Ultimate pH and Storage Related Changes to Sheepmeat Odour and Flavour

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Terence John Braggins

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Abstract

Gas chromatography/mass spectrometry (GC/MS), gas chromatography/olfactometry and a trained sensory panel were used to study the effects of meat ultimate pH on the odour and flavour of cooked sheepmeat. As part of methods development, supercritical carbon dioxide elution was evaluated as an alternative to thermal desorption of volatile compounds from Tenax-TA traps, but this technique did not offer any significant advantage. In a separate study, the sensory panel and an emerging technology, an electronic nose, were used to investigate the changes in cooked meat odour and flavour arising from long term storage of chilled lamb legs in a carbon dioxide atmosphere.

Pre-slaughter injection of adrenalin was used to produce sheepmeats of normal (5.66), moderate (6.26) and high (6.81) ultimate pH. Meat with a moderate or high ultimate pH had a significantly lower overall cooking odour and flavour intensity, as assessed by the sensory panel, than sheepmeat of a more acceptable pH (5.66). Panellists also found that desirable odour and flavour notes decreased and undesirable ones increased as ultimate pH increased. Purge-and-trap GC/MS of fat rendered during cooking identified 57 (out of a total of 325) volatile compounds that significantly decreased in concentration with increasing meat pH. The most common of these compounds were aldehydes. GC/olfactometry identified 54 odour-active compounds, 10 of which were also found to be responsive to changes in meat ultimate pH. Most of these compounds were again aldehydes.

High ultimate pH raw meat which was titrated with hydrochloric acid to pH levels close to that of low ultimate pH had odour and flavour intensities comparable to those of normal (low) ultimate pH meat. Most of the volatile compounds found by GC/MS analysis to decrease in concentration with increasing ultimate pH did not have a reduced concentration after this downward pH adjustment. These results and parallel analyses of soluble protein, free amino acids and free fatty acids led to the conclusion that pH-related changes in the odour and flavour of cooked sheepmeat are probably mediated by direct

pH effects during the cooking process, rather than by a pH-related development of odour and flavour precursors in raw meat before cooking begins.

Meat pH values of lamb legs increased significantly between 4 and 14 weeks storage in the CO_2 aging. Sensory panellist found there were significant storage time effects in "overall", "sheepmeat", and "sweet" odours of cooked meat. Panellists also detected a significant decrease in "sweet" and "roasty" flavours in the CO_2-packed lamb legs stored at -1.5°C compared with vacuum packed controls stored frozen (-35°C). Of particular concern, after 14 weeks chilled storage in CO_2, the intensity of a "livery/offaly" flavour was significantly higher than for the frozen-stored control.

The electronic nose could detect differences in headspace volatiles of both raw and cooked minces from meat subjected to the different storage treatments. The results suggest that, under these experimental conditions, the electronic nose was more discerning and possibly gave more reproducible results than sensory panellists. What relationship the electronic nose's measurements have to human perception of odour has yet to be determined.

The multidisciplinary approach used in this thesis has helped to explain some of the variations in meat flavour quality important to the New Zealand meat industry.

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Chapter 1.

General Introduction

The New Zealand economy is currently heavily dependent on the export meat industry, and there are no significant signs that this will change in the next 20 years. The $4 billion industry contributes 18 percent of the country's total merchandise trade earnings. These are derived almost equally from sheep and cattle. The red meat industry provides jobs for 37,000 farm workers, and around 20,000 people are involved in meat processing.

At the industry's peak in the 1970s, sheep numbers stood at around 70 million. Since then, numbers have steadily declined to the current 48 million. Beef cattle numbers have slowly increased over the years and now stand at around 5 million. Both changes are undoubtedly linked to economic issues. Land for pastoral meat production continues to come under pressure from alternative land uses such as forestry, dairying, horticulture and subdivision to lifestyle farms and for urban development. Reduced financial returns to beef and sheep farmers, increased and more stable returns to dairy farmers and potentially greater returns from forestry, particularly on marginal farm land, are the main causes of the downturn in animal numbers.

The two biggest traditional markets for New Zealand meat are North America, which buys more than 80 percent of all beef and 5 percent of all sheepmeat exported from New Zealand, and Europe, which takes more than 65 percent of all New Zealand's sheepmeat exports. New expanding markets include the Middle East and Asia, where both populations and economies are rapidly expanding.

Market surveys have shown that attitudes to consumption of red meats are changing. Concern over food safety and interest in the nutritional value of red meat is increasing. Consumer life styles are changing such that people have less time to prepare meals and the switch to convenience meals has meant that many people are losing the skill to cook meat suitably. Worldwide, red meat production has remained static over the last 10 years while

chicken and pork production is rising steadily from between 4 and 5 percent annually. This is due, in part, to the incorrect perception that red meats are less healthy to eat than white meats. Given these competitive market pressures, increasingly more discerning and quality and health-conscious consumers require producers to be more knowledgeable of their product and give greater attention to its quality.

New Zealand has a temperate climate with abundant rainfall, and its varied and fertile soils make it well-suited to pastoral farming. Mild winter conditions do not demand indoor sheltering and grain feeding. Most of the country is also blessed with a year round plentiful supply of forage. One corollary of this is that New Zealand red meats have a distinctive pastoral flavour compared with meat from grain-fed animals in many Northern hemisphere countries. Some markets, particularly the markets of North America and Japan, prefer the milder flavour of meat from grain-fed animals over the stronger flavour of pastorally farmed animals.

Another consequence of pastoral farming practices is pre-slaughter stress-induced changes in meat quality. Both psychological stress (mediated through increased circulation of adrenaline) and physical stress before slaughter can cause a rapid decline in muscle glycogen. A similar result, but at a much slower rate, can occur during starvation.

Glycogen is the main carbohydrate energy store for muscle tissue. During exercise, glycogen is broken down by anaerobic glycolysis and the oxygen-dependent citric acid cycle. Upon death of the animal, the oxygen supply is rapidly depleted and glycogen is broken down anaerobically to lactic acid in a vain attempt to resynthesise adenosine triphosphate (ATP) to help maintain structural and functional integrity of the muscle. The subsequent rise in H^+ concentration is manifest as a decline in muscle pH to an ultimate level that is dependent on the initial level of glycogen, creatine phosphate and adenosine triphosphate supply (Lawrie, 1985).

The ultimate pH of meat, the rate of pH decline and the temperature at which the process occurs each contribute to the final physical appearance, functional properties and eating

quality of meat. For example, accelerated pH decline to a low ultimate pH combined with high carcass temperatures can cause a problem, particularly in pigs, of pale, soft and exudative meat (the so-called PSE condition) (Offer, 1991).

A major challenge confronting the meat industry, particularly in New Zealand, is the control of pre-slaughter stress-induced depletion of muscle glycogen. New Zealand farming practices can contribute to this problem. Infrequent contact with humans and farm dogs, and social regrouping and yarding all contribute to the psychological stress of animals during handling pre-slaughter. Excessive physical exercise and mismanagement of animals before slaughter can also reduce muscle glycogen stores. Repletion of muscle glycogen in ruminants is 5 to 10 times slower than in monogastrics (Tarrant, 1988). Low muscle glycogen reserves immediately before slaughter can produce meat with a higher than desirable (5.5) ultimate pH. In its extreme, meat of a dark, firm and dry appearance (DFD) is produced.

