effects of diet and breed, on the volatile profile (Elmore et al., 2005; Elmore, Mottram, Enser, & Wood, 2000) and volatile branched-chain fatty acids (Priolo et al., 2004) of beef meat. The volatile compounds generated from a complex series of heat-induced reactions during cooking, such as lipid oxidations, Maillard browning reactions and thiamine degradation, are responsible for the aroma attributes and characteristic flavours of cooked meat (Mottram, 1998).

Enzymes have been used for centuries to treat meat to make it easier to chew and swallow. The effect of some of these enzymes on the tenderness of meat is well established with the use of several plant proteases, tenderizing solutions and ions. The relative effects of an *Aspergillus oryzae*-expressed aspartic protease and papain on meat proteins and beef tenderness have been reported by Ashie, Sorensen, & Nielsen (2002). Recently papain, ficin, bromelain, homogenized fresh ginger, *Bacillus subtilis* protease, and two *Aspergillus oryzae* proteases have been applied to beef muscle of high and low-connective tissue to determine the extent of tenderization using physical and sensory measurements (Ashie et al, 2002; Sullivan & Calkins, 2010). Murphy & Zerby (2004) reported the use of sodium chloride, phosphate and dextrose solutions to improve

tenderness of pre-rigor beef. Polidori et al (2000) investigated the tenderization of wether lamb meat through pre-rigor infusion of calcium ions. In addition, ginger extract (Naveena & Mendiratta, 2004; Naveena, Mendiratta, & Anjaneyulu, 2004) and kiwifruit juice (Han, Morton, Bekhit, & Sedcole, 2009) have also been used to tenderize meat.

Various techniques have been used to evaluate the volatile compounds in meat using dynamic headspace (DHE), simultaneous distillation–extraction (SDE) and solid-phase microextraction (SPME). Of these three methods, SPME is gaining interest as it is simple, low-cost, solvent-free, and is a relatively fast and sensitive technique for the analysis of volatile compounds with a wide boiling point range without artefact formation (Reineccius, 2007). SPME involves the extraction of volatile compounds out of liquid samples or out of the headspace of solid or liquid samples onto a fused-silica fibre coated with a polymeric phase. Hence the selective phase of fibre and SPME extraction conditions can affect the sensitivity and accuracy of SPME analysis. Headspace SPME has been used in the extraction of volatile compounds from pork (Olivares, Navarro, & Flores, 2011; Ruiz, Cava, Ventanas, & Jensen, 1998); Park, Yoon, Schilling, and Chin, (2009), beef (Bhattacharjee et al., 2011; Machiels & Istasse, 2003; Van Ba, Oliveros, Ryu, & Hwang, 2010), chicken (Goodridge, Beaudry, Pestka, & Smith, 2003), lamb (Almela et al., 2010) and goat (Madruga, Stephe, Dodson, & Mottram, 2009).

1.2 Objectives

Although proteases are widely used in the meat industry to tenderize meat muscle, they can also contribute to the formation of amino acids that can act as precursors in the Maillard reaction and the Strecker degradation. This will result in nonenzymatic browning and production of volatile flavour compounds in cooked meat. As the cooked flavour profile of meat tenderized by proteases has up till now not been reported, this research was carried out to determine the effect of pre-rigor injection of beef with nine proteases from plant and microbial sources, after 1 day and 21days post-mortem storage, on the volatile profile of cooked beef using solid phase microextraction (SPME) in combination with gas chromatography (GC) and mass spectrometry (MS) analysis.

Chapter 2 Literature Review

In this chapter, pre-rigor meat and its benefits will be explained firstly (2.1). The muscle structure and composition of meat will then be described briefly (2.2). Recent research on the use of tenderizing enzymes will then be summarised (2.3). In addition, the volatile compounds from cooked meat (2.4), and the different methods of meat volatile extraction and analysis from previous researches (2.5) will be reviewed.

2.1 Pre-rigor

Meat that is removed from the carcass early post-mortem when it is still physiologically active (responds to electrical stimulation; has high pH and available energy) and has not entered the onset of rigor is termed pre-rigor (Claus & Sørheim, 2006).

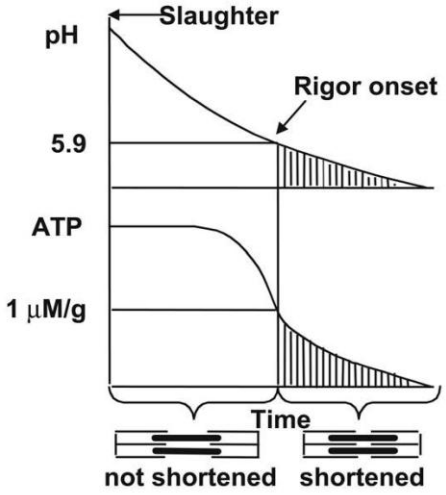

Figure 2-1 Adenosine triphosphate (ATP) and pH decline during rigor mortis development (Alan, 2000)

Adenosine triphosphate (ATP) decreases once an animal dies and the production of lactic acid increases. As shown in Figure 2-1, cell pH decreases from near neutrality to a more acidic pH of about 5.7 (Alan, 2000). This results in the activity of some of the ATP-producing enzymes to decline and further reduces the production of ATP. The muscle cell's ATP concentration also falls below 1μM/g in rigor mortis, and is no longer responsive to nervous or other stimulus. As a result, meat tenderness and juiciness are affected because of the contraction of muscles and decreased water holding capacity.

Compared to post-rigor meat, pre-rigor meat has been shown to have higher emulsifying capacity and greater extractability of salt soluble proteins (myosin, actin, tropomyosin) (Dzudie, Okubanjo, & Sidonie Béatrice, 2000). Ample evidence also showed that changes in the myofibrillar component pre-rigor can markedly influence the tenderness of meat (Newbold & Harris, 1972). Karakaya, et al (2006) concluded that the pre-rigor stage of mutton and goat meat had the most desirable characteristics for emulsion-type meat products. In addition, pre-rigor meat was reported to have improved water-holding capacity and texture in emulsion-type sausages (Wang, Xu, & Zhou, 2009). Therefore, the use of pre-rigor meat can be advantageous for red meat processing. The tenderness of meat is however not entirely due to conditions prevailing during the period between slaughter and the full development of rigor mortis It can also be influenced by meat processing.

2.2 Meat muscle structure related to tenderness

Meat eating qualities including tenderness, juiciness and flavour are considered the most important meat palatability traits by consumers (Lawrie & Ledward, 2006; Warriss, 2000). Although tenderness is considered the most important trait, and consumers are willing to pay more for guaranteed tenderness, up to 20% of steaks sold to consumers are tough (Miller, 2002). Meat tenderness is determined by the amount and solubility of connective tissue, sarcomere shortening during rigor development, and post-mortem proteolysis of myofibrillar and myofibrillar-associated proteins (Koohmaraie & Geesink, 2006; Troy & Kerry, 2010). Myofibrillar (salt-soluble) and connective tissue (fibrous and insoluble) proteins are located intracellularly and extracellularly, respectively (Aberle, Forrest, Gerrard, & Mills, 2001).

The tenderness of meat varies widely among species of animals and among different muscles held for different times post-mortem. Collagen is the most abundant protein in the animal body and has a significant influence on meat tenderness. The relative proportion of connective tissues and muscle fibres differ, and as such contributes to the relative differences in meat tenderness (Kandeepan, Anjaneyulu, Kondaiah, Mendiratta, & Lakshmanan, 2009). There is general agreement that proteolysis of myofibrillar proteins, accelerated by the Calpain’s proteolytic system, is the major contributor to tenderization of beef

during post-mortem storage (Bowker, Fahrenholz, Paroczay, Eastridge, & Solomon, 2008; Huang, Huang, Xue, Xu, & Zhou, 2011). Previous research reported that activation of μ-calpain and m-calpain was responsible for post-mortem proteolysis and tenderization (Polidori et al., 2000). In addition, l-calpain plays an important role in meat tenderization by weakening the structural integrity of the myofibrillar proteins (Geesink, Kuchay, Chishti, & Koohmaraie, 2006; Koohmaraie & Geesink, 2006).

Geesink, et al (2011) suggested that additional intervention methods, such as tenderizing enzymes are needed to improve the tenderness of pre-rigor cooked meat. Exogenous enzyme treatments have been shown to increase tenderness via myofibrillar and collagen protein degradation with no difference among high and low-connective tissue muscles in beef (Sullivan & Calkins, 2010).

2.3 Tenderizing meat

Consumers are willing to pay a premium for increased tenderness (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). Hence it is important to develop safe tenderization methods to improve meat tenderness and consistency efficiently and economically for the meat industry. Tenderization of meat can be carried out chemically or physically. The technologies used to improve meat tenderness include electrical stimulation (Hope-Jones, Strydom, Frylinck, & Webb, 2010; Hopkins & Thompson, 2002), post-mortem ageing (Jayasooriya, Torley, D'Arcy, & Bhandari, 2007), mechanical tenderization (Anna et al., 2007; Bowker et al., 2007), ionic chemical solution (Hunt et al., 2003) and injection of plant enzymes (Ashie et al., 2002; Wada, Suzuki, Yaguti, & Hasegawa, 2002).

Treatment by proteolytic enzymes is the most popular method for meat tenderization. Three common methods of introducing the proteolytic enzymes into meat cuts post-mortem, include dipping the meat in a solution containing proteolytic enzymes, pumping enzyme solution into major blood vessels of the meat cut, and the rehydration of freeze-dried meat in a solution containing a proteolytic enzyme (Gerelt, Ikeuchi, & Suzuki, 2000). The first two methods are somewhat unsatisfactory, since they over-tenderize the surface and produce a mushy texture. As the enzymes are unable to penetrate within the meat, the interior is left unaffected (Lawrie & Ledward, 2006). The rehydration of the freeze-dried meat showed a much better distribution of enzymes than dipping or

perfusion. However this is not ideal, and requires the setting up of a freeze-dryer. Instead of introducing enzymes into meat post-mortem, pre-slaughter injection of the enzymes into live animals have been carried out (Beuk, Hinsdale, Goeser, & Hogan, 1959) and has proved to be the most effective method of introducing the enzymes into meat (Christensen et al., 2009; Gao et al., 2011; Liu, Xiong, & Rentfrow, 2011).

Five exogenous proteases that have been classified as 'Generally Recognized as Safe' (GRAS) by USDA's Food Safety Inspection Service (FSIS) (Payne, 2009): papain, bromelain, ficin, and microbial enzymes sourced from *Bacillus* and *Aspergillus*. These enzymes are shown to have varying degrees of activity against myofibrillar and collagenous proteins. In addition to these GRAS enzymes, enzymes isolated from kiwi fruit (actinidin) and ginger showed potential for future inclusion in meat systems for tenderization (Han, Morton, Bekhit, & Sedcole, 2009; Naveena & Mendiratta, 2004; Wada, Hosaka, Nakazawa, Kobayashi, & Hasegawa, 2004; Wada et al., 2002). Previous research investigating the effects meat tenderness using different treatments and proteases are summarised and presented in Table 2-1. However, to date there has been no research carried out on the volatile compounds generated from the different treatment of proteolytic enzymes for meat tenderization.

Table 2-1 Proteases used in meat tenderization

Protease	Raw material meat	Processing conditions	Effect on meat texture and other quality factors	Reference
Bromelain	Beef round muscles	Injection	Improved tenderness, although salt & phosphate injection was more effective in some cases.	Kolle, McKenna, & Savell, 2004
Papain, Bromelain, Ficin, Bacillus, *Aspergillus* & Ginger	Beef muscles	Injection	Papain had the greatest ability to improve tenderness. Juiciness and textural changes can be negatively affected. Bromelain increased tenderness and degraded collagen more than the contractile proteins. Ginger had potential for tenderizing meat but the injection level was limited due to flavour issues.	Sullivan & Calkins, 2010
Ginger extract	Sheep meat chunks	Marinating	At the level of 3% ginger extract was found to be effective for improving the sensory and keeping qualities of mutton chunks	Mendiratta, Anjaneyulu, Lakshmanan, Naveena, & Bisht, 2000

Table 2-1 (continue)

Protease	Raw material meat	Processing conditions	Effect on meat texture and other quality factors	Reference
Ginger extract	Goat meat chunks	Marinating	Increased protein solubility (especially collagen), tenderness and shelf life	Pawar, Mule, & MacHewad, 2007
Ginger extract & Papain	Buffalo meat	Marinating	Ginger was a better alternative to papain for tenderness. Ginger treated samples received better scores for appearance, flavour, tenderness and overall acceptability.	Naveena & Mendiratta, 2004; Naveena et al., 2004
Pomegranate fruit	Goat meat	Marinating	Pomegranate fruit protease was better for tenderization compared with 0.2% control papain.	Narsaiah et al., 2011
Papain, Pineapple and Ginger	Goose breast	Injection	Addition of 0.12% papain only slightly fractured the muscle fibre as compared to control. 6% pineapple juice and 4% ginger juice seriously ruptured the muscle fibre.	Gao et al., 2011
Papain	Beef meat	Injection	Papain-treated meat received the highest score in tenderness, but had lower flavour scores than control	Schenková et al., 2007

Table 2-1 (continue)

Protease	Raw material meat	Processing conditions	Effect on meat texture and other quality factors	Reference
Bacillus	Beef meat	Marinating	Bacterial protease had marked preference for elastin and collagen. Bacillus protease had same effect on beef meat as papain, based on texture, sensory and structure analyses.	Qihe, Guoqing, Yingchun, & Hui, 2006
Ginger extract	Spent hen	Marinating	3% ginger extract was optimum for meat tenderisation. The tenderisation is achieved through its action on both myofibrillar and connective tissue components.	Naveena & Mendiratta, 2001
Actinidin	Pork	Injection	Improved sensory-assessed tenderness. Juiciness & flavour attributes were not affected.	Christensen et al., 2009
Actinidin	Pork loin muscle	Injection	Tenderness of meat by shear force measurement improved more than 2-fold after kiwifruit juice injection.	Liu et al., 2011
Papain	Beef	Injection	Greatest ability to improve meat tenderness during cooking. No significant changes in tenderness during frozen or refrigerated storage of meat.	Ashie et al., 2002; Gerelt et al., 2000
Kiwifruit juice	Pre-rigor lamb	Infusion	Significant degradation of the myofibrillar proteins. Appearance of new peptides and activation of m-calpain during post-mortem aging.	Han et al., 2009

2.4 Flavour in meat

Flavour is an important eating characteristic when meat products are served (Behrends et al., 2005). Meat flavour is thermally derived, because uncooked meats have little or no aroma and only possess a blood-like taste. During cooking, the volatile compounds generated between non-volatile components of lean and fatty tissues of meat through a complex series of thermally induced reactions, contribute to the aroma attributes and characteristic flavours of meat (Mottram, 1998). Volatile compounds formed during cooking determine the aroma attributes that contribute to the characteristic flavours of meat. A large number of volatile compounds have been identified in beef than in other meats. However this is reflected by the larger number of publications for beef compared to pork, sheep meat or poultry (Warriss, 2000). The variations between different cuts or muscles of meat, sample collection and preparation procedures and cooking conditions may also affect the generation of aroma compounds (Fu & Ho, 1997; Priolo, Micol, & Agabriel, 2001). According to Mottram (1998), over 1000 of the volatile compounds identified in beef, pork, mutton, and chicken can be grouped into chemical groups. These groups include aldehydes, alcohols, ketones, hydrocarbons, phenols, carboxylic acids, esters, lactones, furans, pyrans, pyrroles, pyridines, pyrazines, oxazoles, oxazolines, thiophenes, thiazoles, thiazolines and other nitrogen or sulphur containing compounds. Huang & Ho (2001) reported that meat flavour components are formed from the thermal breakdown of compounds normally found in meat such as fats, proteins and carbohydrates and heating processes that significantly influence overall meat flavour. Biochemical mechanisms involved during post-mortem may also influence the generation of volatile compounds through enzymatic oxidation of unsaturated fatty acids, and further interactions with proteins, peptides and amino acids (Huang & Ho, 2001).

The major precursors of meat flavour can be divided into two categories: water-soluble components and lipids. These precursors provide roast, boiled, fatty and species-related flavours, as well as the characteristic meaty aromas associated with all cooked meats. It has been suggested that the basic meaty aroma is the same for beef, pork and lamb, and is derived from the water-soluble fraction of muscle, whilst the species-specific differences in the aroma of cooked

meats are mainly due to concentration and compositional differences in lipid-derived flavour substances (Song et al., 2011).

2.4.1 Water-soluble components

The main water-soluble flavour precursors are derived from free sugars, sugar phosphates, nucleotide-bound sugars, free amino acids, peptides, nucleotides, and other nitrogenous components, such as pyrroles (Figure 2-2). Studies on the aromas produced when heating mixtures of amino acids and sugars, confirmed the importance of cysteine and ribose in meat flavour formation (Mottram, 1998).