Excessively high ultimate pH meat creates problems during storage and retail display and has reduced organoleptic characteristics. At pH values above 5.8 the keeping quality of fresh chilled meat is adversely affected because of altered bacterial growth. This is due to the lower content of glucose, lactic acid, and pH itself (Gill and Newton, 1981). The resulting reduction in the shelf life of high pH meat makes it unsuitable for the vacuum-packed chilled meat trade. Elevated ultimate pH can also affect eating quality, particularly tenderness (Howard and Lawrie, 1956; Hedrick *et al.*, 1961; Devine *et al.*, 1993). As the ultimate pH increases from 5.5 to 6.0, the tenderness of cooked meat decreases although above pH 6.0 the effect is reversed (Devine *et al.*, 1993). The dark colour of high pH meat makes it less attractive to the consumer, who may suspect that the meat comes from a very old animal or has been badly stored. In fact, the dark appearance is due to increased translucence. Incident light can penetrate high pH meat more deeply and is strongly absorbed by myoglobin, causing the meat to appear dark red (Offer *et al.*, 1989).

Moreover, it has also been suggested that high ultimate pH affects cooked beef flavour (Lawrie, 1985; Purchas, 1986). Sensory studies have shown that high pH beef is less

flavourful (Dransfield, 1981; Purchas *et al.*, 1986), has more "off" flavours (Fjelkner-Modig and Ruderus, 1983) and evokes more negative flavour reactions by panellists than normal pH beef (Dransfield, 1981; Dutson *et al.*,1981).

Studies to date have concentrated only on sensorial effects of high ultimate meat pH on cooked meat flavour. Instrumental measurement of quantitative and qualitative chemical changes in cooking odours caused by high ultimate pH have been overlooked by most flavour researchers. However, in a brief report, Park and Murray (1975) noted large differences in the semi-qualitative composition of the steam-volatile fraction from normal and high pH meat. No mention was made of the species tested or types of compounds involved.

Surveys by Graafhuis and Devine (1994) have revealed that the ultimate pH of beef and sheep slaughtered in New Zealand can be variable, with 30 percent of animals from both species exhibiting pH values greater than 5.8. This variability and the importance of assured production of meat of good eating quality warrant detailed investigation into the effect that pH has on cooked meat odour and flavour.

Accordingly, a major part of this thesis will explore the effects high ultimate pH meat has on the cooked meat odour and flavour, as assessed by sensory panels and instrumental analysis by gas chromatography coupled to mass spectrometry.

A second part of this thesis will explore odour and flavour changes after prolonged chilled storage under carbon dioxide. Historically, New Zealand farmers initially focused on wool production as a major export earner. Lack of refrigeration and geographic position remote from the familiar European markets confined sale of sheepmeat to the local market. The introduction of refrigeration in the 1880s gave New Zealand the opportunity of supplying Northern hemisphere countries, mainly Britain, with large quantities of frozen meat. This stable market continued to grow until the 1970s when Britain joined the European Economic Community. In subsequent years demand from Britain for New Zealand produced sheepmeat decreased along with changes in customer preferences for red meats.

No longer could meat producers export only frozen dressed carcasses. Competition from non-red meats (pork and chicken) on world markets is increasing steadily.

In a drive to market table-ready cuts of New Zealand beef and lamb, scientists at the Meat Industry Research Institute of New Zealand (Inc.,) (MIRINZ) developed a method to transport chilled meat, in a concentrated carbon dioxide (CO_2)-rich atmosphere, to Europe by sea freight.

Carbon dioxide controlled atmosphere packaged (CAP) meat, held chilled between -0.5 and -1.5°C, has a reliable hygienic shelf life of at least 16 weeks. The high partial pressure of oxygen-free CO_2 slows the growth rate of spoilage organisms (Gill and Penny, 1986; Gill, 1990).

However, some potentially negative consequences on long-term stored chilled meat can occur. In a brief report, Gill (1988s) mentioned that carbon dioxide controlled atmosphere "stripped" ovine flavour from sheepmeat and gamey flavour from venison - perhaps seen by some consumers as an enhancement. He also commented that with prolonged storage, red meat develops aged livery flavours probably in response to peptides released during the breakdown of proteins. However, little evidence of this effect in red meats can be found in the scientific literature. Proteolytic changes during prolonged storage can lead to loss of desirable texture characteristics.

In 1995 almost 6 percent of all lamb exported from New Zealand was as CO_2 CAP chilled meat. It is surprising, considering the greater importance the New Zealand meat industry is currently putting on chilled meat exports, that so little work has been done on understanding the biochemical changes that may occur during prolonged storage of red meat in a saturated CO_2 atmosphere, and how these changes may affect cooked odour and flavour. Thus, as part of this thesis, a study was conducted on the effects prolonged storage of raw meat in a CO_2 atmosphere has on the odour and flavour of cooked meat. This work is presented in Chapter 6.

Investigations of factors that affect meat odours and flavours require an objective method to measure volatile compounds liberated during cooking. Identification and quantification are usually done by gas chromatography (GC) combined with either mass spectrometry (MS), flame ionization detection (FID), odour port analysis (OPA) or combinations of these detection methods.

Current methods to collect volatile compounds from foods, fats and oils for instrumental analysis include steam distillation, simultaneous distillation and extraction (SDE) (Likens and Nickerson, 1964) and solvent extraction. These multistage workup methods tend to distort the relative proportions of the volatile compounds and so give only qualitative information. Direct thermal desorption (Dupuy *et al.*, 1973), static headspace sampling (Macku *et al.*, 1988) or dynamic headspace sampling with porous polymer (e.g. Tenax) trapping (MacLeod and Ames, 1986a) are alternative methods. Headspace analysis techniques isolate volatiles in equilibrium with food that are more representative of the compounds noted by the sense of smell.

Dynamic headspace sampling with porous polymer trapping allows concentration of volatiles from small quantities of sample. A popular trapping material is a porous polymer based on 2,6-diphenyl-*para*-phenyleneoxide (Tenax-GC or TA). Tenax-TA has been the porous polymer of choice because of its good trapping efficiency of a wide range of food-derived organic compounds at ambient temperature, and its high thermal stability (up to 450°C), relative low water retention during trapping and low bleed (breakdown) during thermal desorption (Macleod and Ames, 1986a).

Although volatile compounds are trapped at relatively low temperatures (around ambient and below), thermal desorption takes place at temperatures up to 260°C for periods as long as 20 minutes (Mottram *et al.*, 1982). Concerns about possible thermal conversion of labile compounds during thermal desorption of food volatiles from Tenax traps have also been raised (Lewis and Williams, 1980; Snyder and Mounts, 1990).

An alternative to thermal desorption is solvent elution (Vercellotti *et al.*, 1992). However, each eluent must be pre-concentrated before analysis and concentration of solvent contaminants is possible. Recently Hawthorne *et al.* (1986) and Wrong *et al.* (1991) investigated supercritical carbon dioxide as an alternative eluent for Tenax traps.

Compared with thermal desorption, supercritical carbon dioxide (SCO_2) elution has an advantage of eluting volatile compounds at relatively low temperatures, thus avoiding possible thermal degradation effects. Because of this advantage, it was decided to evaluate SCO_2 as an alternative elution procedure to thermal desorption and organic solvent elution of food flavour volatile compounds from Tenax traps. This work is presented in Chapter 3.

Recent advances in chemical sensor technology has results in the commercial production of an electronic volatile chemical sensor or olfactometer - more commonly called an electronic nose. This instrument emulates the sense of smell by combining an array of semiconducting chemical sensors and pattern recognition algorithms to discriminate between samples of different volatile composition or intensity. This new and exciting technology is briefly evaluated as a means of discriminating meat samples stored for prolonged periods in an CO_2 atmosphere. A review of this technology and the results of this study is also presented in Chapter 6.

In summary, this thesis addresses three main issues: first, to evaluate supercritical carbon dioxide as an alternative elution procedure to thermal desorption and organic solvent elution of food flavour volatile compounds from Tenax traps (Chapter 3); second, to explore the effects of the high ultimate pH condition on the cooked meat odour and flavour, as assessed by sensory panels and gas chromatography coupled to mass spectrometry (Chapters 4 and 5); and third, to explore the effects of prolonged storage of raw meat in a CO_2 atmosphere on the odour and flavour of the cooked meat as assessed by sensory panels and an electronic nose (Chapter 6). General conclusions are presented in Chapter 7.

Chapter 2.

The Background of Odour and Flavour Development in Cooked Meat

2.1 Postmortem Changes in Muscle Metabolism

Living muscle uses glycogen stores by the anaerobic glycolysis pathway and the aerobic citric acid cycle, which together supply adequate levels of adenosine triphosphate (ATP) for contraction and maintenance of structural integrity. During bursts of extreme muscular activity, particularly in larger animals, the circulatory system cannot completely sustain aerobic metabolism. Instead, muscles must use their stored glycogen as the fuel to generate ATP anaerobically, with lactate as the product. Later, the lactate is transported by the blood from the muscle to the liver where it is slowly converted back to glucose during a subsequent rest period.

On death, a similar survival mechanism attempts (but in vain) to maintain structural and functional integrity of the muscle. The mechanism is the same but with a significantly different outcome.

Soon after death muscles are deprived of life-supporting oxygen, and anaerobic breakdown of glycogen becomes the main generator of ATP. Anaerobic degradation yields only about one tenth of the aerobic production of ATP, so glycogen stores are consumed about 10 times faster than under aerobic conditions (Bendall, 1973). Lactate is the end product, as with living muscle, and is accompanied by the production of one proton (H^+) per lactate molecule. Without a circulating blood supply, accumulating lactic acid cannot be transported to the liver for resynthesis to glycogen. The result is that the pH of the meat falls considerably (from 7.0 to around 5.5).

Bodwell *et al.* (1965) followed various chemical changes in beef muscle from 10 minutes after slaughter until 20 days postmortem. Their results, plotted over the first 48 hours

(figure 2-1). Initially, ATP is generated from high energy reserve creatine phosphate (CP). Simultaneously, ATP is hydrolysed by ATPase enzymes (particularly myosin-ATPase) to ADP (Bendell, 1973). Rapid depletion of CP reserves and subsequent build up of ADP initiates catabolism of glycogen. During this time of rapid glycolysis, lactic acid concentrations rise with a concomitant fall in pH. At a critical point of about pH 6.2, glycolytic enzymes are inhibited causing a net loss in ATP. Once ATP concentration decreases below 0.1 mM, stiffening and loss of extensibility of the musculature occurs. This process, rigor mortis, is due to insufficient levels of available ATP to keep the principal contractile proteins, actin and myosin, apart. They unite irreversibly to form inextensible actinomyosin (Lawrie, 1992). Sometimes ATP can still be present in muscle after rigor development as a rapid decline in pH can inhibit ATPase, preventing hydrolysis of ATP to ADP, and separation of the actinomyosin complex (Pearson and Young, 1989).

The rate and degree of postmortem pH fall is related to many inherent factors such as animal species and genotype, diet, age, the type of muscle and the intramuscular location. External postmortem factors such as temperature, time, and electrical stimulation (Chrystall and Hagyard, 1976), also affect the rate of glycolysis and thus pH fall.

The rate of pH decline can be around twice as fast in pigs compared with rabbit, sheep and beef muscle at the same temperature (Hallund and Bendall, 1965; Greaser, 1986) and even faster than horse muscle (Lawrie, 1985). The rate of pH decline can also be faster than is desirable. In some pig genotypes, rapid pH fall at elevated temperatures produces meat that is pale, soft and exudative (PSE). This condition is caused by early denaturation of muscle protein, particularly myosin, and depends on an interaction between the rate of pH fall, final pH and the chilling regime (Offer, 1991).

Rate of pH decline also varies between muscle types and is related to physiological functionality of individual muscles and muscle fibres. Muscles fall conveniently into two major classes according to their colour - red and white. This colour difference is due to the relative content of the pigment myoglobin, which acts as an oxygen reservoir. Red muscle fibres contain more respiratory enzymes whereas white muscle fibres undertake

fast, intermittent, and largely anaerobic contraction. Thus, white muscle contains greater stores of glycogen, high ATPase activity and a high content of CP to replenish ATP from ADP (Lawrie, 1992).

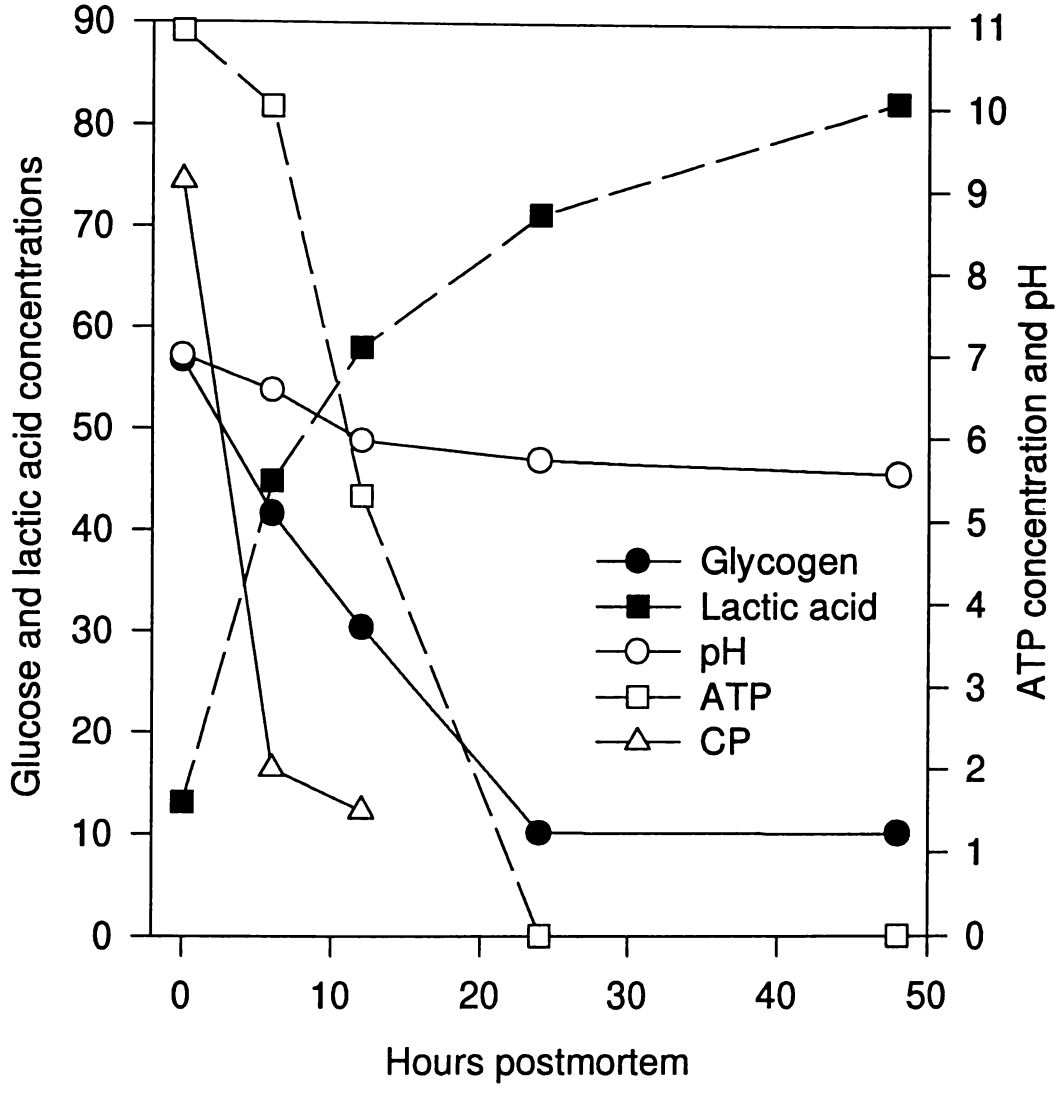


Figure 2-1.
Values for pH and chemical constitutents in beef muscle at various times postmortem. From Bodwell *et al.* (1965). Values are average observations on longissimus dorsi muscle from five carcasses and are expressed in umol g^{-1} fresh tissue.