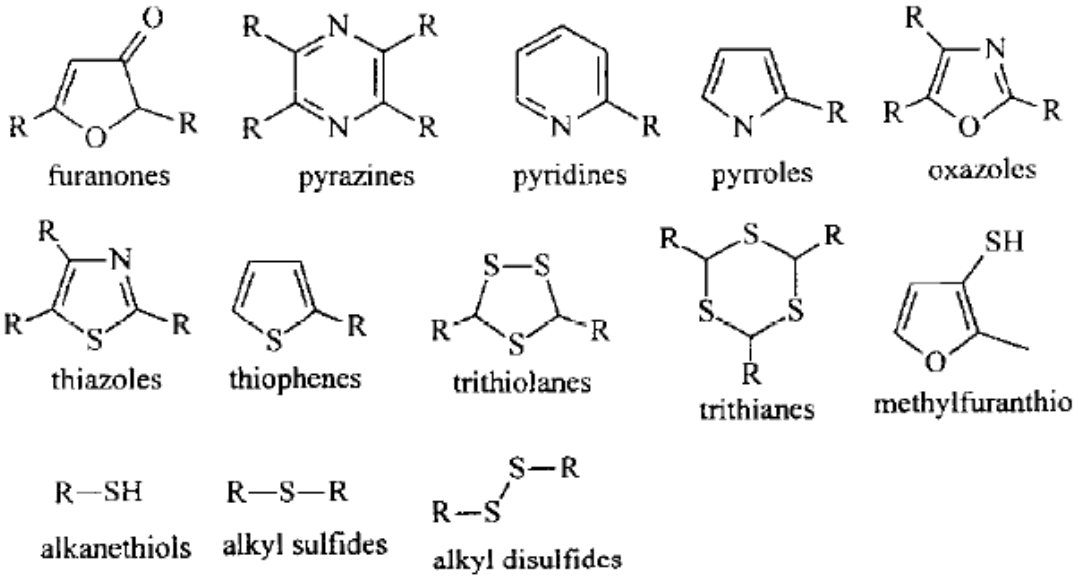

Figure 2-2 Compounds derived from water-soluble precursors (Mottram, 1998)

2.4.2 Lipid-derived volatiles

Several hundred volatile compounds derived from lipid degradation have been found in cooked meat, including aliphatic hydrocarbons, aldehyde, ketone, alcohols, carboxylic acids and esters (Figure 2-3). Some aromatic compounds, hydrocarbons, as well as oxygenated heterocyclic compounds such as lactones and alkylfurans have been reported. In general, these compounds result from the oxidation of the fatty acid components of lipids. When meat is cooked, lipid degradation occurs quickly and provides a different profile of volatiles which contribute to desirable flavours. Unsaturated fatty acids undergo auto-oxidation much more readily than those, which are saturated. Lipid-derived volatiles are quantitatively dominant and it is only in meat grilled under severe

conditions, where the Maillard-derived volatiles are the major components (Mottram, 1998). These compounds can provide aroma directly or undergo further Maillard reaction to produce major cooked flavour volatiles (Zamora & Hidalgo, 2005).

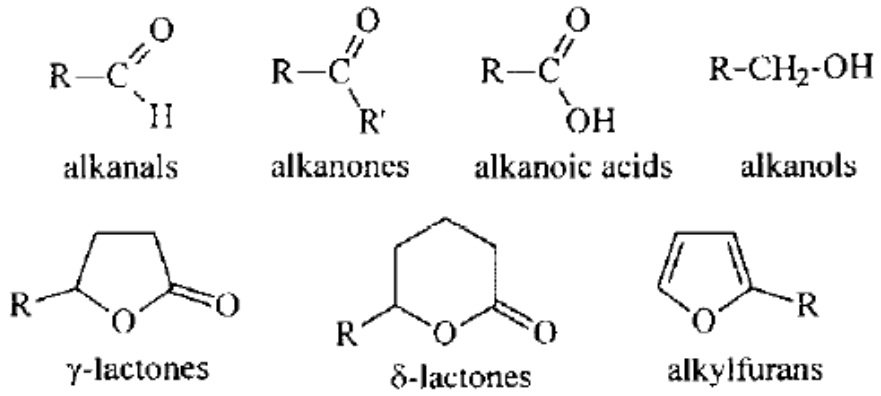

Figure 2-3 Compounds derived from lipid precursors. (Mottram, 1998)

Hydrocarbons are derived from the oxidation of fatty acids. Hwang (1999) reported that hydrocarbons were not major contributors to meat aroma in pork, bacon and ham. Drumm & Spanier (1991) reported that hydrocarbons had no significant impact on flavour due to their relatively high odour threshold values. Ketones, which are mainly derived from fatty acid oxidation, are generated in reasonably large amounts during the cooking of beef (Rochat & Chaintreau, 2005). However, Wettasinghe et al (2001) reported that ketones did not contribute much to chicken and beef meat flavour.

Saturated and unsaturated aldehyde, such as hexanal, heptanal, octanal, nonanal, are important lipid oxidation products. Aldehydes, in particular, have meaty, tallowy odours (Rowe, 2002). These not only contribute to the odour of meat, but can also react with other compounds to produce flavour through amino-carbonyl reactions (Adams, Kitryte, Venskutonis, & De Kimpe, 2009). The characteristic flavour of different meat species is derived from lipid sources. Aldehydes, as major lipid degradation products, are probably involved in certain species characteristics. The higher proportion of unsaturated fatty acids in the triglycerides of pork and chicken, compared with beef or lamb, gives more unsaturated volatile aldehydes in these meats that may contribute to the specific aromas of these species (Mottram, 1998). Hexanal, heptanal, octanal and

nonanal, which are derived mainly from oleic acid and linoleic acid are important contributors to cooked beef flavour (Machiels et al, 2004). The aroma of these aldehydes is described as green, fatty, and tallowy. 2, 4-decadienal has been reported to have an aroma of fat-fried food. It is therefore likely that aliphatic aldehydes can contribute to the fatty flavours of cooked meat (Song et al., 2011).

Alcohols are also derived from lipid oxidation and are the most abundant compounds in cooked meat (Estévez, Morcuende, Ventanas, & Cava, 2003; Wettasinghe et al., 2001). 1-octen-3-ol, for example, is derived from linoleic acid oxidation. It has been identified as having a marked mushroom flavour, and contributes to overall flavour due to its low threshold (Muriel, Antequera, Petrón, Andrés, & Ruiz, 2004).

2.4.3 The volatiles from the Maillard reaction

The Maillard reaction, which occurs between amino compounds and reducing sugars, is one of the most important routes for the generation of flavour compounds in cooked foods, including meat. This reaction is complex and provides a large number of compounds which contribute to flavour. The initial stages of the reaction have been studied in some detail and involve the condensation of the carbonyl group of a reducing sugar with an amino compound, to give a glycosylamine. Subsequently, this rearranges and dehydrates, via deoxyosones, to various sugar dehydration and degradation products such as furfural and furanone derivatives, hydroxyketone and dicarbonyl compounds (Mottram, 1998). Table 2-2 summarizes the important aroma compounds in thermally treated meat that are formed from the Maillard reaction.

The subsequent stages of the Maillard reaction involve the interaction of these compounds with other reactive components such as amines, amino acids, aldehyde, hydrogen sulfide and ammonia. It is these steps, which provide the aroma compounds, which characterise cooked foods. An important associated reaction is the Strecker degradation of amino acids by dicarbonyl compounds formed in the Maillard reaction. The amino acid is decarboxylated and deaminated forming an aldehyde, while the dicarbonyl is converted to an α-aminoketone or aminoalcohol. If the amino acid is cysteine, Strecker degradation can also lead to the production of hydrogen sulfide, ammonia and

acetaldehyde (Mottram, 1998). These compounds, together with carbonyl compounds produced in the Maillard reaction, provide a rich source of intermediates for further flavour-forming reactions. These lead to many important classes of meat flavour compounds including furans, pyrazines, pyrroles, oxazoles, thiophenes, thiazoles and other heterocyclic compounds.

Table 2-2 Important aroma compounds derived from Maillard reaction in thermally treated meat

No	Compounds	Oudour description	Detected in	Reference
A	**Compounds containing sulfur**			
	2-Methyl-3-furanthiol	Meaty,sweet, sulfury	Beef, Pork	Carrapiso, Ventanas, & García, 2002; Moon, Cliff, & Li-Chan, 2006
	2-Furfurylthiol	Roasty,sulfury	Beef, Pork, Lamb	Carrapiso et al., 2002; Kerscher & Grosch, 1998
	3-Mercapto-2-pentanone	Sulfury, catty	Beef, Pork, Lamb	Carrapiso et al., 2002; Kerscher & Grosch, 1998
	Methional	Cooked potato-like	Pork, Boar	Lammers, Dietze, & Ternes, 2009; Pham et al., 2008
	2-Acetyl-2-thiazoline	Roasty, popcorn-like, burnt	Lamb	Bueno et al., 2011
B	**Compounds containing oxygen**			
	2/3-Methylbutanal	Malty, cocoa-like	Goat, Beef, Pork	Machiels & Istasse, 2003; Madruga et al., 2009; Ruiz et al., 1998
	2-Methylpropanal	Malty, fruity, pungent	Pork	Jurado, Carrapiso, Ventanas, & García, 2009; Martín, Jurado, García, & Carrapiso, 2010
	Phenylacetaldehyde	Honey-like, sweet, flowery	Pork	Martín et al., 2010
	Acetaldehyde	Solvent-like	Beef, Pork	Rivas-Cañedo, Juez-Ojeda, Nuñez, & Fernández-García, 2011a, 2011b
	2,3-Butanedione	Buttery	Beef, Pork	Rivas-Cañedo et al., 2011a, 2011b
C	**Compounds containing nitrogen**			
	2-Acetyl-1 -pyrroline	Earthy,Roasty	Pork	Cadwallader & Song, 2008

The Maillard reaction is mainly responsible for the large number of heterocyclic compounds reported in the volatiles of cooked meat that are responsible for savoury, roast and boiled flavours (Mottram & Mottram, 2002). Pentoses, in particular ribose from meat ribonucleotides, and the sulphur-containing amino acid, cysteine, are important precursors for these reactions in meat. Furanthiols and furan sulfides and disulfides are important flavour compounds, with exceptionally low odour threshold values, which are responsible for characteristic meaty aromas. Sulphur volatile compounds contribut to meat flavour due to their low thresholds of sensory detection (Drumm & Spanier, 1991). Sulphur-compounds, derived from ribose and cysteine, are particularly important for the characteristic aroma of meat (Mottram, 1998).

2.4.4 Pastoral flavour

Animal diet affects the flavour of muscle foods. Pasture diets made up of a mixed sward of grasses, green legumes and other broadleaf plants develop a characteristic meat flavour termed as ‘pastoral’ flavour (Young, Lane, Priolo, & Fraser, 2003). α-pinene, and limonene which, have been detected in sheep/lamb are the main discriminating compounds of grass fed animals (Insausti, Goñi, Petri, Gorraiz, & Beriain, 2005; Ruiz, Ventanas, Cava, Andrés, & García, 1999). Priolo et al (2004) also confirmed the presence of terpenes in subcutaneous fat from lambs fed on pasture.

2.4.5 Enzymatic generation of flavour in meat

Enzymatic action is also responsible for the flavour of processed meat. The main enzymatic reactions affecting meat flavour or formation of flavour precursors are proteolysis and lipolysis. Both these reactions are either due to the contribution of endogenous proteases, lipases and enzymes of microbial origin naturally present in the product or enzymes added during the manufacturing process. In the ripening processing of fermented sausages meat protein hydrolysis is mainly catalyzed by endogenous enzymes, and lipolysis that contribute to aroma formation (Toldrá, 1998). Phospholipases and lipases hydrolyze phospholipids and triacylglycerols to form free fatty acids. Unsaturated fatty acids can be further oxidized to aroma volatile compounds. This oxidation may lead to the formation of aliphatic hydrocarbons, alcohols, aldehyde and ketone. Further alcohols react with free fatty acids that can form some esters (Fernández,

Ordóez, Bruna, Herranz, & De la Hoz, 2001). The flavour characteristics of dry sausages are thought to result from meat endogenous enzyme activities (Ordóñez, Hierro, Bruna, & Hoz, 1999). The flavour characteristics of dry sausages are thought to result from meat endogenous enzyme activities (Ordóñez et al, 1999). The acceleration of ripening and flavour in the process of fermented sausages involve the addition of lipases and proteinases, e.g. lipases from *Candida cylindracea* and *Rhizomucor miehei*, pancreatic lipase, serine proteinase from *Lactobacillus paracasei*, pronase from *Streptomyces griseus*, aspartyl proteinase from *Aspergillus oryzae* and papain from *Carica papaya* (Arnau, Serra, Comaposada, Gou, & Garriga, 2007). Results have shown that it is possible to accelerate proteolysis and lipolysis, but only a slight flavour improvement was obtained in some cases. The softening effect of proteases was found to be more important than their effect on flavour, and at high concentrations, an excessive softening is observed.

2.4.6 Effect of post-mortem storage on the flavour of cooked meat pre-treated with proteases

Although longer post-mortem storage improves tenderness, it is important to understand the effects of aging on flavour (Gruber, Belk, Tatum, Scanga, & Smith, 2006). The effect of aging up to 14 days in cooked beef has been shown to increase sensory characteristics like fatty flavour, positive flavour notes such as “beefy”, “brothy”, “sweet” and “browned caramel”, as well as some negative attributes such as “painty”, “cardboard”, “bitter” and “sour” (Bruce, Beilken, & Leppard, 2005; Gorraiz, Beriain, Chasco, & Insausti, 2002; Spanier, Flores, McMillin, & Bidner, 1997). Stetzer et al (2007, 2008) reported that positive flavour compounds decreased with aging and negative compounds increased. Endogenous μ- and m-calpain enzymes known primarily for textural changes during the post-mortem period can influence flavour by producing peptides. These enzymes correlate with increases in rancid, sour and salty flavours (Toldrá & Flores, 2000)

Aging increases carbonyls derived from lipid oxidation, some of which may contribute noticeable off-flavours. Aging for more than >21 days may decrease flavour identity and aging for 35 days may increase metallic flavour (Yancey, Dikeman, Hachmeistert, Chambers Iv, & Milliken, 2005). Aging in a higher

oxygen environment results in a burnt, toasted off-odour. Derivatives of 2-methyl-3-furanthiol provide a more aged beef aroma (Rowe, 2002). In addition, dry-aging increases beef flavour more than aging in vacuum or in carbon dioxide (Campbell, Hunt, Levis, & Chambers Iv, 2001; Jeremiah & Gibson, 2003; Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006).

Increased concentration of cysteine and ribose may be responsible for increased flavour intensity in aged meat (Koutsidis et al., 2008). The reaction between cysteine and ribose when meat is cooked results in the formation of potent sulphur-containing compounds, such as 2-methyl-3-furanthiol, 2-furanmethanethiol and various thiophenes. These compounds have been reported as being crucial in cooked meat aroma (Mottram, 1998). Free amino acids, such as leucine, isoleucine, serine, threonine, valine and phenylalanine, increased during conditioning, particularly between days 7 and 14 (Koutsidis et al., 2008). These amino acids are important in the formation of Strecker aldehydes, such as 2- and 3-methylbutanals, and other aroma compounds such as pyrazines.

2.5 Meat volatiles extraction techniques

Although hundreds of volatile constituents have been identified, only a few of these compounds play a significant role in the overall aroma quality (Rochat & Chaintreau, 2005). Studies on meat flavour have been conducted using gas chromatography (GC) coupled with a flame ionization detector (FID) or mass spectrophotometer (MS) to identify volatile compounds in red meat (Elmore, Mottram, & Hierro, 2001; Moon et al., 2006; Wettasinghe et al., 2001). However, the different extraction techniques used have resulted in different flavour volatiles being identified in cooked meat (Table 2-3). The choice of extraction technique, which produces a volatile profile of the sample extract that is representative of the original sample, is an important consideration for food volatile analysis. In the following section, the use of dynamic headspace extraction (DHE), solid phase microextraction (SPME) and simultaneous steam distillation and extraction (SDE) in the analysis of meat volatiles will be reviewed. The potential use of stir bar sorptive extraction (SBSE) will also be discussed.

Table 2-3 Major volatile compounds and extraction methods used to isolate and concentrate aroma compounds in cooked meat prior to gas chromatography analysis.