A useful classification scheme is based on contraction rate and energy metabolism. Identified muscle can be classified into three major functional groups: slow-twitch-oxidative (Type I), fast-twitch-oxidative-glycolytic (Type IIA), and fast-twitch-glycolytic (Type IIB) (Peter *et al.*, 1972). Their relative distribution varies within and between muscles and helps explain variations in the rates of pH fall. Aalhus and Price (1991a) found that muscles with a high proportion of glycolytic fibres had slow pH declines whereas muscles with a low proportion of glycolytic fibres had a rapid pH decline. This is contrary to what could be expected since muscles with a high porportion of glycolytic fibres have high glycolytic and lactate dehydrogenase (converts pyruvate to lactic acid) activity. Aalhus and Price (1991a) postulate that muscles with a high glycolytic activity also have a high buffering capacity (Monin, 1981; Rao and Gault, 1989) which would also reduce the rate of pH decline.

Probably the most important influence on the rate of postmortem glycolysis is temperature. As with most biological reactions in warm blooded organisms, a 10°C rise in temperature roughly doubles the reaction rate and this rule also hold true in postmortem muscle metabolism.

Postmortem glycolysis is most rapid at normal mammalian body temperatures (37 - 39°C) and explains some variations in the rate of pH fall found between muscles at different locations within a cooling carcass (Aalhus and Price, 1991a). Muscles found deep within the carcass cool more slowly than their topical counterparts, so one would expect faster rates of glycolysis at the former regions (Bendall, 1978). Overall variation in postmortem pH decline is collectively related to initial glycogen stores, rate of cooling, and the fibre type composition of the muscle (Jeremiah, 1978).

The temperature range in which postmortem glycolysis occurs can have detrimental effects on the eating quality of meat. Shortening of the muscle can occur at extremes of temperature. Shortening can occur if rigor is allowed to develop at high temperatures - termed 'high temperature rigor'. Another condition, called 'cold-shortening' can also occur if rigor is allowed to develop below 10°C before post mortem glycolysis has bought

the pH below about 6.0 (Locker and Hagyard, 1963; Honikel *et al.,* 1983). The toughness created by cold-shortening can be prevented by electrically stimulating the carcass immediately after slaughter (Carse, 1973; Chrystall and Hagyard, 1976). Electrical stimulation rapidly reduces ATP and creatine phosphate by inducing muscular contraction. Rapid fall in postmortem pH and onset of rigor mortis before the muscle cools below a critical temperature are the main benefits (Davey *et al.,* 1976). This process is now common practice within slaughter houses in New Zealand and allows faster carcass processing without loss of product quality.

Prevention of cold shortening is not the only benefit of electrical stimulation. Smulders and Eikelenboom (1986) showed that electrical stimulation improved tenderness of meat not exposed to cold shortening. Improvement in muscle colour and meat flavour has also been reported (Savell, 1979). These improvements may be due to the release of lysosomal enzymes (Dutson *et al.,* 1980) or tearing of the myofibres (Sorinmade *et al.,*1982).

The rate of glycolysis is not the only factor involved in quality during converting muscle to meat. The extent to which glycolysis proceeds has also a major influence on the physical and chemical attributes of meat.

During postmortem glycolysis, glycogen in muscle is metabolized to lactic acid in an approximately stoichiometric manner (Bodwell *et al.,* 1965). Therefore, the ultimate pH of meat is principally related to preslaughter glycogen stores.

The concentration of glycogen found in muscles is dependent on the animal's muscle type and nutritional status. Talmant *et al.* (1986) found a wide variation in ultimate pH (5.55-6.15) in 18 bovine muscles. Ultimate pH correlated negatively with ATPase activity and 'glycolytic potential' (i.e. the amount of compounds transformable into lactic acid). Devine and Chrystall (1989) reported differences between 9 individual muscles for 10 lambs. The pH values varied from 5.65 (*longissimus dorsi*) to 6.08 (*infraspinatus*). This difference is probably associated with muscle fibre type. The former represents a red, fast-twitch oxidative glycolytic (Type IIB) muscle and the latter represents a slow red muscle (fast-twitch oxidative, Type I).

Preslaughter muscle glycogen levels can be depleted by poor nutrition or stress. Physical or psychological stresses are the main causes of muscle glycogen depletion in farmed animals as starvation is highly exceptional. From a meat quality point of view, a normal ultimate pH is around 5.5 - the pH at which muscle proteins reach their isoelectric point. However, in practice, this is not always the case. The result of low muscle glycogen immediately preslaughter is an elevated ultimate pH. The consequences for this condition, at pH > 5.8, is that the meat has a dark, purplish appearance, sticky to touch when cut and is less acceptable to the consumer. This condition is known as 'dark cutting' or 'dark, firm and dry' (DFD).

White muscles may show a greater potential for low ultimate pH because of their greater capacity for glycogen storage, but they are also at greatest risk from glycogen loss (Lister, 1988). As one might expect, the powerful muscles in the back and hind legs would have the higher ultimate pH, arising from repetitive strenuous use before slaughter (Tarrant, 1988).

Psychological stress is mediated by the adrenergic mechanism, eventually converting inactive phosphorylase *b* into the active, *a*, form. Phosphorylase *a* catalyses the first step in the breakdown of glycogen to lactic acid. Stressors such as social grouping, transportation, preslaughter holding condition and handling conditions may be a combination of physical and emotional effects (Lacourt and Tarrant, 1985; Tarrant, 1988).

The effect of preslaughter stressors may be of greater importance in those countries where 'free range' pastoral farming (e.g. New Zealand and Australia) as opposed to intensively-managed feed-lot farming practices are employed (e.g. in North America and Europe). In the former farming practice, infrequent contact with humans and farm dogs, and social regrouping and yarding all contribute to the psychological stress of animals during handling preslaughter (Shorthose, 1988).

Lower energy forage feeding as opposed to higher energy feed-lot diets may also influence the flavour quality of meat. In a recent experiment, cattle finished on grain for 8 weeks

preslaughter gave similar ultimate pH values (5.6) to those of animals finished on pasture alone (Daly, 1996). However, the grain finished animals had almost twice as much post-rigor muscle glucose and 5 times more residual glycogen than their pasture fed counterparts. Similar changes in the level of glucose and changes in meat aroma and flavour of meat from cattle finished on corn has also been reported (Melton *et al.*, 1982). Free sugars are important in the production of flavour compounds when they react with amino acids in the Maillard reaction (discussed in detail later in this chapter).

Whatever the cause of high ultimate pH meat,-stress or diet-, it does create many product quality problems. Customers tend to reject DFD meat because of its dark colour and sticky feel. They believe that it is from old animals and will be tough or has poor eating qualities (McIntyre, 1989). In fact, DFD meat is generally more tender (Dransfield, 1981) than meat with an ultimate pH range of between 5.8 and 6.0 (Devine *et al.*, 1993; Purchas and Aungsupakorn, 1993). High ultimate pH meat also has a higher water holding capacity (WHC) and less water loss when cooked than normal pH meat (Dezeure-Wallays *et al.*, 1984). Increased WHC is an advantage for many processed meat products, because these are required to bind water well. However, the variability of meat pH causes problems. The cured colour stability and WHC of sausages made of the same formulation but with different pH meat will be quite different.

But, any perceived advantages high pH meat has in relation to functional properties of processed meat products is far out weighed by disadvantages of poor colour, susceptibility to microbial spoilage and, as is examined in later Chapters, cooked meat odour and flavour. DFD meat contains little or no glucose so under aerobic storage conditions, amino acids are degraded by microorganisms causing earlier-than-normal accumulation of bacterial metabolites responsible for spoilage odours. High pH meat also affects the storage of vacuum-packed meat. Meat of normal pH is spoiled under anaerobic conditions by the dominant organism, *Lactobacillus* fermenting glucose. Slow accumulation of some fermentation products inhibit growth of competing organisms. The absence of glucose in DFD meat means that the lactobacilli are depleted of their normal substrate and are unable to grow to a density that would prevent the growth of competing organisms. Competing

organisms such as *Enterobacter liquefaciens* are known to produce spoilage odours at low cell densities on DFD meat. Green discolouration, caused by *Alteeromonas putrefaciens*, of vacuum-packed DFD meat also reduces the products' shelf life (Gill and Newton, 1981).

The focus of this thesis is the effect ultimate pH has on the odour and flavour quality of cooked meat. It has been shown that high ultimate pH affects cooked beef flavour (Boulton *et al.*, 1957). A review by Ford and Park (1980) cites many unpublished studies at the Meat Research Laboratory, CSIRO, Australia between 1956 and 1976. In one study, meat from a group of 108 lambs cooked as stewed minces showed a highly significant negative correlation with ultimate pH for both intensity ratings and hedonic scaling of acceptability. Intensity of 'different' aroma showed a smaller but highly significant positive correlation with ultimate pH. They also reported gross qualitative and quantitative changes in the composition of flavour volatile fractions of cooked beef, mutton and lamb from muscles of high ultimate pH (6.3-7.0). In a brief report, Park and Murray (1975) noted large differences in the semi-qualitative composition of the steam-volatile fraction from normal and high pH meat. No mention was made of the species tested or types of compounds involved.

Studies of dark-cutting beef by Dransfield (1981) showed a wide variation in flavour scores of trained panellists assessing steer and bull beef over a wide pH range. Average hedonic flavour scores only fell 3 points of the 14 point scale (-7, dislike extremely; +7 like extremely) when pH was raised from 5.5 to 7.0. However, analysis of individual panellist scores revealed that 8 of the 9 panellists disliked the flavour of DFD meat (although only four panellists showed significant trends). Using flavour intensity scales on grilled beef, revealed a negative relationship between pH and flavour intensity at four of five sites where samples were tested (Langford, U.K.; Dublin, Ireland; Roskilde, Denmark; Kumbach, Germany; and Theix, France.). All sites except Kumbach, showed a negative correlations of flavour intensity with ultimate pH. However, only two sites (Langford and Theix) showed a statistically significant relationship. Consumer trials done by the same author showed DFD meat had poorer flavour.

In the most extensive study to date, Fjelkner-Modig and Ruderus, (1983) used different pre-slaughter handling procedures to generate three groups of bull beef meat: normal (≤5.8), medium (5.9-6.2), and DFD (≥6.2). Meat flavour intensity decreased significantly with increasing pH for vacuum-packed *longissimus dorsi* and *semimembranosus* stored for 0, 5 or 14 days at +4°C. Flavour intensity of normal pH meat increased with storage time, whereas medium pH and DFD meat decreased in flavour intensity from 5 to 14 days storage after a initial increase from 0 to 5 days storage. Panellists also noted an off-flavour and sulphide-like odour in DFD samples after 14 days of storage. However, the contribution of spoilage organisms is unknown as no microbial assessment was made. A similar reduction in beef flavour intensity with pH has been reported by Purchas *et al.*, (1986).

In a recent study Young *et al.* (1993) compared odour and flavour differences between Coopworth and Merino lambs grazed on similar pasture and unexpectedly found a species-related meat pH effect. They suggested that pH, rather than breed, might be the dominant factor affecting cooked meat odour and flavour characteristics. Panellists also registered several negative flavour and odour descriptors for the high pH meat.

Contrasting the above results is a study by Devine *et al.* (1993) on the effect of growth rate and ultimate pH on meat quality of lambs. Various preslaughter stress regimes were applied to 75 entire male lambs to produce a wide range (5.36-6.78) of ultimate pH values. These researchers could find no significant change in aroma and flavour with increasing pH. This may have been due in part to averaging of panellists' hedonic sensory scores (1 = dislike intensely to 9 = like extremely) that may only show personal preferences of the group of panellists (Dransfield, 1981).

Although considerable sensory panel work has been performed, little work has been done to investigate the postmortem biochemical changes that contribute to ultimate pH related changes in meat flavour. Lawrie (1985) suggested reduced flavour intensity of high pH meat is due to the meat's swollen structure interfering with access to the palate of the substances involved. Laser-Reutersward *et al.* (1981) found the concentration of inosine

and hypoxanthine were lower, whereas inosine-monophosphate (IMP) and glycolytic metabolites (glucose, glucose 6-phosphate and fructose-6-phosphate) were higher in normal pH (≤5.8) than in DFD (pH ≥6.21). They suggested that the increased concentration of these metabolites in normal pH meat would contribute to the enhanced flavour compared with DFD meat. The flavour-intensifying effect of IMP and the contribution of inosine and hydroxanthine to bitter notes have been observed for chicken flavour (Kazeniac, 1961).

A more recent suggestion is the pH may have a direct effect on the formation of meat flavour compounds (Farmer, 1992). Generation of heterocyclic disulphides, compounds that frequently possess strong meaty aromas, decrease with increasing pH (Farmer and Mottram, 1990). This theory will be addressed in more detail later in the thesis.

Factors I have addressed thus far have mainly related to the biochemical changes that occur as muscle turns into meat over the first 24 to 48 hours post-slaughter when the ultimate pH is initially stabilized. Yet, most meat is stored for far greater periods before it is consumed. Accordingly, a study of the changes in muscles biochemistry and possible relationships with aroma and flavour of aged meat is appropriate.

It has long been established that meat aging-the biochemical steps leading to tenderization-commences during the very early postmortem period (Joseph and Connolly, 1977) and continues during long-term storage. Much effort has been focused on the effect of aging on meat tenderization (Ouali, 1990) but because of the complexity of the process, it is far from fully understood. Most attention has been applied to two of the many known proteolytic system in muscle because of their likely involvement in muscle protein degradation, thus, tenderization. These two systems are the calcium-dependent neutral proteinases, also called calpains, which play a principal role in tenderization, and the acidic lysosomal proteinases (cathepsins D, B, H, and L). Cathepsin D's role in meat tenderisation is questionable since its pH optimum is between pH 2.8-5.0 (Zeece, 1986).

Far less attention has been given to proteolysis and its effect on flavour changes during ageing and long-term storage. Davey and Gilbert (1966) calculated that 2.3% of sarcoplasmic meat protein is converted by proteolysis into non-protein nitrogen when meat is aged for 32 days at 2°C. Similar increases in non-protein nitrogen and increases in free amino acids were also observed in beef stored over 13 days at 2°C (Parrish *et al.*, 1969). In both cases this proteolysis was independent of observed meat tenderisation. Doty and Pierce (1961) suggested that the increase in the non-protein nitrogen during ageing could be an important factor in flavour changes associated with ageing processes. A more interesting group of proteinases are referred to as the "multicatalytic proteinase complex" and have been shown to hydrolyze sarcoplasmic proteins (Dahlmann *et al.*, 1985). Perhaps this "multicatalytic proteinase complex", is involved in the generation of non-protein nitrogen and free amino acids during ageing.