Cooked meat products	Volatile extraction technique	Instrumental technique	Major volatile compounds	Reference
Belgian beef	Simultaneous distillation and extraction (SDE)	GC-MS	aldehydes, ketones, pyrazines and furans,	Raes et al., 2003
Goat	SDE	GC-MS	Aldehyde; phenols and alicyclic sulphides	Madruga et al., 2009
Beef	Dynamic headspace entrainment (DHE)	GC-MS	Aldehydes, ketones, hydrocarbons and sulphur compounds	Insausti et al., 2005
Goat	Solid-phase microextraction (SPME)	GC-MS	Aldehyde and alcohol	Madruga et al., 2009
Goat	DHE	GC-MS	Aldehyde; thiazoles, alcohol and pyrazines	Madruga et al., 2009
Beef	SPME	GC-MS	Esters; Heterocycles; Terpene derivatives; Fatty acids; Propanoic acid	Rivas-Cañedo et al., 2011b
Beef	DHE	GC-MS	Alcohols,aldehydes, ketones	Rivas-Cañedo et al., 2011b
Pork loin	SPME	GC-MS	Aldehydes, alcohols and ketones	Park et al., 2009
Sausage	SPME	Selected ion flow tube mass spectrometry (SIFT-MS) and GC-MS	Aldehyde, alcohol and carboxylic acid	Olivares et al., 2011

2.5.1 Dynamic headspace extraction (DHE)

DHE, often referred to as ‘‘purge and trap” is an extraction and pre-concentration technique in which the volatile compounds in the gas phase are continuously absorbed into an inert material or concentrated in a trap. This method involves moving the analytes away from the sample matrix into the headspace. The air around the sample material is constantly swept away by a flow of carrier gas, taking the volatile analytes with it. The extraction of volatile compounds in goat and beef meat using DHE has been reported (Madruga et al., 2009; Rivas-Cañedo et al., 2011b). Although DHE has been used in the extraction of meat flavour, Elmore et al (1997) suggested this method of dynamic headspace trapping is more suitable for trace analysis, and recommended the use of SPME for the analysis of major volatile components in meat.

2.5.2 Simultaneous Steam Distillation and Extraction (SDE)

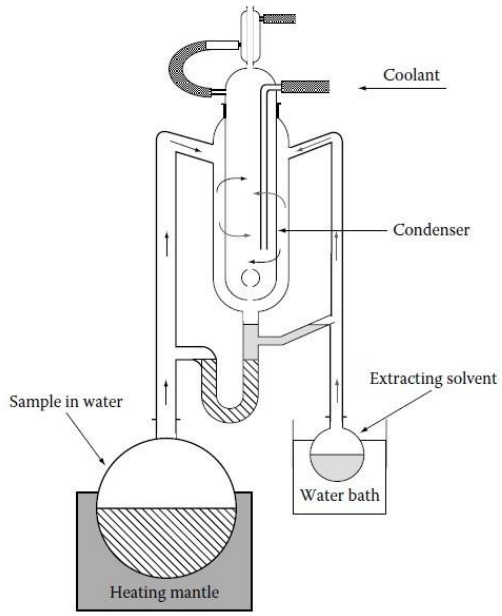

Figure 2-4 Likens–Nickerson apparatus for simultaneous steam distillation/extraction (Elmore, 2010)

Simultaneous distillation and extraction (SDE) is one of the most widely used techniques for isolation of volatiles from food matrices or aromatic plants using the Likens–Nickerson apparatus (Figure 2-4). In SDE the compounds are first distilled and then extracted into an organic solvent. This makes SDE an attractive extraction concentration technique for volatile compounds. It is a simple technique, which involves a small amount of solvent that effectively strips

the volatiles, and allows quantitative recovery of many volatile compounds (Reineccius, 2007).

This technique is used less today than in the past, but still has great value. Studies have reported the use of SDE to analyze aroma compounds in beef fat (Ohnishi & Shibamoto, 1984), pork (Garcia-Esteban, Ansorena, Astiasaran, Martin, & Ruiz, 2004; Xie, Sun, Zheng, & Wang, 2008), and goat (Madruga et al., 2009). A drawback of SDE is that when the extract is concentrated by distilling off the solvent, low-boiling volatile compounds can be lost. These compounds include 2-butanone, 2- and 3-methylbutanal, which are often present at high levels in the headspace of lamb and beef extracts. Furthermore, artefacts can be formed as a result of the high temperatures used and volatiles can be generated when samples are overcooked during extraction through enhanced lipid oxidation (Elmore, Mottram, & Dodson, 2004). Garcia-Esteban et al (2004) concluded that although SDE was capable of analyzing high molecular weight and low volatility compounds, the high temperatures during distillation may lead to the formation of compounds not present originally. This may result in a different SDE volatile profile from the original one.

2.5.3 Stir Bar Sorptive Extraction (SBSE)

Stir bar sorptive extraction was first introduced by Baltussen and colleagues as a new and improved sample preparation technique (Baltussen, Sandra, David, & Cramers, 1999). As shown in Figure 2-5, the use of SBSE has linearly increased in the past 10 years, reaching up to 400 publications in August 2011 (Summarized from a Scopus search).

SBSE has been successfully applied to trace analysis of environmental samples and has the analytical reproducibility needed in the analysis of volatile and semi-volatile components of biological mixtures (Sánchez-Rojas, Bosch-Ojeda, & Cano-Pavón, 2009).

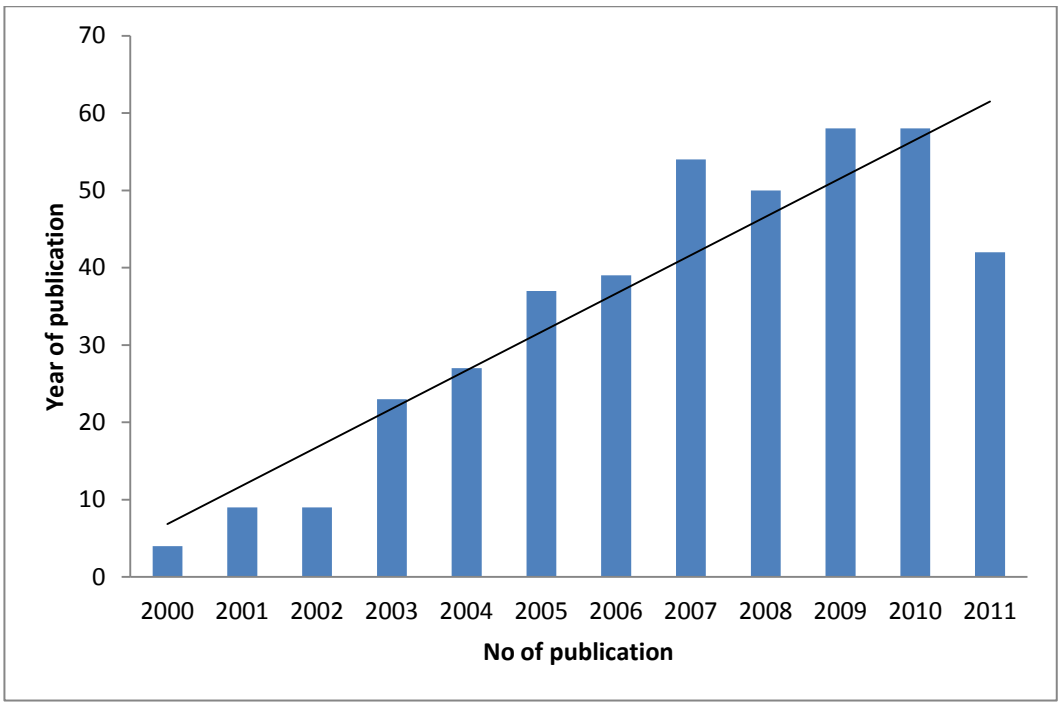

Figure 2-5 The number of publications using SBSE as a method of extraction in the last decade

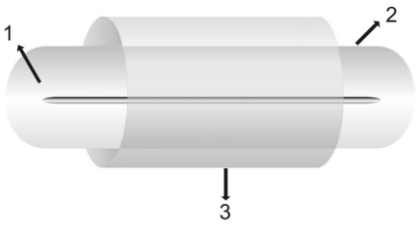

Figure 2-6 SBSE 1. Magnetic rod; 2. glass jacket; 3. PDMS coating (Lancas, Queiroz, Grossi, & Olivares, 2009)

It is based on a thick film of polydimethylsiloxane (PDMS) coated onto a glass-enveloped magnet (Figure 2-6). SBSE like SPME is a solventless sample preparation technique. Organic adsorbents like Tenax often lead to poor blanks due to thermal decomposition and have significant catalytic activity, which prevents their use with chemically labile compounds (Baltussen, Cramers, & Sandra, 2002). SBSE is considered to be more sensitivity and accurate than SPME for determinations at trace level in difficult matrices. It has been demonstrated that a wide range of volatile and semi-volatile substances can be retained on a polymer coated magnetic bar (Ochiai et al., 2001; Tobiszewski, Mechlinska, Zygmunt, & Namiesnik, 2009).

Guerrero et al (2007) compared SBSE with a previous SPME method for extraction of volatile compounds in vinegar. Although performance characteristics obtained for both methodologies were similar, SBSE showed lower detection and quantitation limits and better repeatability reproducibility values. Several papers have described the analysis of polycyclic aromatic hydrocarbons and reported the higher extraction efficiency obtained by SBSE in comparison to SPME (Baltussen et al., 1999; Popp, Bauer, Hauser, Keil, & Wennrich, 2003; Popp, Bauer, & Wennrich, 2001). However, the extraction of volatile compounds in meat using SBSE has not been reported.

2.5.4 Solid Phase Microextraction (SPME)

Solid phase microextraction (SPME) is a syringe shaped device with a fused-silica fiber in the needle that adsorbs volatile compounds inside a sealed vessel and is allowed to stand until equilibrium of volatile compounds is established between the headspace and the food sample (Wampler, 2001).The fiber is coated with various polymeric phases to adsorb different polar and non-polar groups of volatiles. During adsorption, the amount of volatile compounds are concentrated by the fiber, and then injected into a gas chromatography injection port for desorption and analysis.

This technique is sensitive, selective, fast, low-cost, solvent-free, easy to handle and also compatible with low detection limits. In addition, because low extraction temperature can be used, SPME could give a better estimation of the aroma profile as perceived by the human nose (Brunton, Cronin, Monahan, & Durcan, 2000). SPME involves two basic steps: the partitioning of analytes between the extraction phase and the sample matrix, and desorption of concentrated extracts into an analytical instrument. Appropriate selection and optimization of SPME extraction parameters, such as fiber coating selection, extraction time, agitation, addition of salt, and extraction temperature are crucial when improving the sensitivity and reproducibility of this method (Balasubramanian & Panigrahi, 2011).

2.5.4.1 Selection of fiber

The physical and chemical properties of the fiber, such as length, coating thickness and material, affect the sensitivity of SPME. The affinity of the fiber for an analyte depends on the principle of ‘like dissolves like’, and coating fibers

having different properties or thickness are selected in accordance with different compounds analysed (Kataoka, Lord, & Pawliszyn, 2000).

The fused-silica fiber is coated with a relatively thin film of several polymeric stationary phases. Shirey (2000) stated that fibres containing adsorbents, such as Carboxen and divinylbenzene (DVB), extracted more volatiles than fibres composed of a liquid stationary phase. DVB had been shown to have a high affinity for amines but in general Carboxen was a more effective coating than DVB for low-molecular mass compounds. A number of researches showed that the DVB/CAR/PDMS fiber had higher reproducibility than other fibres and reported the adsorption of more higher boiling point compounds (Elmore et al., 2001; Ho, Wan Aida, Maskat, & Osman, 2006; Machiels & Istasse, 2003).

2.5.4.2 Extraction conditions

In HS-SPME, extraction time and the concentration of analyte are important factors. The addition of salt to the sample, agitation, and changing the pH and temperature can improve extraction of analyte. Extraction time depends on the agitation rate and the partition coefficient of the analyte between the fiber coating and sample matrix. The maximum sensitivity of SPME is at the equilibrium point. Addition of soluble salts, such as sodium chloride to the sample will improve the extraction efficiency. In principle, super saturation of the sample with salts is the most effective due to the salting-out effect (Kataoka et al., 2000). Heating during extraction can also increase the concentration of the analytes in the gaseous phase in HS-SPME, which causes an increase in extraction rate, and simultaneously a decrease in the distribution constant. Therefore, the appropriate temperature and extraction time should be considered when optimizing a SPME method.

2.5.4.3 Desorption conditions

The temperature and desorption time are two major factors to consider during thermal desorption of volatiles. Generally, temperatures between 200-250 °C can be used as a desorption temperature (Chin et al., 2007; Ong et al., 2008; Roberts, Pollien, & Milo, 2000). Steenson et al (2002) concluded that a higher desorption temperature increased the amount of volatile compounds extracted from the SPME fiber and decreased desorption time. Machiels & Istasse (2003)

reported that highly volatile compounds were not affected by desorption time, and that less volatile compounds needed more time to desorb.

2.5.4.4 Limitations of headspace

Although there are several benefits of HS-SPME in volatile analysis, its limitation should also be considered. The major problem lies in the use of a fragile fiber which can be easily broken and the use of different fibers of same phase type can result in more than 20% variation (Chin et al., 2007). Thus, the same fiber should be used in replicate analytes to generate reproducible results (Yang, Huang, & Smetena, 2002) and should be used no more than 100 times. In addition, artefact peaks can form, such as thermal oxidation occurring during the desorption step (Lestremau, Andersson, & Desauziers, 2004) or siloxane formation (Perera, Marriott, & Galbally, 2002).

2.5.4.5 Applications of SPME in meat analysis

Originally, the SPME technique was developed for analysis of pollutants in environmental water samples. It has now been increasingly applied to flavour analysis of foods such as cheese (Delgado, González-Crespo, Cava, & Ramírez, 2011), edible oils (Gromadzka & Wardencki, 2011), coffee (Budryn, Nebesny, Kula, Majda, & Krysiak, 2011) and wines (Weldegergis, Crouch, Górecki, & de Villiers, 2011).

Several researches have used SPME to examine the volatile constituents of meat. It has been used to measure the volatile flavour profile in goat meat (Madruga et al., 2009), levels of nitrosamines in smoked ham (Sen et al, 1997), heterocyclic amines in cooked foods and meat extracts (Skog, Solyakov, Arvidsson, & Jägerstad, 1998), volatile components in cooked beef meat (Machiels & Istasse, 2003) and volatile aroma compounds in cooked pork (Elmore et al., 2001). The technique can eliminate solvent artefact formation during extraction, although fiber artefacts may also cause some interference. As SPME fibre type, adsorption time, adsorption temperature, and salt addition will influence reproducibility as explained earlier, these factors were considered in this research when optimizing the conditions to analyze volatile compounds in cooked beef meat.

Chapter 3 Materials and Methods

In this chapter, the preliminary methods trialled for beef volatile extraction using purge and trap and Stir Bar Sorptive Extraction (SBSE) will be explained (3.1). The use of solid phase microextraction coupled to GC-FID or MS for cooked beef volatile extraction is also described (3.2 to 3.5).

3.1 Preliminary methods for the extraction of cooked beef volatile compounds

3.1.1 Purge and Trap

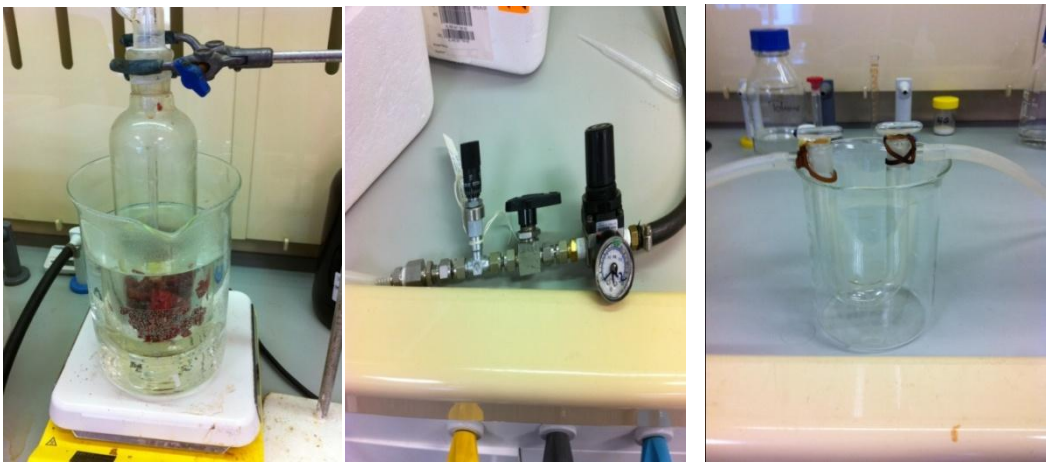

Figure 3-1 Purge and trap apparatus used in this study

The purge and trap used in this research is shown in Figure 3-1. Beef meat (20 g) in a 250 mL bottle was placed in a 100 °C water bath. The gas valve was opened after 30 min of cooking and all the vapour in the bottle was passed through a U-tube immersed in dry-ice. The aqueous sample from the U-tube was injected into the GC. The resulting chromatogram did not show much volatile compounds being extracted from the cooked beef.