Flavour changes also occur during storage of meat. Sink and Smith (1972) observed an increase in the levels of lipid soluble carbonyls in beef stored over 14 days at 3°C. Although no sensory evaluation was done in this experiment, the fact carbonyls are involved in the Maillard reaction (discussed below) and have odour in their own right suggest they play a part in the development of meat flavour during storage. Vacuum-packed beef stored for up to 4 weeks at 0.6-3.3°C showed total organic volatiles extracted from cooked meat increased with ageing period (Coppock and MacLeod, 1977). Increases in concentrations of alkanes, benzenoid compounds, furans, pyrroles, pyridines and pyrazines also coincided with odour descriptions by a trained sensory panel that indicated a trend from weak, bland, and unappetising to strong, savoury, appetising and roasted.

More recently, Nishimura *et al.* (1988) studied both taste and taste components of beef, pork and chicken aged at 4°C for 12, 6 and 2 days respectively. Concentrations of free amino acids were higher after ageing for all three meats tested. The differences were more pronounced from pork and chicken even though they were aged for shorter periods compared with beef. In contrast, oligopeptide levels decreased for beef and increased for pork and chicken. These chemical changes matched sensory panel observations that a

'brothy' taste intensity was unchanged for stored beef, but significantly stronger for stored pork and chicken. Their results also showed a decrease in the concentration of nucleotides, ADP, AMP, and IMP and an increase in inosine and hypoxanthine after conditioning for all meats tested. Several nucleotide products have pronounced 'savoury' flavours.

A frequent observation, but one given little explanation, is a gradual increase in meat pH during long term storage (Bodwell *et al.*, 1965). Parrish *et al.* (1969) reported an increase of 0.2 pH units between 3 and 28 days storage of raw beef at 2°C. Boakye and Mittal (1993) studied the changes in pH of beef during ageing over 16 days at 2°C and observed a greater increase in pH of meat stored in vacuum packaging compared with meat stored in unpacked. They proposed that ageing of meat in a vacuum package excluded oxygen transfer which may prevent the increase in acidity. However, no explanation for the increase in pH of meat stored without packaging was given.

The effects of increasing pH after long-term ageing has on cooked aroma and flavour also needs investigation. This will be addressed in Chapter 6 of this thesis.

2.2 Chemical Reactions Involved in Odour and Flavour Development in Cooked Meat

Before investigating the effects ultimate pH has on the odour and flavour of cooked meat, a brief review of the composition of meat is appropriate to help understand the basic chemical reactions that occur when meat is cooked, and possible interactions of precursors responsible for flavour development.

Lean meat comprises, on average, 75% water, 19% protein, 2.5% fat and 3.5% soluble, non-protein substances (Lawrie, 1985). The latter, water-soluble fraction contains some precursors for meat flavour development. These are principally, free amino acids, peptides, nucleotides, reducing sugars that, individually and in combination, are responsible for the formation of many heterocyclic compounds contributing to meaty flavour. The lipid fraction includes triacylglycerols (with saturated and unsaturated fatty

acid components) that form the major component of intramuscular fat, and phospholipids that are an integral part of cell membrane structure. Phospholipids, although a minor component of total lipid, contain a high proportion of unsaturated fatty acids.

On heating, interaction between compounds derived from both the lipid and lean components contribute to the overall flavour. Fat can also serve as a solvent for the aroma compounds formed, so affecting the release of flavour from the lean fraction (Wasserman and Spinelli, 1972).

Meat can be cooked over a wide range of temperatures from mild roasting and boiling to high temperature grilling. Depending on cooking time and the size of the piece of meat, large temperature gradients can be found from the outside to the centre of cooking meat. The cooking temperature has a significant influence on the quality and quantity of volatile compounds liberated from cooked meat (MacLeod and Ames, 1986b; Spanier *et al.*, 1994).

Phospholipids and their fatty acids and lipid oxidation products are responsible for many meat volatiles. The products are principally aliphatic compounds such as hydrocarbons, aldehydes, alcohols, ketones, carboxylic acids. Lactones, furans, aromatic and alicyclic hydrocarbons and ketones also play a part.

More compounds have been identified in the volatiles of cooked meat than in almost any other food. A review by Shahidi *et al.* (1986) lists a total of 995 volatile compounds found in beef, pork, and sheepmeat. However, only some of these compounds contribute to the overall aroma of cooked meat. Research to date has identified two chemical reactions that play a major role in meat aroma formation. These are the Maillard reaction, involving amino acids (or peptides) and reducing sugars, and lipid oxidation.

The Maillard reaction

The French chemist, Louis Maillard, first described the formation of brown pigments (melanoidins) when solutions of glucose and glycine were heated. Although Maillard

browning can occur when mixtures of reducing sugars and amino acids are stored at refrigerated temperatures for long periods, reaction to yield flavour-active compounds requires cooking temperatures. The reaction proceeds more readily at low moisture levels such as on the exterior surface of roasting or grilling meat where evaporative water loss is greatest (van den Ouweland *et al.,* 1978).

The first stage of the Maillard reaction does not cause browning or produce flavour compounds. In the case of an aldose sugar, it involves condensation between the carbonyl group of the reducing sugar (or aldehyde) and the free amino group of an amino acid, amine, peptide, protein or ammonia (figure 2-2a). Water is rapidly eliminated from the resulting condensation product (addition compound) and converted into a Schiff's base. Cyclization to the corresponding N-substituted glycosylamine occurs before conversion to the 1-amino-1-deoxy-2-ketose by the acid catalysed Amadori rearrangement (MacLeod and Ames, 1988). This rearrangement involves the transition from an aldose to a ketose sugar derivative (Hurrell, 1982). Reaction of a free amino group with a ketose sugar results initially in formation of an N-substituted ketosylamine, which then forms a Heyns compound (figure 2-2b).

Although the Amadori and Heyns compounds are nonvolatile, they are thermally labile and form a large range of volatile compounds. Their rearrangement and decomposition form the second stage of the Maillard reaction (figure 2-3a). Two paths for the Amadori compound are possible. One path involves an irreversible 2,3-enolization. This is favoured at higher pH values and is more pronounced with Amadori reaction products from strongly basic amino acids. Many dicarbonyl or hydroxycarbonyl compounds are formed via a 1-deoxyosone intermediate. These highly reactive fragmentation products include glyceraldehyde, pyruvaldehyde, hydroxyacetone, diacetyl, acetoin, and hydroxydiacetyl. They contribute significantly to aromas in their own right, but are capable of further reaction. The second path, favoured at lower pH, involves 1,2-enolisation and subsequent degradation into a 3-deoxyosone that, on dehydration, form flavour compounds such as 2-furaldehydes. These compounds will also form via the 3-deoxyosone intermediate after 1,2-enolization of a Heyns compound (MacLeod and Ames, 1988). Alternative routes to

enolization of Amadori reaction products, such as direct dehydration of carbohydrates, have also been proposed to explain the formation of compounds during the thermal decomposition of carbohydrates (Yaylayan, 1990).

The Maillard reaction produces a plethora of interactive products such as furanones, furfurals, and dicarbonyls, that can react with substances such as aldehydes, ammonia, hydrogen sulphide, amino acids, amines and thiols. The products of these latter reactions include important classes of flavour compounds such as pyrazines, thiophenes, thiazoles and sulphur-containing heterocyclic compounds. Generally, Maillard reaction rate is increased by an increase in pH (Hurrell, 1982). Using a model mixture containing lysine and glucose, Leahy and Reineccius (1989), showed the rate of production of pyrazines and the number of types of alkylpyrazines, increased as pH was raised from 5.0 to 9.0. Pyrazines are important flavour compounds of meat, particularly in cooked beef (MacLeod and Ames, 1987).

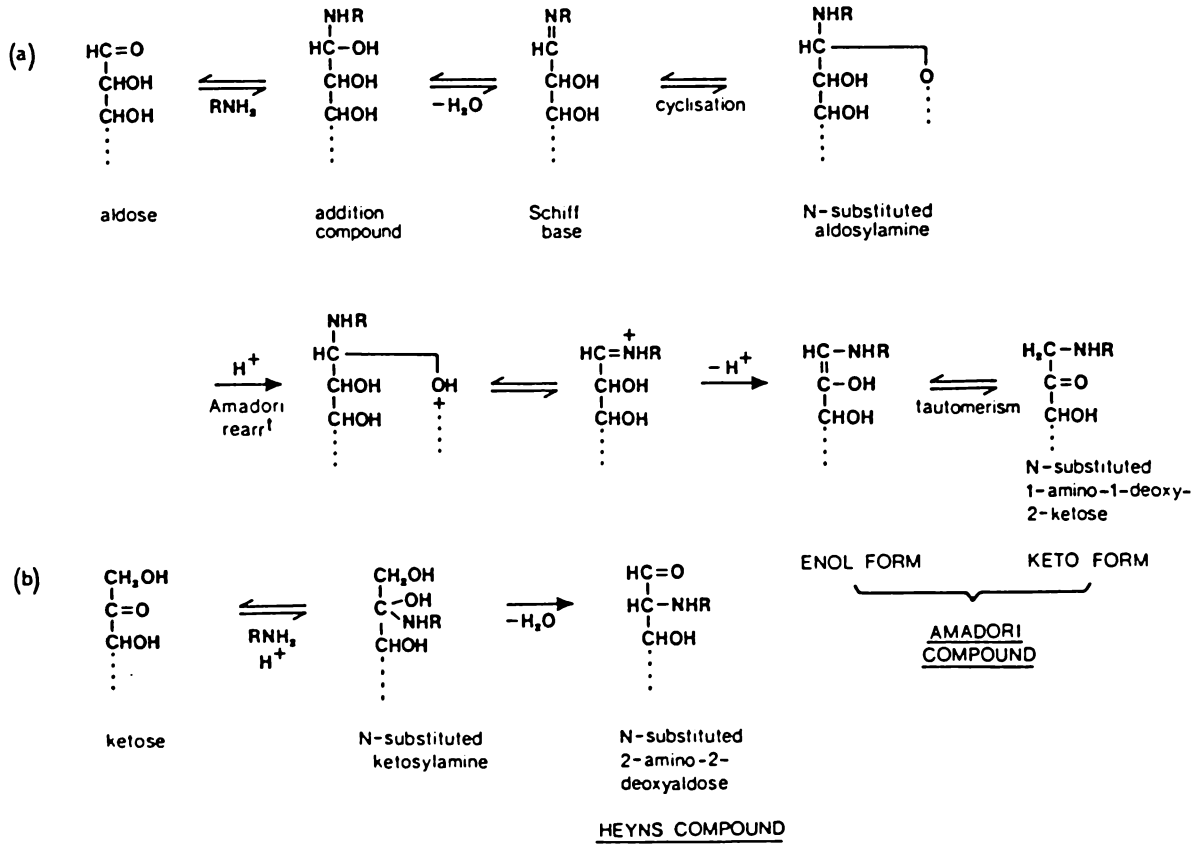


Figure 2-2.
Formation of Amadori and Heyns compounds.

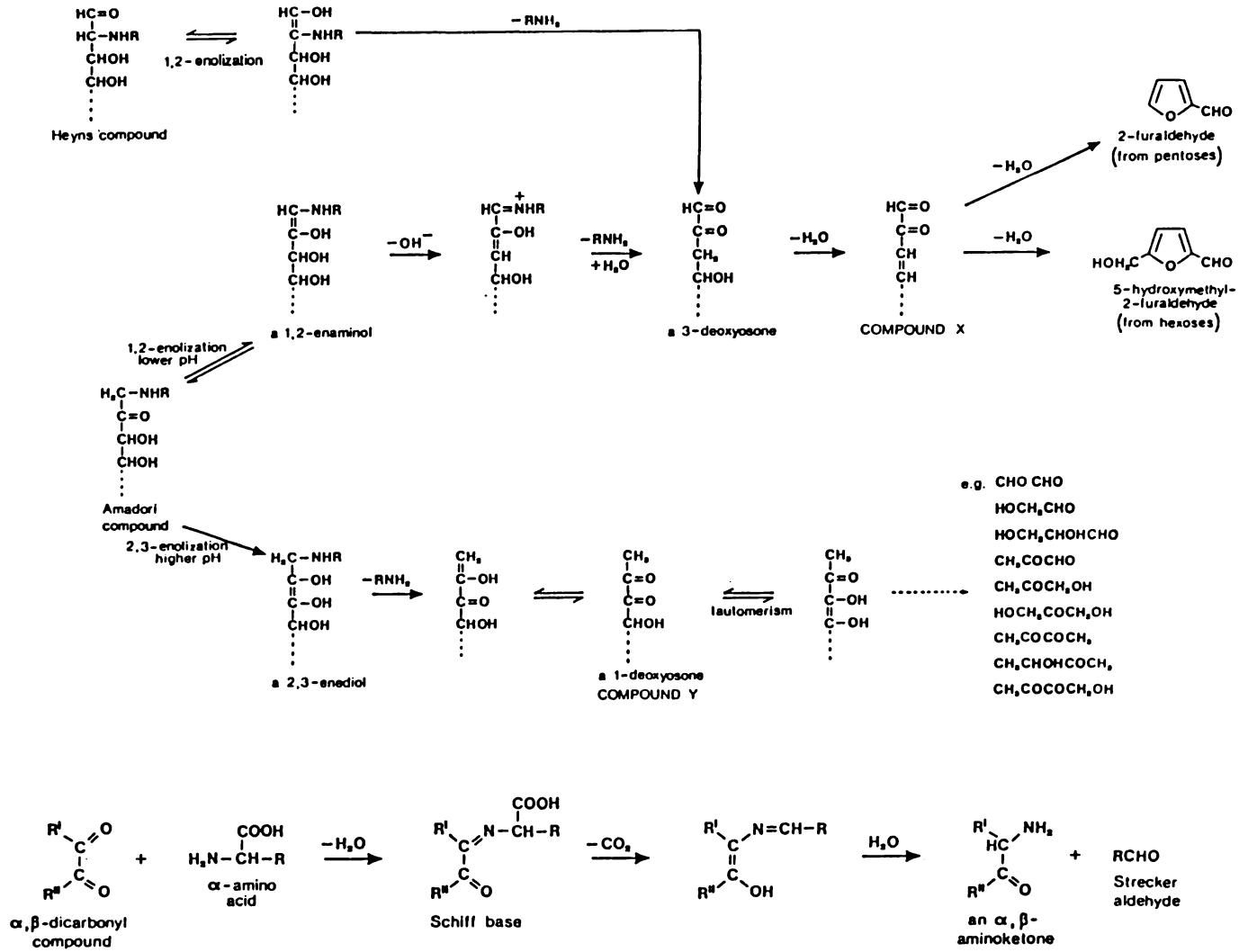


Figure 2-3.

(a) Decomposition of Amadori and Heyns compounds. (MacLeod and Ames, 1988); (b) Strecker degradation of α-amino acids. (MacLeod and Ames, 1988)

Associated with the Malliard reaction is the Strecker degradation of α-amino acids in the presence of dicarbonyl compounds (figure 2-3b). Oxidative deamination and decarboxylation of this mixture produces an aldehyde containing one less carbon atoms than the original amino acid plus an α-aminoketone. Some Strecker aldehydes and their parent amino acids are listed in Table 1.