3.1.2 Stir Bar Sorptive Extraction (SBSE)

The stir bars (Gerstel GmbH, Germany) was placed in the injector liner of the GC at 250 °C for 30 min prior to extraction. The bar was then placed in a 20 ml headspace vial containing cooked beef (3.5 ± 0.1g). After 30 min, the bar was removed for desorption. Two types of desorption methods can be used: thermal desorption (TD) and liquid desorption (LD). LD was trialled in this research using

2ml of either carbon disulfide, chloroform or methanol that were added to the stir bar in a vial. The vial was stirred at 25°C on magnetic plate for 60 min. However none of the three solvents were successful in extracting the volatiles from meat. A second trial involved the thermal desorption of stir bars within the liner of a GC injector port at 250°C.

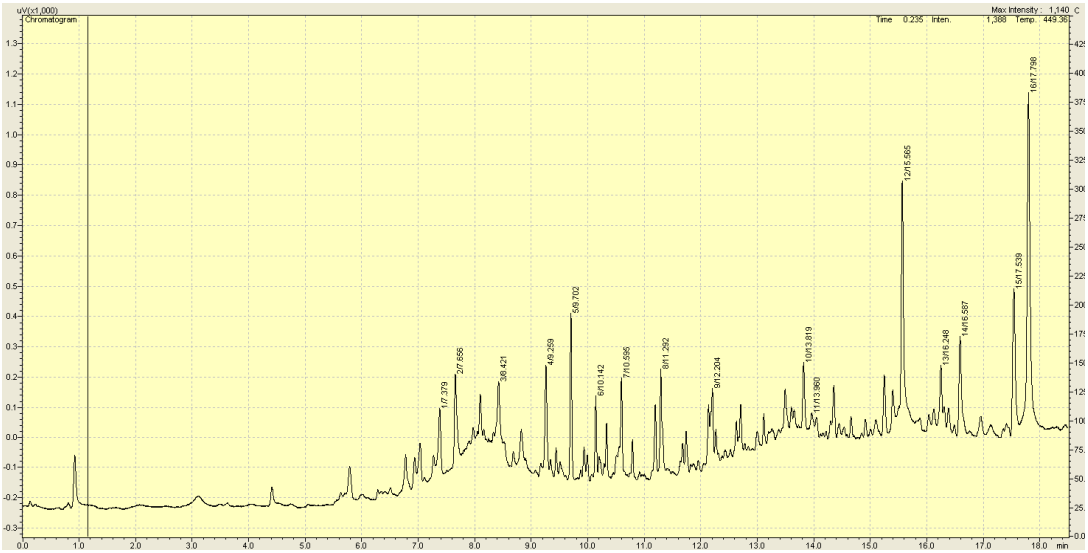

Figure 3-2 Chromatography of beef volatile compounds using SBSE

Although a significant amount of volatile compounds were detected by GC-FID (Figure 3-2), the lack of a dedicated thermal desorption unit was a major drawback in further development of this technique.

3.2 Preparation of the beef samples used in this study

Hot-boned topsides from dairy cows (>5 years old) were randomly selected on the day of slaughter (approximately 2-3 hours following slaughter) from Alliance Group Ltd (Pukeuri Plant, Oamaru). For logistic reasons, topsides from both sides of 22 carcasses (44 topsides in total) were collected over 3 consecutive days. Some basic information on the hot carcass weight, the carcass grade and the topside weights are shown in Table 3-1.

Table 3-1 Carcass grade, hot carcass weight and the weight of topsides used in the present study

Animal	Tag No.	Slaughter date	Wt(kg)		Grade	Hot carcass weight (kg)	Topsides weight (kg)
			Left side	Right side			
1	500001	4/05/2010	97.00	96.60	CWM	193.60	8.77
2	500002	4/05/2010	107.40	109.10	CWM	216.50	8.41
3	500003	4/05/2010	98.50	96.10	CWM	194.60	7.32
4	500004	4/05/2010	77.90	76.60	CWM	154.50	5.65
5	500005	4/05/2010	122.80	117.00	CWM	239.80	9.22
6	500006	4/05/2010	93.70	94.20	CWM	187.90	6.16
7	500007	4/05/2010	85.30	86.30	CWM	171.60	6.50
8	500008	4/05/2010	79.70	81.00	CWM	160.70	5.72
9	500001	5/05/2010	92.90	93.00	CWM	185.90	6.35
10	500002	5/05/2010	66.60	67.80	CWM	134.40	4.60
11	500003	5/05/2010	105.80	109.40	CWM	215.20	8.28
12	500004	5/05/2010	123.10	127.90	HL2	251.00	8.62
13	500005	5/05/2010	79.20	81.70	CWM	160.90	6.20
14	500006	5/05/2010	85.10	87.10	CWM	172.20	6.22
15	500007	5/05/2010	64.20	64.90	CWM	129.10	4.44
16	500021	6/05/2010	106.30	104.00	HL3	210.30	7.13
17	500022	6/05/2010	85.00	84.60	CWM	169.60	6.26
18	500023	6/05/2010	82.50	82.50	HA3	165.00	5.36
19	500024	6/05/2010	71.60	71.80	CWM	143.40	4.69
20	500025	6/05/2010	88.00	86.20	CWM	174.20	6.65
21	500026	6/05/2010	122.00	127.40	CWM	249.40	9.77
22	500027	6/05/2010	69.80	71.00	CWM	140.80	5.30

The topsides were packed in Styrofoam boxes and the temperature of samples was recorded using temperature loggers. The topsides were transported to University of Otago within 1.5-2 hours of boning and sample treatment was performed 2-3 hours after arrival at the laboratory. Topsides were halved and

cut into steaks (average weight ± SD was 305.1 ± 58.8 g) that were assigned to 1 day post-mortem (PM) treatment, and meat blocks of about 9x9x20 cm (average weight ± SD was 1677.6 ± 488.5 g) that were assigned to 21 days PM vacuum packed storage period. All of the samples (steaks and blocks) were subjected to needle injection (to 10% of the original weight) with a series of enzyme tenderisers (Table 3-2) according to the manufacturers' recommendations or at experimental level for the two in-house extracts prepared as described by (Bekhit, Han, Morton, & Sedcole, 2007).

Table 3-2 Description of proteases used in the present trial and their sources

Code	Product/Treatment	Concentration	Manufacturer
C	None (control)	-	-
PA	Papain 25,000 MG	0.01g/L	Supplier A
BR	Bromelain	0.05g/L	Supplier A
AC	Kiwifruit PE (Actinidin)	10g/L	Supplier B
ZI	Digest Easy (Zingibain)	100 mL/L	Supplier C
F31	Fungal protease 60k	0.01g/L	Supplier A
F60	Fungal protease 31k	0.02g/L	Supplier A
BA	Bacterial protease G	0.08g/L	Supplier A
KJ	Kiwifruit crude juice	20%	Prepared in house
ASP	Asparagus crude juice	50%	Prepared in house

The samples were vacuum-packed, stored at 2°C for the designated PM time (1 or 21 days). Samples for volatiles analysis were frozen in liquid nitrogen, vacuum packed and stored at -30°C until analysis. Frozen samples were air freighted to the laboratory at AUT University and the sample treatment codes are shown in Table 3-3.

Table 3-3 Sample treatment codes in this study

Treatment	Treatment code	
	1 day post-mortem	21 days post-mortem
Control	C2	C2 w3
	C3	C3 w3
	C6	C6 w3
	C8	C8 w3
Papain	PA-1	PA-1 w3
	PA-4	PA-4 w3
	PA-6	PA-6 w3
	PA-8	PA-8 w3
Bromelain	BR-1	BR-1 w3
	BR-2	BR-2 w3
	BR-3	BR-3 w3
	BR-8	BR-8 w3
Actinidin	AC-2	AC-2 w3
	AC-3	AC-3 w3
	AC-4	AC-4 w3
	AC-7	AC-7 w3
Zingibain	ZI-1	ZI-1 w3
	ZI-2	ZI-2 w3
	ZI-3	ZI-3 w3
	ZI-7	ZI-7 w3
Fungal 31	F31-2	F31-2 w3
	F31-3	F31-3 w3
	F31-5	F31-5 w3
	F31-7	F31-7 w3
Fungal 60	F60-3	F60-3 w3
	F60-6	F60-6 w3
	F60-7	F60-7 w3
	F60-8	F60-8 w3
Bacterial	BA-2	BA-2 w3
	BA-4	BA-4 w3
	BA-6	BA-6 w3
	BA-8	BA-8 w3
Kiwi juice	KJ-1	KJ-1 w3
	KJ-2	KJ-2 w3
	KJ-3	KJ-3 w3
	KJ-5	KJ-5 w3
Asparagus	ASP-2	ASP-2 w3
	ASP-6	ASP-6 w3
	ASP-7	ASP-7 w3
	ASP-8	ASP-8 w3

3.3 Optimization of cooked beef volatile compounds by HS-SPME

Samples of minced beef (2.0 ± 0.1) with 6% salt were placed in a 20 ml flat bottom headspace vial (Chromatography Research Supplies, Inc, USA) with a PTFE/Silicone septum and crimp cap (Supleco, USA). The head space vial was heated using a plate heater at 80 °C for 10 min. Volatile components in the samples were adsorbed onto a 30/50um layer of divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Co., Bellefonte, USA). The stainless steel needle housing the fiber penetrated the septum, and after equilibration at 60 °C for 5 min, the SPME fiber was exposed to the headspace of the samples for 40 min. The SPME fiber was preconditioned prior to analysis at 250 °C for 30 min. 1, 2-Dichlorobenzene (2 µl, 1.3ppm) was used as an internal standard and added to a 250 µl flat bottom insert, which was placed in the head space vial containing the sample.

3.4 GC-FID and GC-MS analysis

After extraction, the SPME device was removed from the head space vial and inserted directly into the injection port of the GC. The SPME fiber was thermally desorbed at 250 °C in the injector port of both the GC-FID and GC-MS for 30 min in the splitless mode (a split ratio of 10).

GC-FID: The Shimadzu GC-17A was equipped with a flame ionization detector (FID) (Japan). The GC-FID was installed with a ZB-5 low bleed/MS fused-silica capillary column (5%-Phenyl-95%-Dimethylpolysiloxane Phase, 30m×0.53mm×1.50um) (Phenomenex, Inc, Torrance, USA). Nitrogen was used as the carrier gas, which was set at 43 Pa, total flow was 7ml/min, and oven was held for 2 min at 40°C, heated to 250 °C at 5 °C/min, and held 3 min at this temperature.

GC-MS: The Trace GC Ultra (Thermo Scientific, USA) was equipped with a Mass Spectrophotometer (Thermo Scientific, DSQ Series, USA). The GC-MS was installed with a VF-5 ms low bleed/MS fused-silica capillary column (5%-Phenyl-95%-Dimethylpolysiloxane Phase, 60m×0.25mm×0.25um) (Phenomenex, Inc, Torrance, USA). Helium was used as carrier gas with a constant flow of 1.5 ml/min in the GC-MS. Chromatographic conditions were as

follows: oven was held for 2 min at 40 °C, heated to 250 °C at 5 °C/min, and held 3 min at this temperature. The mass spectrometer operated in the electron impact mode with a source temperature of 200 °C, an ionising voltage of 70eV, and the transfer line temperature was 250 °C. The mass spectrometer scanned masses from m/z 48 to m/z 400 at a rate of 3.41 scan/s.

Peak identification was carried out by comparison of their mass spectra with spectra from authentic compounds previously analysed, NIST/EPA/NIH Mass Spectral Database (Version 2.0a, 2002), or NIST web book (http://webbook.nist.gov/chemistry/). To confirm the identification of volatile compounds, the retention index (RI) was calculated for each volatile compound using the retention times of a homologous series of C7-C30 n-alkanes (1000ug/ml in hexane from Supelco Co., Bellefonte, USA) and comparing the RI with compounds analysed under similar conditions in previous literature. The approximate quantities of the volatiles were estimated by comparison of their peak areas with that of the 1,2-dichlorobenzene internal standard using a response factor of 1.

3.5 Statistical analysis

The chromatographic data were collated using Microsoft Office Excel 2007 and subjected to statistical analysis using the XLSAT MX software release 2010. One-way analysis of variance (ANOVA) was used to volatile compounds in each treatment and different post-mortem with same treatment with the difference being considered as significantly at $p<0.05$. The volatile compounds for all 1 day and 21 days post mortem were analysed by principal component analysis (PCA).

Chapter 4 Results and Discussion

In this chapter, the development of a reliable HS-SPME method for monitoring the volatile compounds of cooked beef will be firstly discussed. This will then be followed by a discussion on the effects of pre-rigor injected of beef with nine plant and microbial proteases and at two different post-mortem storage on the volatile profile of cooked beef using SPME in combination with gas chromatography (GC) and GC-mass spectrometry (MS) analysis.

4.1 Effect of HS-SPME variables on cooked beef volatile extraction

In this section, variables affecting SPME were investigated to improve the precision and accuracy of this method for cooked beef volatile analysis. To investigate this, seven key odour-active compounds in cooked beef meat were used. The compounds included 2, 5-dimethylpyrazine, methylpyrazine, 3-methylbutanal and 2-acetylthiazole that contribute to roasty note of cooked beef; (E)-2-nonenal associated with a fatty note; as well as hexanal and heptanal that give a grass, fruity note to the aroma profile of cooked beef (Song et al., 2010). A 50/30 um CAR/DVB/PDMS SPME fibre was chosen because of its higher reproducibility, especially in the analysis of flavour compounds with larger levels of higher boiling point compounds (molecular weight between 40 and 275) (Elmore et al., 2001; Ho et al., 2006; Machiels & Istasse, 2003). Each experiment was done in triplicates.

4.1.1 Effect of extraction temperature and time

High temperature provides enough energy for volatile compounds to overcome energy barriers, which bind them to the matrix, and increases vapour pressure for the mass transfer process (Zhang & Pawliszyn, 1995). This facilitates the release of volatile compounds into the headspace. The effects of temperature and extraction time were evident from the chromatograms of the extractions performed at 40 and 60 °C for 20 and 40 min (Figure 4-1). An increase in the peak chromatographic area was found, especially with the less volatile compounds at higher temperature and longer extraction time. The increase in

volatile compounds can be explained by the fact that higher temperature tends to drive the volatiles from the sample matrix to the fiber coating.

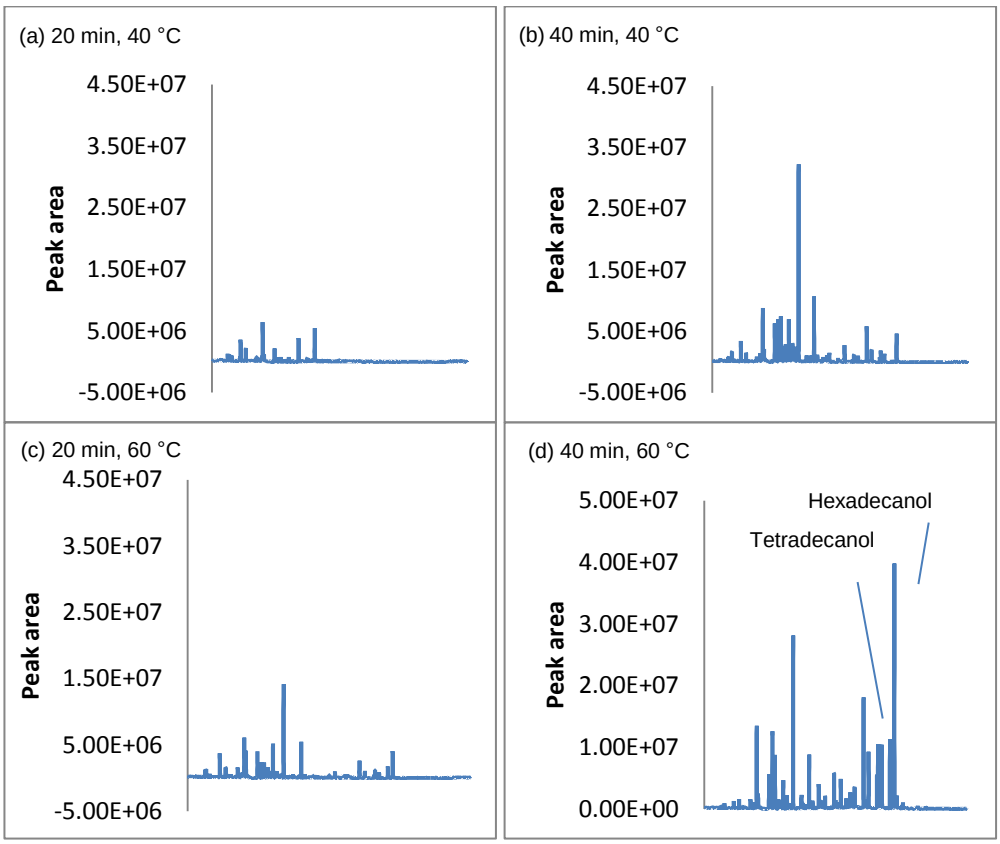

Figure 4-1 Gas chromatography-mass spectrometry chromatograms of the volatiles in cooked beef meat using SPME

As seen Figure 4-1 (d), high boiling point compounds such as tetradecanol and hexadecanol, had higher peak areas at higher temperature and longer extraction time. Headspace sampling was further carried out at 60 °C for 20, 30, 40 and 50 minutes. Longer extraction time did not have much effect on the low boiling point volatile compounds, such as 3-methylbutanal and methylpyrazine (Figure 4-2). 2-acetylthiazole continued to increase after 40 min but (E)-2-nonenal, heptanal and hexanal decreased after 40 min. After these preliminary experiments, HS-SPME extraction was carried out for 40 min at 60 °C.

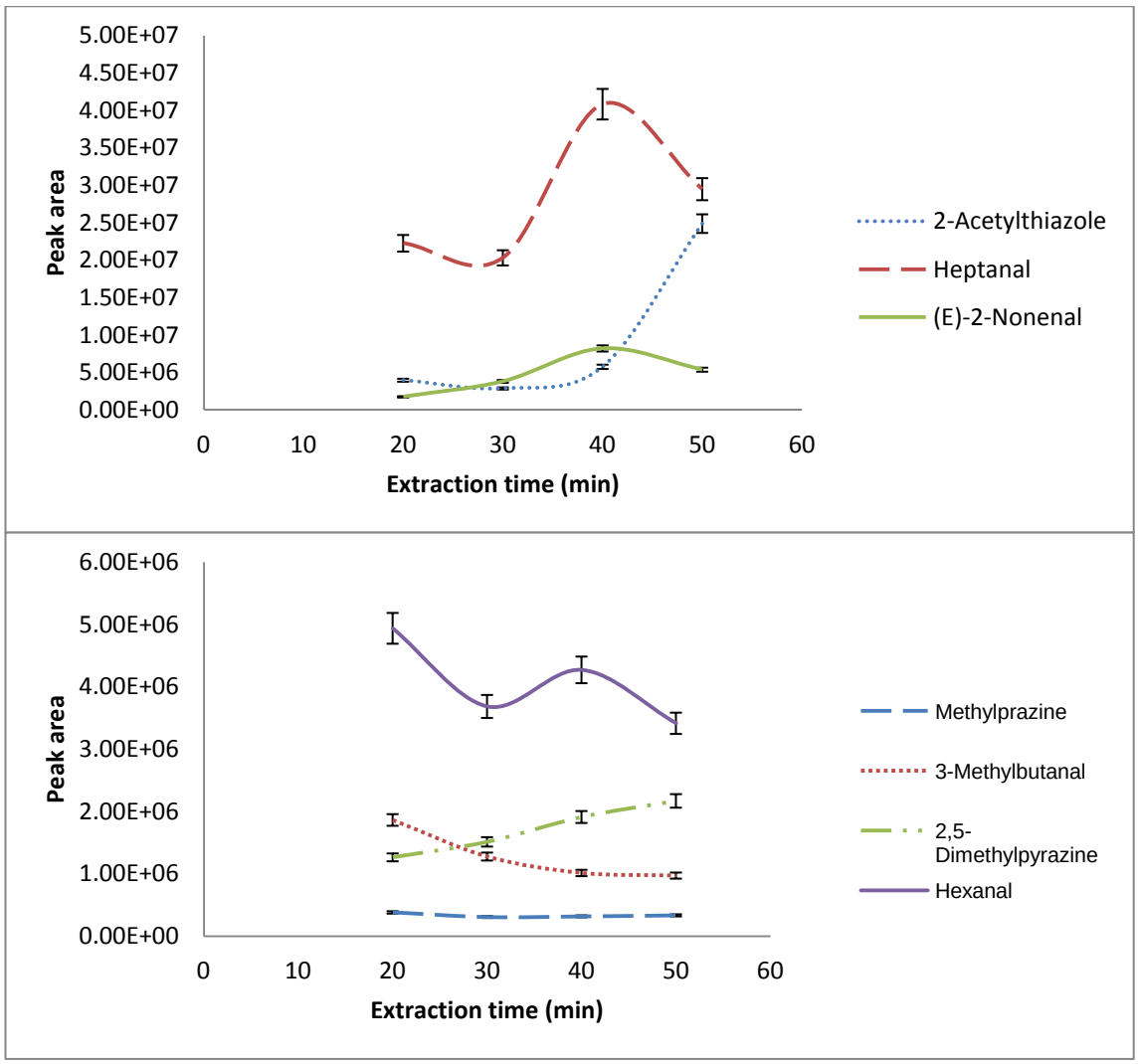

Figure 4-2 The peak area of key volatile compounds at different extraction times (20, 30, 40 and 50 min)

4.1.2 Effect of desorption time

In order to determine the optimum desorption time, the fibre was desorbed at different times (30 s, 2 min, 30 min) in the injection port at 250 °C. Peak areas were compared for target compounds and a blank fibre extraction was performed after each analysis to check for remaining analytes. A desorption time of 30 s was sufficient to remove the volatiles completely from the fibre. Machiels & Istasse (2003) reported that highly volatile compounds were not affected by desorption time, and less volatile compounds needed more time to desorb.

4.1.3 Effect of salt addition to meat sample

Dissolution of salt into sample matrix has been reported to stimulate adsorption of the volatile components from samples by changing the phase border properties and decreasing the solubility of hydrophobic components in the aqueous phase (Yang & Peppard, 1994). This is known as the “salting out” effect. Lee et al (2003) suggested that between 20–30% (w/w) concentrations of salt affected most flavour compounds during extraction. In addition, 0–10% (w/w) concentration of salt did not affect adsorption efficiency of volatiles such as ethyl isovalerate and isoamyl acetate, while adsorption of both compounds significantly increased at 20% salt concentration (Liu & Yang, 2002). In this study, 0.12g of salt (6% w/w) was added to the sample matrix and compared with control, because a high concentration of salt was known to stimulate denaturation of proteins (Cheftel, Cuq, & Lorient, 1985).

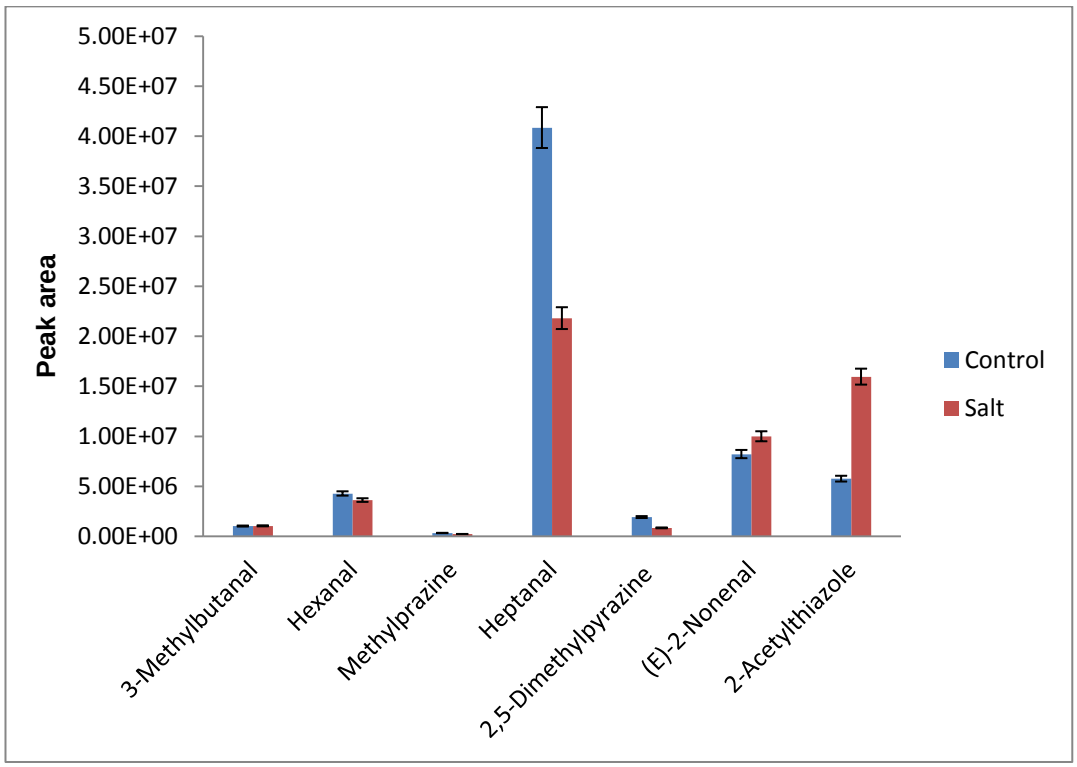

Figure 4-3 Salting-out effect on SPME efficiency

In this study, sodium salt addition improved SPME absorption of most monitored compounds, such as (E)-nonanal and 2-acetylthiazole (Figure 4-3). However, reductions in the amount of hexanal and heptanal were evident when the sample matrix was saturated with salt. Roberts et al (2000) reported that the enhancing effect of salt was not similar for all volatiles. The decrease in absorption of these

volatile compounds could be either attributed to competition with other high concentration volatiles distributed in headspace or the fact that concentration exceeds the maximum of linear range for the fiber coating (Roberts et al., 2000; Zhang & Pawliszyn, 1993).

4.2 Headspace volatile profile of cooked pre-rigor beef meat

The volatile compounds of cooked beef meat can be divided into two groups, which are formed by lipid oxidation and Maillard reactions. Volatile compounds from the Maillard reaction include nitrogen, sulphur and non-heterocyclic compounds. Compounds from lipid oxidation include aldehydes, ketones, hydrocarbons, alcohols and alkylfurans. In this study, a total of 56 key volatile compounds were found in headspace of cooked pre-rigor beef meat using the SPME–GC–MS method. This included 23 aldehydes, 5 ketones, 3 furans, 8 nitrogen and sulphur compounds, 4 alkanes, 7 alcohols and 6 terpenes (Table 4-1). Additionally, another 40 unknown volatile compounds were detected. These unknown volatiles were present in relatively small amounts, and could not be tentatively identified by comparison of the MS spectra and RI data with the NIST EPA/NIH Mass Spectral library database. A one way analysis of variance was carried out on the quantitative data for each volatile compound (Table 4-1) present in meat subjected to different protease treatments after 1 day and 21 days post-mortem storage.

Table 4-1 Volatile compounds extracted from cooked pre-rigor beef injected with proteases using HS-SPME

No	RI [a]	Compounds	Identification[b]	Functional group	Characteristic flavours/aromas [c]
1	656	3-Methylbutanal	MS+RI	Aldehyde	Pungent, apple-like odour, malt, fatty
2	664	2-Methylbutanal	MS+RI	Aldehyde	Burnt, fermented,maltsweet, slightly fruity, chocolate-like taste
3	691	2,3-Pentanedione	MS+RI	Ketone	Buttery diacetyl-like, fermented dairy and creamy,popcorn buttery
4	694	n-Pentanal	RI	Aldehyde	Pungent, acrid, lightly fruity and nut-like
5	696	2-Ethyl-furan	MS+RI	Furan	Smoky burnt
6	718	Dimethyl disulfide	MS+RI	Nitrogen and Sulphur Compound	A diffuse, intense onion odour non-lacrhrymatory
7	763	Toluene	MS 85%	Aldehyde	No description
8	797	Octane	MS+RI	Alkane	No description
9	800	Hexanal	MS+RI	Aldehyde	Fatty-green, grassy
10	825	Methylpyrazine	MS+RI	Nitrogen and Sulphur Compound	Nutty, cocoa, green, roasted, chocolate, meaty odour
11	838	Furfural	MS+RI	Furan	Almond,meat,fatty,oily
12	862	2-Furanmethanol	MS+RI	Alcohol	Very mild, warm, oily, “burnt” odour and a cooked sugar taste

Table 4-1 (continue)

No	RI [a]	Compounds	Identification[b]	Functional group	Characteristic flavours/aromas [c]
13	871	1-Hexanol	MS 85%	Alcohol	Herbaceous, woody, fragrant, mild, sweet, green fruity odour and aromatic flavour
14	892	2-Heptanone	MS+RI	Ketone	Fruity, spicy, cinnamon, banana, slightly spicy odour, burnt meat, vitamin
15	896	o-Xylene	MS+RI	Aldehyde	No description
16	901	Heptanal	MS 85%	Aldehyde	Oily, fatty, rancid, unpleasant
17	911	3-Methylthiopropanal	MS+RI	Nitrogen and Sulphur Compounds	Sharp, pungent odour
18	920	2,5-Dimethylpyrazine	MS+RI	Nitrogen and Sulphur Compounds	Earthy odour, potato-like odour
19	937	α-Pinene	MS+RI	Terpene	Piney, fruity, citrus, turpentine
20	953	Camphene	MS+RI	Terpene	No description
21	965	Benzaldehyde	MS 85%	Aldehyde	Volatile almond oil, bitter almond
22	975	Dimethyl trisulfide	MS+RI	Nitrogen and Sulphur Compounds	Powerful, diffusive, penetrating odour reminiscent of fresh onion
23	979	1-Octen-3-ol	MS+RI	Alcohol	Mushroom-like
24	982	2-Methyl-3-octanone	MS+RI	Ketone	No description

Table 4-1 (continue)

No	RI [a]	Compounds	Identification[b]	Functional group	Characteristic flavours/aromas [c]
25	989	6-Methyl-5-hepten-2-one	MS+RI	Ketone	Fatty, green, citrus-like
26	992	2-Pentylfuran	MS+RI	Furan	Green bean, metallic, vegetable odour
27	1003	Octanal	MS 85%	Aldehyde	Fatty, citrus, honey odour
28	1023	2-Acetylthiazole	MS+RI	Nitrogen and Sulphur Compounds	Grass, nutty, roast
29	1029	2-Ethyl-1-hexanol	MS+RI	Alcohol	A mild, oily, sweet, slightly floral odour
30	1033	Limonene	MS+RI	Terpene	Pleasant lemon-like, turpentine, citrus, fruity, fresh, light
31	1037	1,8-Cineole	MS+RI	Terpene	Camphoraceous odour and fresh, pungent, cooling taste.
32	1049	Benzeneacetaldehyde	MS 85%	Aldehyde	Cocoa aromapungent, bitter flavour, turning sweet and fruit-like, floral
33	1060	(E)-2-Octenal	MS+RI	Aldehyde	Peculiar green-leafy odour, orange,honey-like, cognac-like aroma.
34	1070	1-Octanol	MS+RI	Alcohol	Fresh, orange-rose
35	1090	2-Nonanone	MS+RI	Ketone	Rue odour, rose and tea-like flavour
36	1098	Undecane	MS+RI	Alkane	No description

Table 4-1 (continue)

No	RI [a]	Compounds	Identification[b]	Functional group	Characteristic flavours/aromas [c]
37	1104	Nonanal	MS 85%	Aldehyde	Fatty, citrus-like flavour.
38	1162	(E)-2-Nonenal	MS 85%	Aldehyde	Orris-like, waxy
39	1171	2,3-Diethyl-5-methylpyrazine	RI	Nitrogen and Sulphur Compounds	Nutty, roasted, vegetable aroma.
40	1198	Terpineol	MS+RI	Terpene	Lilac odour with a sweet tast
41	1205	Decanal	MS 85%	Aldehyde	Waxy, floral, citrus, pronounced fatty
42	1228	Citronellol	MS+RI	Alcohol	Rose- and lemon-like odour
43	1241	Benzothiazole	RI	Nitrogen and Sulphur Compounds	Rose-like
44	1243	Citral	RI	Aldehyde	Lemon-like odour
45	1257	Geraniol	MS 85%	Alcohol	Rose-like odour
46	1263	(E)-2-Decenal	MS 85%	Aldehyde	Waxy, orange
47	1270	Geranial	MS 85%	Aldehyde	Lemon-like odour
48	1306	Undecanal	MS+RI	Aldehyde	Sweetish, fatty

Table 4-1 (continue)

No	RI [a]	Compounds	Identification[b]	Functional group	Characteristic flavours/aromas [c]
49	1395	Tetradecane	MS+RI	Alkane	No description
50	1408	Dodecanal	MS+RI	Aldehyde	Fatty
51	1491	α-Curcumene	MS 85%	Aldehyde	No description
52	1501	Zingiberene	MS 85%	Terpene	Spicy
53	1508	α-Farnesene	MS 85%	Aldehyde	A fruity, herbaceous odour
54	1533	β-Sesquiphellandrene	MS 85%	Alkane	Herbal fruity woody
55	1610	Tetradecanal	MS+RI	Aldehyde	Fatty, orris-like
56	1814	Hexadecanal	MS+RI	Aldehyde	No description

[a] RI on a VF-5MS column, was calculated in relation to the retention time of *n*-alkane ($C–C_{30}$) series

[b] MS, tentative identification by comparison of mass spectrum with NIST library spectrum (over 85%); MS+RI, mass spectrum identified using NIST mass spectral database and RI agree with literature values (Machiels & Istasse, 2003)

[c] Characteristic flavours/aromas was obtained ‘Fenaroli's handbook of flavour ingredients’ (Burdock & Fenaroli, 2010)

4.2.1 The effect of protease treatments

Enzymatic action is responsible for the flavour of processed meat. The main enzymatic reactions affecting meat flavour or formation of flavour precursors are proteolysis and lipolysis. The action of proteases may correlate with increases in rancid, sour and salty flavours (Toldrá & Flores, 2000). In addition, the source of proteases, such as kiwi juice and ginger, may possibly influence flavour. The effect of nine protease-treated pre-rigor beef samples on the volatile compounds of cooked beef will be discussed in this section.

As shown in Table 4-2, 11 volatile compounds including camphene (20), 1, 8-cineole (31), terpineol (40), citronellol (42), citral (44), geraniol (45), geranial (47), α-curcumene (51), zingiberene (52), α-farnesene (53) and β-sesquiphellandrene (54) were only detected in meat treated with zingibain (ZI) at 1 day and 3 post-mortem storage. Sullivan & Calkins (2010) reported that the ginger-treated meat had greater off-flavour ratings ($p< 0.0001$) in beef muscle of both high and low-connective tissues. These off-flavours may be attributed to the characteristic flavour of ginger essential oil (Bartley & Jacobs, 2000; Wohlmuth, Smith, Brooks, Myers, & Leach, 2006). Another study reported that 5% v/w ginger treatment resulted in desirable flavour of buffalo meat, compared to 3% and 7% ginger extract treatment in India (Naveena & Mendiratta, 2004).

Terpenes are recognized constituents of spices (Chevance & Farmer, 1999), and might be derived from feeding (Ruiz et al., 1999). Priolo et al (2004) reported that terpenes accounted for a small percentage of volatile in adipose tissue of sheep. They also reported that certain terpene compounds were characteristic of animals fed with green forage diets. Although limonene was generally found in all protease treated samples stored 1 day and 21 days post-mortem. There was only significantly higher ($p<0.05$) limonene in ZI treated samples compared to the other treatments for at 1 day post-mortem.

Most aldehydes including 3-methylbutanal, n-pentanal, heptanal, benzaldehyde, and nonanal, were present in fungal protease treated samples. These aldehydes were present in F60 treated samples at significantly high levels ($p<0.05$) compared to BR treated samples at 1 day post-mortem. As for the fungal protease, F31 treated sample at 21 days post-mortem storage, there was a significantly ($p<0.05$) higher concentration of benzaldehyde, octanal and

hexanal than control. Hexanal, together with other volatile aldehydes such as heptanal, octanal and nonanal, are important in cooked beef meat flavour, and may impart a pleasant fruity flavour in low concentration (Machiels et al., 2004).

Alcohols are mainly derived from lipid oxidation. In this study, 1-hexanol and 2-furanmethanol, were significantly ($p<0.05$) higher in bacterial protease (BA) treated sample than control at 21 days post-mortem. These aroma compounds have been previously associated with smoke or smoked food products (Varlet, Knockaert, Prost, & Serot, 2006). The compound 2-furanmethanol, which exhibit burnt meat and vitamin-like aromas, has been suggested to be formed from the Maillard reaction (Mottram, 1998). In addition, 2-furanmethanol was one of the aroma impact compounds that positively impacted on consumer acceptability of American dry-cured ham (Pham et al., 2008). Compared to BR, KJ and ZI treated samples, 1-octen-3-ol was significantly ($p<0.05$) higher level in papain (PA) and F31 treated samples at 1 day and 21 days post-mortem storage, respectively. This volatile compound is derived from linoleic acid oxidation, and has a marked mushroom flavour, that can contribute to overall flavour due to its low threshold (Muriel et al., 2004).

Ketones are generated in reasonably large amounts during cooking of beef due to lipolysis (Rochat & Chaintreau, 2005). In this study, the F60 treated sample showed higher levels of 2, 3-pentanedione and 2-heptanone ($p<0.05$) compared to the control sample at 1 day post-mortem. 2, 3-pentanedione has a fermented dairy odour. However, Wettasinghe et al (2001) reported that the contribution of ketones to the flavour of meat was lower than that of aldehydes in chicken meat, and did not contribute much to the flavour of meat from beef shoulder muscles.

4.2.2 The effect of post-mortem storage

Post-mortem aging is a critical management practice that can improve the consistency of beef tenderness (Tatum, Belk, George, & Smith, 1999). However, it is important consider the effects of aging on flavour. Stetzer et al (2007, 2008) concluded that positive flavour compounds decreased with aging and negative compounds increased. The changes in volatile compounds of cooked beef meat that were pre-treated with proteases at one day and 21 days post-mortem storage will be discussed.

Aldehydes, in general, are not stable and can easily react with other compounds to produce different compounds which have different flavours (Mottram, 1998). In this study, the aldehydes were consistently present at high levels for all protease-treated samples (Table 4-2). Nine aldehydes (2- and 3-methylbutanal n-pentanal, hexanal, heptanal, benzaldehyde, benzeneacetaldehyde, octanal and nonanal) tentatively identified in this study. Benzeneacetaldehyde was however not present at high levels in AC and ZI protease-treated samples. These aldehydes have been reported in cooked beef (Machiels et al., 2004) and lamb ham (Paleari et al., 2006). Benzaldehyde and benzeneacetaldehyde result from proteolysis and amino acid degradation rather than lipid degradation. Benzaldehyde significantly increased ($p<0.05$) only in treatments F31and ASP from 1 day to 21 days post-mortem storage. This may be attributed to increased protein degradation in meat during longer post-mortem storage.

The Strecker degradation of amino acids is a key reaction in the generation of potent aroma compounds during Maillard-type processes (Mottram, 1998). In this study, a significant increase ($p<0.05$) in 3-methylbutanal was observed in KJ, BA, BR and F31 treated samples at 21 days post-mortem storage. This compound has been identified in cooked beef (Machiels & Istasse, 2003) and lamb (Madruga et al., 2009). It has been described as malty and fatty (Burdock & Fenaroli, 2010) and is an important volatile compound in dry-cured ham products. In addition, Ruiz et al (1999) reported that 3-methylbutanal was found in high concentrations in longer aged hams.

At 21 days post-mortem storage, hexanal levels increased ($p<0.05$) in BR, F31, KJ and ASP treated samples. Although hexanal generated initially in meat can be continuously oxidized (Calkins & Hodgen, 2007) and contribute positively to

beef flavour, it may produce undesirable flavours at higher concentrations (Melton, 1990). Hexanal is the most prominent volatile compound in cooked meat. Its amount is directly proportional to thiobarbituric acid reactive substances (TBARS), a measure of oxidation, and inversely proportional to flavour acceptability (Calkins & Hodgen, 2007).

1-octen-3-ol significantly increased ($p<0.05$) in BR, KJ and F31 samples, from 1 day to 21 days post-mortem storage. The increase in 1-octen-3-ol during the storage days (0 to 4 days) at 4 °C was were reported in cooked chicken meat (Byrne, Bredie, Mottram, & Martens, 2002). This volatile has been described as having a mushroom like odour (Table 4-1).

Mottram (1998) reported that sulphur volatile compounds are derived from sulphur-containing amino acid degradation. Koutsidis et al (2008) concluded that the concentration of cysteine increased threefold during storage, which may also be responsible for increased flavour intensity in aged meat. The reaction between cysteine and ribose when meat is cooked can form potent sulphur-containing compounds. These compounds play an important role in the flavour of cooked beef since some heterocyclic sulphur compounds have been described as possessing meat like aromas. 2-Acetylthiazole increased significantly ($p<0.05$) in the F31 treated sample from 1 day to 21 days post-mortem storage. Dimethyl trisulphide significantly ($p<0.05$) increased in KJ and AC treatment during 1 day and 21 days post-mortem, while dimethyl disulfide also showed significant ($p<0.05$) increased in KJ treatment during 1 day and 21 days post-mortem. Even though their levels were low, sulphur compounds are potent contributors to the meat flavour (Drumm & Spanier, 1991). Hogan (2002) reported that although low levels of sulphur-containing compounds are meaty, high levels are objectionable.

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Table 4-2 Volatile compounds in cooked beef treated with protease at 1 day and 21 days post-mortem

Volatile compounds	Post-mortem (day)	Treatment[e]									
		C	PA	BR	AC	ZI	F31	F60	BA	KJ	ASP
3-Methylbutanal	1	2.28^{abcx}	3.04^{abcx}	1.53^{cx}	1.52^{cx}	2.21^{abcx}	1.80^{bcx}	3.99^{ax}	3.94^{ax}	2.55^{abcx}	3.88^{abx}
	21	7.51^{bcx}	4.52^{bcx}	5.78^{bcy}	4.38^{bcx}	3.46^{cx}	10.38^{aby}	6.54^{bcx}	10.08^{aby}	16.06^{ay}	10.27^{abx}
2-Methylbutanal	1	1.58^{abcx}	2.04^{abcx}	0.98^{cx}	1.15^{bcx}	1.59^{abcx}	1.15^{bcx}	2.82^{ax}	2.83^{ax}	1.25^{bcx}	2.60^{abx}
	21	4.39^{bcx}	2.67^{bcx}	3.50^{bcy}	2.75^{bcx}	2.10^{bcx}	6.40^{aby}	4.28^{bcx}	6.63^{abx}	8.67^{ay}	6.35^{abx}
2,3-Pentanedione	1	1.25^{bx}	2.31^{abx}	1.28^{bx}	1.31^{bx}	1.98^{abx}	1.20^{bx}	3.08^{ay}	3.04^{ax}	1.11^{bx}	2.18^{abx}
	21	1.45^{abx}	0.58^{by}	1.10^{abx}	1.50^{abx}	0.92^{bx}	2.20^{ax}	1.41^{abx}	1.69^{abx}	1.30^{abx}	1.52^{abx}
Pentanal	1	0.47^{bcx}	0.64^{bcx}	0.27^{cx}	0.57^{bcx}	0.69^{abcx}	0.78^{abcx}	1.46^{ax}	0.73^{abcx}	1.19^{abx}	0.36^{cx}
	21	0.73^{cx}	1.05^{bcx}	0.90^{bcy}	0.90^{bcx}	0.99^{bcx}	2.21^{ax}	1.14^{bcx}	1.05^{bcx}	1.37^{bx}	1.33^{bx}
2-Ethyl-furan	1	1.09^{abx}	1.37^{abx}	0.50^{bcx}	1.55^{ax}	0.61^{abcx}	1.17^{abx}	1.13^{abx}	1.16^{abx}	Nd^{cx}	1.11^{abx}
	21	1.55^{ax}	0.93^{abx}	0.84^{abx}	1.04^{abx}	0.51^{bx}	0.81^{abx}	0.97^{abx}	1.08^{abx}	0.84^{aby}	1.13^{abx}
Dimethyl disulfide	1	0.68^{abx}	0.84^{abx}	1.44^{ax}	0.41^{bx}	0.46^{bx}	0.31^{cx}	1.17^{abx}	1.45^{abcx}	0.38^{bx}	0.46^{bx}
	21	0.72^{abcx}	0.33^{bcx}	0.97^{abx}	0.56^{bcx}	0.60^{bcx}	1.66^{ax}	Nd^{cy}	0.51^{bcx}	1.12^{aby}	1.01^{abx}
Toluene	1	2.72^{abcx}	4.63^{ax}	0.56^{cx}	2.42^{abcx}	2.07^{bcx}	2.81^{abcx}	3.24^{abx}	2.74^{abcx}	1.42^{bcx}	2.31^{bcx}
	21	2.45^{bcx}	2.90^{abcx}	2.14^{cx}	5.10^{abx}	2.28^{cx}	5.29^{ax}	2.63^{abcx}	3.17^{abcx}	3.16^{abcx}	2.96^{abcx}
Octane	1	0.40^{bcx}	0.40^{bcx}	0.42^{bcx}	0.22^{cx}	0.57^{bcx}	0.68^{bx}	1.19^{ax}	0.70^{bx}	0.39^{bcx}	0.52^{bcx}
	21	0.68^{ax}	0.91^{ax}	0.72^{ax}	0.42^{ax}	0.51^{ax}	0.65^{ax}	0.74^{ax}	0.77^{ax}	0.62^{ax}	0.55^{ax}
Hexanal	1	2.54^{abx}	5.47^{ax}	1.56^{bx}	4.16^{abx}	2.14^{bx}	2.04^{bx}	3.84^{abx}	2.46^{bx}	1.25^{bx}	1.81^{bx}
	21	2.42^{bcx}	2.17^{bcx}	2.39^{bcy}	2.37^{bcx}	1.95^{bc}	4.36^{ay}	1.30^{cx}	2.67^{bcx}	2.77^{abcy}	3.31^{aby}

Table 4-2 (continue)

Volatile compounds	Post-mortem (day)	Treatment[e]									
		C	PA	BR	AC	ZI	F31	F60	BA	KJ	ASP
Methylprazine	1	0.14^{ax}	0.19^{ax}	Nd^{ax}	0.15^{ax}	Nd^{ax}	0.14^{ax}	0.14^{ax}	0.13^{ax}	Nd^{ax}	0.08^{ax}
	21	0.15^{abcdx}	0.12^{bcdx}	0.08^{cdx}	0.30^{ax}	0.17^{abcdy}	0.27^{abx}	Nd^{dx}	0.14^{abcdx}	0.20^{abcy}	0.14^{abcdx}
Furfural	1	0.25^{abx}	0.28^{abx}	0.22^{abx}	0.19^{bx}	0.46^{abx}	0.18^{bx}	0.34^{abx}	0.36^{ax}	0.59^{abx}	0.47^{ax}
	21	0.68^{aby}	0.20^{cx}	0.50^{abcy}	0.34^{bcx}	0.16^{cx}	0.08^{cx}	0.87^{ax}	0.69^{abx}	0.92^{ax}	0.12^{cx}
2-Furanmethanol	1	0.02^{ax}	0.11^{ax}	Nd^{ax}	0.06^{ax}	0.13^{ax}	0.24^{ax}	0.08^{ax}	0.26^{ax}	0.14^{ax}	0.10ax
	21	0.27^{bx}	0.21^{bx}	0.20^{bx}	0.23^{bx}	0.49^{bx}	0.18^{bx}	0.23^{bx}	0.64^{ax}	0.35^{abx}	0.26^{abx}
1-Hexanol	1	0.09^{bcdx}	0.77^{ax}	Nd^{dx}	0.51^{abx}	$0.06c^{dx}$	0.06^{cdx}	0.60^{ax}	0.43^{abcx}	Nd^{dx}	0.12^{bcdx}
	21	0.32^{bcx}	0.55^{bcx}	0.35^{bcy}	0.25^{bcx}	Nd^{cx}	0.89^{bcx}	0.07^{cx}	1.81^{ax}	0.96^{by}	0.27^{bcx}
2-Heptanone	1	0.06^{bx}	0.37^{ax}	0.18^{abx}	0.27^{abx}	0.32^{abx}	0.24^{abx}	0.37^{ax}	0.16^{abx}	0.26^{abx}	0.25^{abx}
	21	0.23^{bx}	0.19^{bx}	0.17^{bx}	0.35^{abx}	0.26^{abx}	0.73^{ax}	0.28^{abx}	0.07^{bx}	0.27^{bx}	0.33^{abx}
o-Xylene	1	Nd^{bx}	0.03^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.81^{abx}	1.08^{ax}	1.35^{ax}	Nd^{bx}	1.12^{ax}
	21	Nd^{dx}	1.77^{aby}	0.92^{bcdy}	Nd^{dx}	0.50^{cdx}	1.75^{abx}	1.17^{abcx}	1.61^{abx}	1.99^{ay}	1.19^{abcx}
Heptanal	1	2.27^{bx}	2.63^{bx}	1.43^{bx}	2.28^{bx}	2.81^{bx}	2.29^{bx}	4.95^{ax}	3.06^{abx}	1.79^{bx}	2.05^{bx}
	21	2.06^{bx}	3.26^{abx}	2.93^{abx}	2.35^{bx}	2.92^{abx}	5.03^{ax}	2.86^{abx}	3.22^{abx}	2.84^{abx}	2.51^{bx}
3-Methylthiopropanal	1	0.34^{ax}	Nd^{cx}	0.16^{bx}	0.19^{bx}	Nd^{cx}	0.23^{bx}	Nd^{cx}	nd^{cx}	Nd^{cx}	Nd^{cx}
	21	0.07^{abx}	0.05^{abx}	0.08^{abx}	0.20^{ax}	0.09^{abx}	0.09^{abx}	0.18^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}
2,5-Dimethylpyrazine	1	Nd^{ax}	0.03^{ax}	Nd^{ax}	Nd^{ax}	0.12^{ax}	0.09^{ax}	0.21^{ax}	0.10^{ax}	0.37^{ax}	Nd^{ax}
	21	0.04^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.04^{bx}	Nd^{bx}	Nd^{bx}	0.21^{ax}	Nd^{bx}	Nd^{bx}

Table 4-2 (continue)

Volatile compounds	Post-mortem (day)	Treatment[e]									
		C	PA	BR	AC	ZI	F31	F60	BA	KJ	ASP
a-Pinene	1	Nd^{bx}	0.03^{bx}	Nd^{bx}	0.08^{bx}	0.40^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.10^{bx}	0.38^{ax}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.17^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
Camphene	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	1.05^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.36^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
Benzaldehyde	1	5.36^{abx}	5.28^{abx}	4.20^{bx}	5.06^{abx}	6.68^{abx}	5.09^{abx}	7.89^{ax}	7.38^{abx}	6.28^{abx}	6.73^{abx}
	21	6.31^{cx}	7.16^{bcx}	6.38^{cx}	6.88^{bcx}	6.02^{cx}	13.46^{ay}	7.94^{bcx}	7.82^{bcx}	12.54^{ax}	10.57^{aby}
Dimethl trisulfide	1	0.19^{abx}	0.58^{ax}	0.21^{abx}	0.36^{abx}	0.46^{abx}	0.41^{abx}	0.56^{abx}	0.39^{abx}	0.14^{bx}	0.15^{bx}
	21	0.52^{abcx}	0.64^{abx}	0.48^{bcx}	0.84^{aby}	0.54^{abcx}	1.02^{ax}	0.51^{abcx}	0.34^{bcx}	0.43^{bcy}	Nd^{cx}
1-Octen-3-ol	1	0.67^{abx}	1.29^{ax}	0.32^{bx}	0.76^{abx}	0.41^{bx}	0.49^{bx}	0.73^{abx}	0.77^{abx}	0.18^{bx}	0.54^{bx}
	21	0.99^{abx}	1.06^{abx}	0.73^{by}	1.00^{abx}	0.73^{bx}	1.24^{ay}	0.68^{bx}	0.80^{abx}	0.59^{by}	0.90^{abx}
2-Methyl-3-octanone	1	0.20^{abx}	0.52^{ax}	0.04^{bx}	0.35^{abx}	0.27^{abx}	0.35^{abx}	0.41^{abx}	0.35^{abx}	0.36^{abx}	0.20^{abx}
	21	0.24^{bx}	0.69^{abx}	0.26^{by}	0.35^{bx}	0.25^{bx}	0.92^{ax}	0.44^{bx}	0.34^{bcx}	0.44^{bx}	0.28^{bx}
6-Methyl-5-hepten-2-one	1	0.10^{bx}	0.17^{bx}	Nd^{bx}	0.24^{bx}	2.60^{ax}	0.19^{bx}	0.19^{bx}	0.07^{bx}	0.16^{bx}	0.11^{bx}
	21	0.17^{bx}	0.23^{bx}	0.16^{by}	0.27^{bx}	1.29^{ax}	0.45^{bx}	0.26^{bx}	0.20^{bx}	0.24^{bx}	0.29^{bx}
2-Pentylfuan	1	0.98^{cx}	2.41^{abx}	1.15^{cx}	1.79^{bcx}	1.28^{bcx}	1.06^{cx}	3.05^{ax}	2.17^{abcx}	1.56^{bcx}	1.69^{bcx}
	21	1.87^{abx}	1.78^{abx}	1.97^{abx}	1.39^{bx}	1.76^{abx}	2.10^{abx}	1.32^{bx}	1.83^{abx}	2.53^{ax}	2.28^{abx}
Octanal	1	1.88^{bcx}	2.75^{abcx}	1.43^{cx}	2.02^{bcx}	2.29^{bcx}	2.65^{bcx}	4.13^{ax}	3.20^{abx}	1.90^{bcx}	2.12^{bcx}
	21	2.08^{bx}	3.60^{abx}	2.43^{abx}	2.37^{abx}	2.39^{abx}	4.27^{ax}	2.98^{abx}	2.51^{abx}	2.53^{abx}	2.41^{abx}

Table 4-2 (continue)

Volatile compounds	Post-mortem (day)	Treatment[e]									
		C	PA	BR	AC	ZI	F31	F60	BA	KJ	ASP
2-Acetylthiazole	1	1.87^{abx}	2.66^{abx}	1.67^{bx}	2.02^{abx}	2.57^{abx}	2.14^{abx}	3.76^{ax}	1.45^{bx}	2.77^{abx}	2.83^{abx}
	21	2.98^{bx}	2.44^{bx}	3.44^{bx}	2.64^{bx}	2.82^{bx}	7.26^{ay}	3.88^{bx}	2.60^{bx}	3.74^{bx}	3.39^{bx}
2-Ethyl-1-hexanol	1	0.37^{cdx}	0.88^{abx}	0.30^{cdx}	0.55^{abcx}	0.66^{abcx}	0.69^{abcx}	0.98^{ax}	0.62^{abcx}	0.08^{dx}	0.48^{bcdx}
	21	0.43^{cdx}	0.79^{bcx}	0.38^{dx}	0.53^{cdx}	0.54^{cdx}	1.26^{ax}	1.01^{abx}	0.52^{cdx}	0.54^{cdy}	0.60^{cdx}
Limonene	1	0.37^{bx}	0.46^{bx}	0.10^{bx}	0.54^{bx}	1.23^{ax}	0.44^{bx}	0.65^{bx}	0.25^{bx}	0.08^{bx}	0.40^{bx}
	21	0.56^{abcdx}	0.51^{abcdx}	0.27^{dx}	0.87^{abx}	0.48^{abcdx}	1.02^{ax}	0.27^{cdx}	0.42^{bcdx}	0.91^{ay}	0.63^{abcx}
1,8-Cineole	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	2.70^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	1.11^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
Benzeneacetaldehyde	1	0.64^{abx}	0.72^{abx}	0.43^{bx}	0.63^{abx}	0.88^{abx}	0.63^{abx}	1.06^{abx}	1.22^{ax}	0.94^{abx}	1.27^{ax}
	21	2.59^{cy}	2.28^{cy}	2.50^{cy}	2.78^{cx}	1.87^{cx}	5.17^{aby}	3.10^{bcy}	3.36^{bcy}	7.00^{ay}	3.90^{bcy}
(E)2-Octenal	1	0.04^{abx}	0.20^{ax}	Nd^{bx}	0.11^{abx}	0.12^{abx}	0.07^{abx}	0.13^{abx}	Nd^{bx}	Nd^{bx}	0.07^{abx}
	21	0.05^{ax}	0.16^{ax}	0.06^{ax}	0.20^{ax}	0.17^{ax}	0.22^{ax}	0.21^{ax}	0.16^{ax}	0.14^{ax}	0.11^{ax}
1-Octanol	1	1.32^{bx}	1.83^{abx}	1.11^{bx}	1.38^{bx}	1.36^{bx}	1.61^{abx}	2.48^{ax}	1.90^{abx}	1.17^{bx}	1.37^{bx}
	21	1.39^{cx}	2.53^{ax}	1.54^{bcx}	1.39^{cx}	1.36^{cx}	2.40^{abx}	1.52^{bcx}	1.49^{cx}	1.39^{cx}	1.42^{cx}
2-Nonanone	1	0.07^{ax}	0.16^{ax}	Nd^{ax}	0.03^{ax}	0.12^{ax}	0.30^{ax}	0.15^{ax}	Nd^{ax}	0.19^{ax}	0.26^{ax}
	21	0.20^{ay}	0.10^{ax}	0.13^{ay}	0.16^{ax}	0.25^{ax}	0.32^{ax}	0.36^{ax}	0.20^{ayx}	0.36^{ax}	0.30^{ax}
Undecane	1	0.35^{abx}	0.53^{ay}	Nd^{cx}	0.42^{abx}	0.42^{abx}	0.34^{bx}	0.46^{abx}	0.32^{bx}	Nd^{cx}	0.41^{abx}
	21	0.29^{bx}	Nd^{cx}	0.36^{aby}	0.41^{abx}	0.49^{ax}	0.39^{abx}	0.39^{abx}	0.38^{abx}	0.37^{ab}y	0.36^{abx}

Table 4-2 (continue)

Volatile compounds	Post-mortem (day)	Treatment[e]									
		C	PA	BR	AC	ZI	F31	F60	BA	KJ	ASP
Nonanal	1	6.52^{bcx}	8.53^{bcx}	5.28^{cx}	6.70^{bcx}	7.89^{bcx}	9.12^{bcx}	14.64^{ax}	10.46^{abx}	7.04^{bcx}	8.32^{bcx}
	21	7.32^{cx}	13.40^{ax}	8.61^{abcx}	8.11^{abcx}	7.48^{bcx}	13.25^{abx}	8.77^{abcx}	9.11^{abcx}	8.35^{abcx}	8.62^{abcx}
(E)-2-Nonenal	1	0.19^{abx}	0.33^{bx}	0.01^{bx}	0.30^{abx}	0.16^{abx}	0.23^{abx}	0.42^{ax}	0.18^{abx}	0.13^{abx}	0.24^{abx}
	21	0.32^{ax}	0.56^{ax}	0.33^{ax}	0.61^{ax}	0.51^{ax}	0.74^{ax}	0.52^{ax}	0.51^{ax}	0.46^{ax}	0.25^{ax}
2,3-Diethyl-5-methylpyrazine	1	0.22^{abcx}	0.43ax	0.04^{cx}	0.28^{abcx}	0.20^{abcx}	0.16^{bcx}	0.37^{abx}	0.20^{abcx}	0.13^{bcx}	0.24^{abcx}
	21	0.28^{ax}	0.33^{ax}	0.22^{ax}	0.35^{ax}	0.23^{ax}	0.34^{ax}	0.29^{ax}	0.25^{ax}	0.30^{ax}	0.29^{ax}
Terpineol	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.94^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.50^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
Decanal	1	0.55^{abx}	0.62^{abx}	0.35^{bx}	0.59^{abx}	0.72^{abx}	0.64^{abx}	0.98^{ax}	0.78^{ax}	0.63^{abx}	0.73^{abx}
	21	0.57^{ax}	0.71^{ax}	0.66^{ax}	0.56^{ax}	0.55^{ax}	0.89^{ax}	0.67^{ax}	0.58^{ax}	0.70^{ax}	0.71^{ax}
Citronellol	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.78ax	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.24^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
Benzothiazole	1	0.07^{abcx}	0.08^{abcx}	Nd^{cx}	0.03^{bcx}	0.03^{bcx}	nd^{cx}	0.08^{abcx}	0.16^{abx}	Nd^{cx}	0.19^{ax}
	21	0.09^{abx}	0.16^{abx}	0.03^{bx}	0.06^{abx}	0.07^{abx}	0.12^{abx}	0.08^{abx}	Nd^{bx}	0.26^{ax}	Nd^{bx}
Neral	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.37^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.15^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
Geraniol	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.65^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.27^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}

Table 4-2 (continue)

Volatile compounds	Post-mortem (day)	Treatment[e]									
		C	PA	BR	AC	ZI	F31	F60	BA	KJ	ASP
(E)-2-Decenal	1	0.12^{cx}	0.23^{abcx}	Nd^{cx}	0.18^{abcx}	0.47^{ax}	0.20^{abcx}	0.45^{abx}	0.12^{cx}	0.13^{cx}	0.15^{bcx}
	21	0.21^{bcx}	0.33^{bcx}	0.17^{bcx}	0.23^{bcx}	0.30^{bcx}	1.05^{ay}	0.35^{bcx}	0.44^{bcx}	0.56^{abx}	Nd^{cy}
Geranial	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.47ax	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.15ax	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
Undecanal	1	0.11^{ax}	0.23^{ax}	0.19^{ax}	0.01^{ax}	0.15^{ax}	Nd^{ax}	0.22^{abx}	0.23^{ax}	0.21^{ax}	0.01^{ax}
	21	0.09^{abx}	Nd^{by}	0.16^{abx}	0.26^{ax}	0.13^{abx}	Nd^{bx}	0.12^{abx}	0.06^{bx}	0.15^{abx}	0.13^{abx}
Tetradecane	1	0.61^{abx}	0.57^{abx}	0.41^{abx}	0.66^{abx}	0.63^{abx}	0.38^{abx}	0.79^{ax}	0.71^{abx}	0.33^{bx}	0.37^{bx}
	21	0.22^{by}	0.36^{abx}	0.28^{aby}	0.46^{abx}	0.31^{aby}	0.47^{abx}	0.50^{ax}	0.34^{abx}	0.42^{abx}	0.34^{abx}
Dodecanal	1	0.25^{abx}	0.25^{abx}	0.24^{abx}	0.24^{bx}	0.38^{abx}	0.17^{bx}	0.51^{ax}	0.38^{abx}	0.16^{bx}	0.35^{abx}
	21	0.21^{ax}	0.28^{ax}	0.35^{ax}	0.32^{ax}	0.31^{ax}	0.33^{ax}	0.36^{ax}	0.39^{ax}	0.31^{ax}	0.36^{ax}
ar-Curcumene	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	1.97^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.97^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
Zingiberene	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	1.50^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.32^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
(E,E)-a-Farnesene	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	1.09^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.28^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
b-Sesquiphellandrene	1	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	1.09^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}
	21	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	0.15^{ax}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}	Nd^{bx}

Table 4-2 (continue)

Volatile compounds	Post-mortem (day)	Treatment[e]									
		C	PA	BR	AC	ZI	F31	F60	BA	KJ	ASP
Tetradecanal	1	0.56^{abx}	0.53^{abx}	0.41^{bx}	0.50^{abx}	0.73^{abx}	0.38^{bx}	0.85^{ax}	0.70^{abx}	0.44^{bx}	0.73^{abx}
	21	0.54^{abx}	0.47^{abx}	0.57^{abx}	0.63^{abx}	0.50^{abx}	0.71^{aby}	0.73^{abx}	0.39^{bx}	0.37^{bx}	0.85^{ax}
Hexadecanal	1	6.21^{abcx}	3.72^{bcx}	4.39^{bcx}	5.14^{abcx}	9.25^{ax}	3.83^{bcx}	6.98^{abcx}	7.00^{abcx}	2.92^{cx}	7.60^{abx}
	21	4.29^{bcdx}	4.69^{bcdx}	5.16^{bcdx}	5.51^{bcdx}	1.98^{dy}	7.46^{abcy}	7.65^{abx}	3.37^{cdx}	3.13^{dx}	9.70^{ax}

[a,b,c,d]: mean values with different superscripts within the same row (different treatment in same volatile compounds) differ significantly ($p<0.05$).

[x,y]: mean values with different superscripts within the same column (different post-mortem in same volatile compounds) differ significantly ($p<0.05$).

[e]: ratio to internal standard; C: control; PA: papain; BR: bromelain; AC: actinidin; ZI: zingibain; F31: fungal 31 ; F60: fungal 60; BA: bacterial; KJ: kiwi juice; ASP: *asparagus*.

Nd: not identified.

4.2.3 Multivariate study of cooked beef volatiles profile after pre-treatment with proteases at 1 day and 21 days post-mortem storage

PCA was carried out to assess the variation in the volatile compounds from cooked beef subjected to pre-rigor injection with nine proteases at 1 day and 21 days post-mortem. In order to illustrate differences between each treatment on the basis of individual volatile compounds, PCA was carried out. PCA shown in Figure 4-4 described 29.57% and 23.60% of the total variation of factor 1 (F1) and factor 2 (F2), respectively. F31 21 days and F60 1 day samples had high positive scores and were separated from the other protease treated samples along F1. Sample F31 21 days had corresponding high positive loadings of benzeneacetaldehyde (32), 2-acetylthiazole (28), benzaldehyde (21), and 1-octen-3-ol (23). In addition, 2, 3 pentanedione (3) and 2-heptanone (14) were associated with the F60 1 day treated sample. All these volatile compounds were found to be significantly different ($p< 0.05$) as shown in Table 4-2 It was found that a total of twelve volatile compounds were associated with ZI treated samples (ZI 1 day and 21 days) that had high negative scores along F1 and were separated from the other protease treated samples (Figure 4-4). These volatile compounds included camphene (20), 1, 8-cineole (31), terpineol (40), citronellol (42), citral (44), geraniol (45), geranial (47), α-curcumene (51), zingiberene (52), α-farnesene(53), and β-sesquiphellandrene (54) that had negative loadings along F1. These volatile compounds have been reported in ginger (Bartley & Jacobs, 2000; Onyenekwe & Hashimoto, 1999). Zingiberene and 1,8-cineole have been described as being spicy and pungent respectively. Sullivan & Calkins (2010) further reported that the tenderizing of beef meat with ginger extracts gave significantly greater off-flavour ratings ($p< 0.0001$).

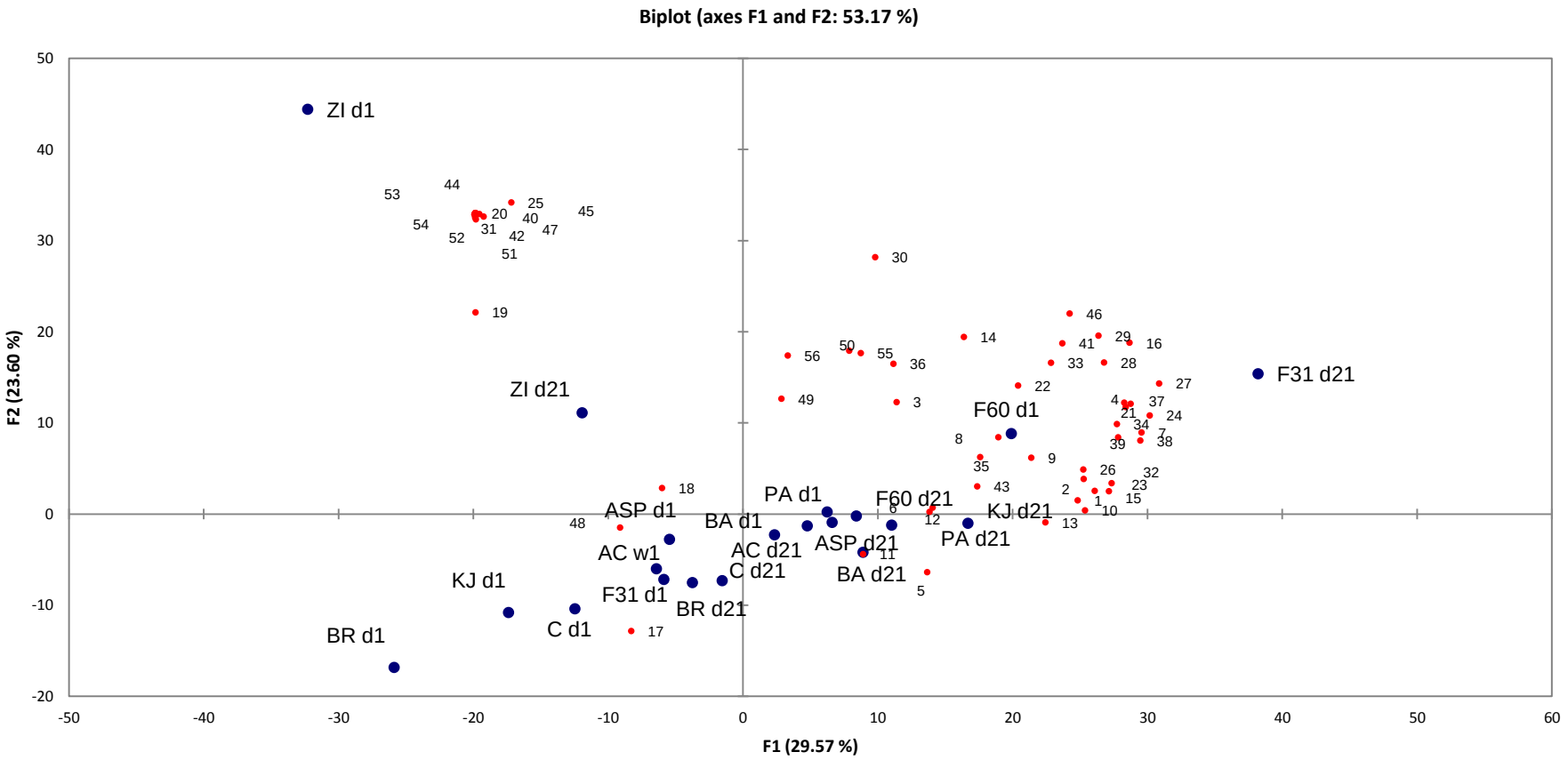

Figure 4-4 Bi-plots of F1 and F2 for the volatile compounds of cooked beef pre-treated with proteases and control

The variables are numbered the same as in Table 4-1, d1: 1 day post-mortem storage, d21: 21 days post-mortem storage

C: control; PA: papain; BR: bromelain; AC: actinidin; ZI: zingibain; F31: fungal 31; F60: fungal 60; BA: bacterial; KJ: kiwi juice; ASP: *asparagus*

In order to clearly illustrate differences between 1 day and 21 days post-mortem samples for all treatments, PCA was carried out without the ZI treated sample group (1 day and 21 days). The PCA shown in Figure 4-5 described 37.40% and 12.96% of the variation for F1 and F2, respectively. The 1 day (except F1，KJ treatment) and 21 days (except AC treatment) post-mortem groups were clearly separated along F2, with 1 day samples having negative scores and 21 days having positive scores. The positive loadings along F2 were described by 3-methylbutanal (1), 2-methylbutanal (2), benzeneacetaldehyde (32), and benzaldehyde (21). These compounds were significantly different ($p<0.05$) for 1 day and 21 days post-mortem samples (Table 4-2). Benzeneacetaldehyde (32) and benzaldehyde (21) are derived from proteolysis and amino acid degradation (Pham et al, 2008). 3-methylbutanal (1) and 2-methylbutanal (2) can be formed from the Strecker degradation (Mottram, 1998). Koutsidis et al (2008) reported that free amino acids, such as leucine, isoleucine, serine, threonine, valine and phenylalanine, increased during conditioning, particularly between days 7 and 14 and are important in the formation of strecker aldehydes, such as 2- and 3-methylbutanal and other aroma compounds such as pyrazines.

The PCA biplot shown in Figure 4-6 described 12.96% and 10.21% of the variation for F2 and factor 3 (F3), respectively. The KJ and AC treated samples were separated along F3 with the former having positive scores and the latter negative scores. The KJ 21 days treated sample corresponded to high positive loadings of 3-methylbutanal (1) and benzaldehyde (21). Han et al (2009) reported that lamb infused with kiwi fruit juice was associated with significant degradation of the myofibrillar proteins, appearance of new peptides and activation of m-calpain during post-mortem aging. Jordan et al (2002) reported 3-methylbutanal (1) as an aroma active compound that contributed to the flavour of aqueous kiwi fruit essence. In addition, Garcia et al (2011) reported the presence benzaldehyde in baby kiwi fruits. Benzaldehyde has been associated with a strong almond odour (Table 4-1).

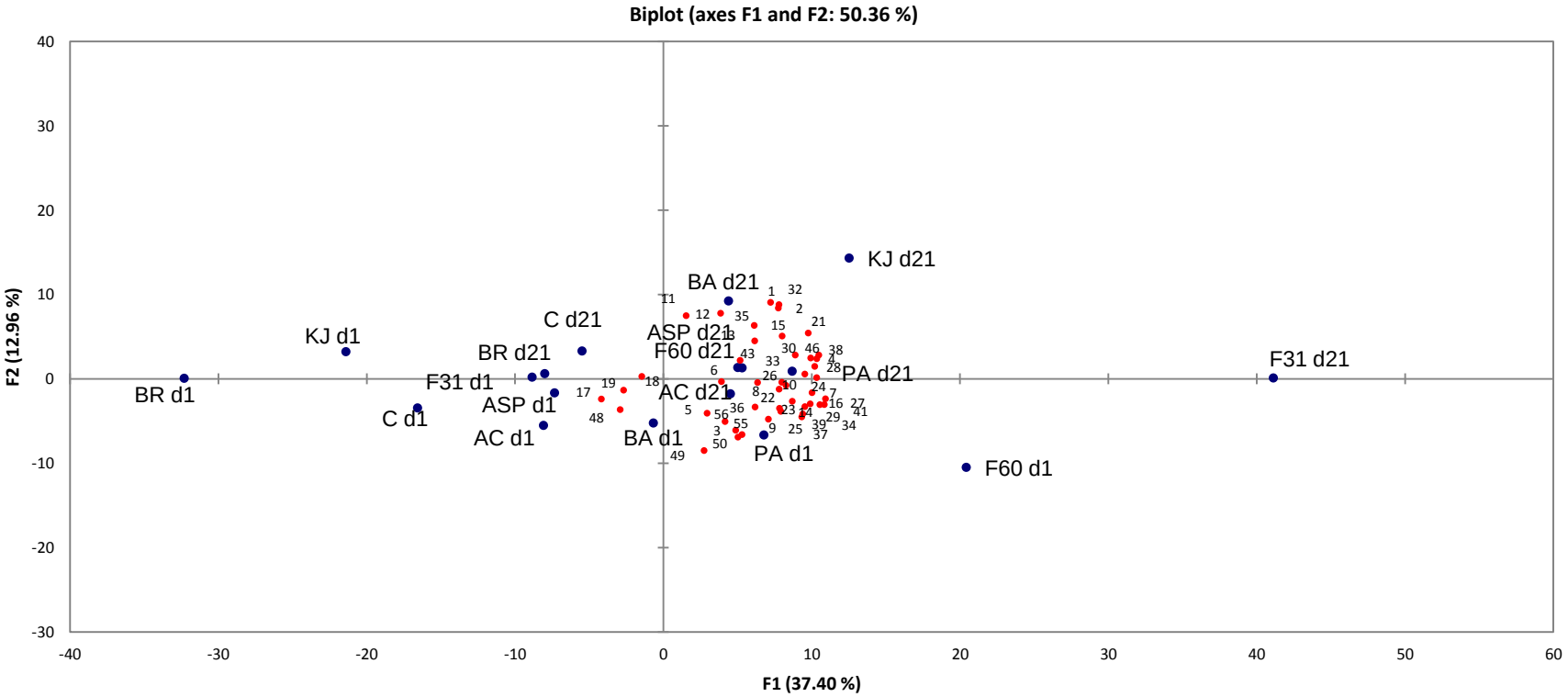

Figure 4-5 Bi-plots of F1 and F2 for the volatile compounds of cooked beef pre-treated with proteases and control

The variables are numbered the same as in Table 4-1, d1: 1 day post-mortem storage, d21: 21 days post-mortem storage

C: control; PA: papain; BR: bromelain; AC: actinidin; ZI: zingibain; F31: fungal 31; F60: fungal 60; BA: bacterial; KJ: kiwi juice; ASP: *asparagus*

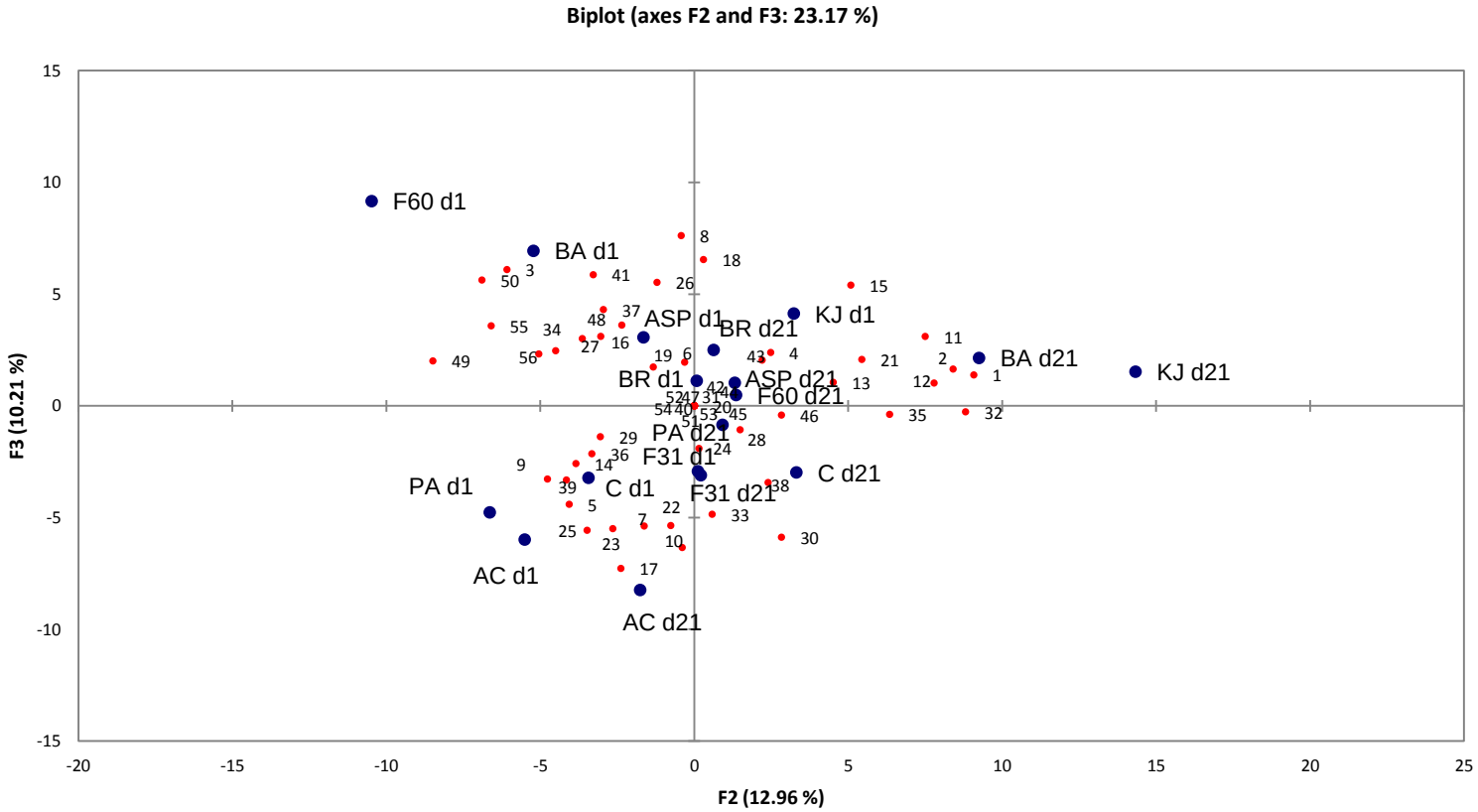

Figure 4-6 Bi-plots of F2 and F3 for the volatile compounds of cooked beef pre-treated with proteases and control

The variables are numbered the same as in Table 4-1, d1: 1 day post- mortem storage, d21: 21 days post-mortem storage

C: control; PA: papain; BR: bromelain; AC: actinidin; ZI: zingibain; F31: fungal 31; F60: fungal 60; BA: bacterial; KJ: kiwi juice; ASP: *asparagus*

Chapter 5 Conclusion

5.1 Conclusion

The effects of pre-rigor injection of topside beef meat with nine proteases from plant and microbial sources, after one day and twenty-one days post-mortem storage, on the volatile profile of cooked beef revealed some significant changes in the flavour profile of beef meat. Beef meat injected with BR, PA, ASP, AC, and KJ (except KJ 21 days) proteases at post-mortem for meat tenderization resulted in fewer changes in volatile compounds compared to the control sample. This indicated that the use of these proteases was unlikely to influence the characteristic cooked beef meat flavour. Eleven volatile compounds characteristic of ginger flavour were only detected in meat treated with ZI at 1 day and 21 days post-mortem storage. Generally there was an increase in most volatiles at 21 days post-mortem storage and this could be attributed to an increase in the total free amino acid pool as meat ages. Quantitatively, the most significant changes were observed for aldehydes as a result of post-mortem storage. Such a change was likely due to increased Maillard reaction-derived flavour compounds. Benzaldehyde concentration increased in F31 and ASP treated samples from 1 day to 21 days post-mortem storage. An increase in 3-methylbutanal was also observed in KJ, BA, BR and F31 treated samples at 21 days post-mortem storageIn addition, 3-methylbutanal and benzaldehyde increased in the KJ 21 days treated samples. Although post-mortem aging process is identified with enhancement of beef sensory quality due to enhanced tenderization this may not be true for the overall flavour of meat. Hence further work is required to examine the effects of protease and post-mortem storage on beef sensory characteristics. This would allow further correlation between volatile compounds and sensory flavour characteristics to be examined in order to understand the effects of meat tenderizing proteases on the flavour quality of beef at different post-mortem storage.

5.2 Limitations of this research

Eleven treatments, including control, water injected and nine proteases injected, were included in the experimental design. However as the water injected samples were accidentally thawed, the new water injected samples that came from a different batch of experiment were significantly different ($p<0.05$) to control and other treatments. As a result comparisons across the treatment could not be made against the water injected samples. It would also have been better statistically if each replicate sample for the all treatments came from the same animals to reduce variability in the results.

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