Table 2-1.

Aldehydes formed by the Strecker degradation of parent amino acids.

Parent amino acid	Volatile Strecker aldehyde
alanine	acetaldehyde
aminobutyric	propanal
valine	methylpropanal
isoleucine	2-methylbutanal
leucine	3-methylbutanal
phenylalanine	phenylacetaldehyde
methionine	methional (3-methyl-thiopropanal)

Carbonyl compounds generated from lipid oxidation can also participate in Maillard-type reactions.

Lipid oxidation

Volatile flavour compounds from lipids are formed by either free radical-initiated or lipoxygenase-mediated oxidation. The reaction of unsaturated lipids with oxygen to form hydroperoxides is a free radical process involving initiation, propagation and termination steps (Chan, 1987).

Initiation	RH	→	$R^{\bullet} + H^{\bullet}$
Propagation	$R^{\bullet} + O_2$	→	$ROO^{\bullet}$
	$RH + ROO^{\bullet}$	→	$ROOH + R^{\bullet}$
	$ROOH$	→	$RO^{\bullet} + {}^{\bullet}OH$

The first steps involve generation of an alkyl radical ($R^{\bullet}$) that then reacts with oxygen to form the peroxy radical ($ROO^{\bullet}$). Abstraction of hydrogen by alkylperoxy radicals from the allyl methylene group ($-CH_2-$) next to a double bond of another fatty acid (RH) then occurs resulting in the formation of hydroperoxide (ROOH). The lipid-free radical can also further react with oxygen to form a peroxy radical.

Termination of the chain reaction involves free radical combination forming stable end products, but this is of minor significance in relation to the fate of the formed hydroperoxides.

Hydroperoxides of unsaturated fatty acids formed by autoxidation are very unstable, particularly at high temperatures, and decompose to give an alkoxy radical and a hydroxy radical ($RO^{\bullet}$ and $^{\bullet}OH$). The alkoxy radical then forms products depending on the structure of R. Finally several common classes of chemicals are produced, including aldehydes, alkenes, alkanes, alcohols, ketones and furans (figure 2-4). The rate of autoxidation increases with the degree of unsaturation. For example, linoleate (C18:2) is oxidised 10 times faster than oleate (C18:1).

The high thermal instability of hydroperoxides may explain differences in the relative amounts and types of volatile products formed during cooking of meat (desirable flavours) compared with long-term storage of uncooked or precooked meat (undesirable, rancid flavours). The concentration of hydroperoxides is unable to build up in heated fat because of their thermal instability, whereas at storage temperatures a significant concentration may build up before decomposition into undesirable rancid off-flavours products (Mottram, 1991).

Saturated fatty acids are relatively stable at low temperatures. However, at higher temperatures (> 60°C) random oxidation of saturated lipids occurs. Monohydroperoxide intermediaries are further fragmented to produce, principally, aldehydes and methyl ketones and acids, hydrocarbons, γ-lactones and alcohols (Grosch, 1982) (figure 2-5).

Lipoxygenase enzymes are ubiquitous in plant, animal and fish tissue, and catalyse the insertion of oxygen into polyunsaturated fatty acids to generate hydroperoxides. Although most research on lipoxygenase has involved those of plant origin, their involvement in flavour generation in meat should not be overlooked. Lipoxygenase action may be responsible for some oxidative and rancidity changes during frozen storage of chicken (Skaln and Tenne, 1984) and fish (McGill *et al.,* 1977), and for the generation of fresh fish aroma (Josephson *et al.,*1984).

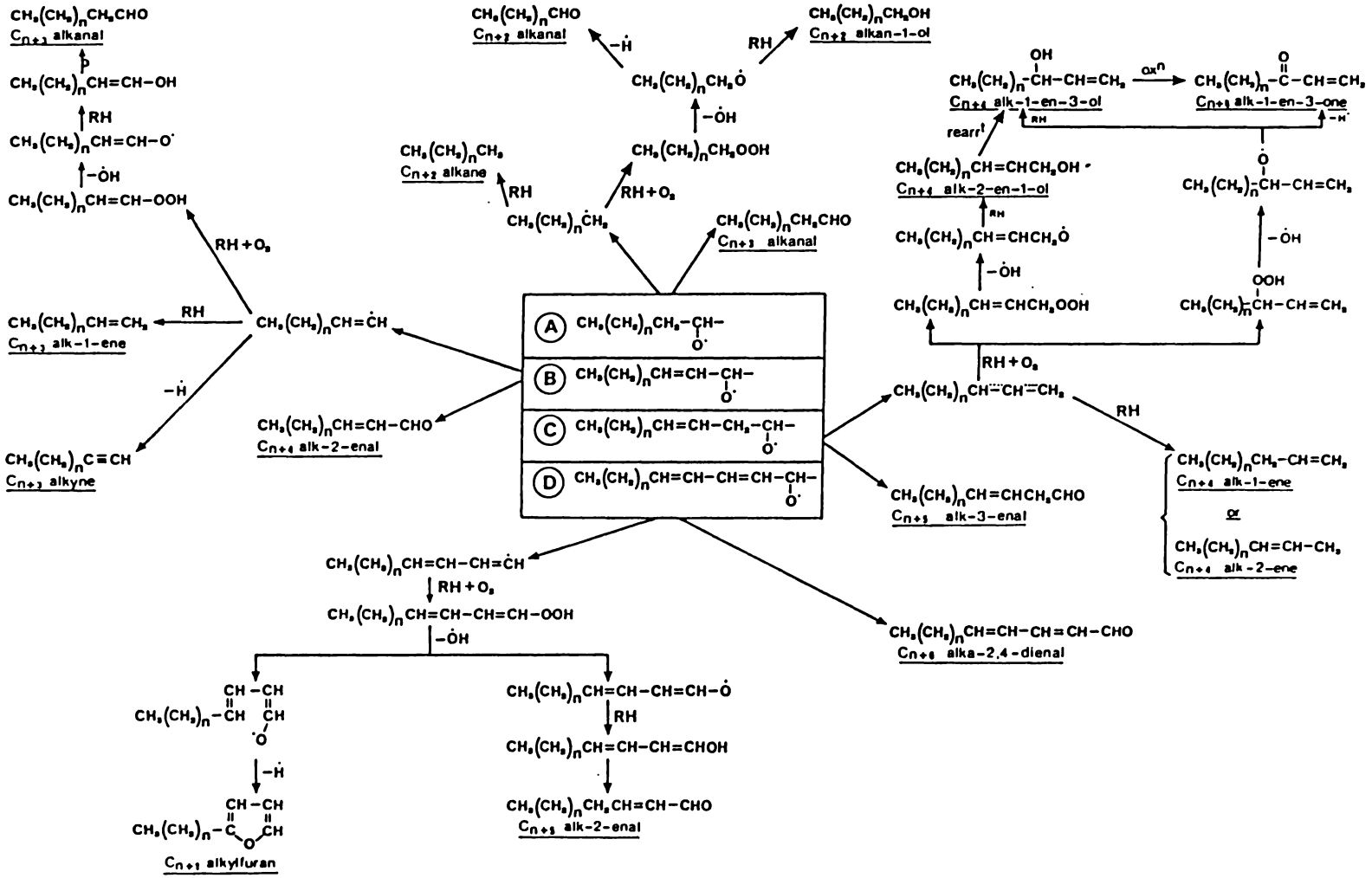


Figure 2-4.

Decomposition of alkoxy radicals ($RO^{\bullet}$) derived from monohydroperoxides and forming aroma volatiles. A means R is saturated; B, R is one double bond; C, R is an allylic system; D, R is a diene. (MacLeod and Ames, 1988).

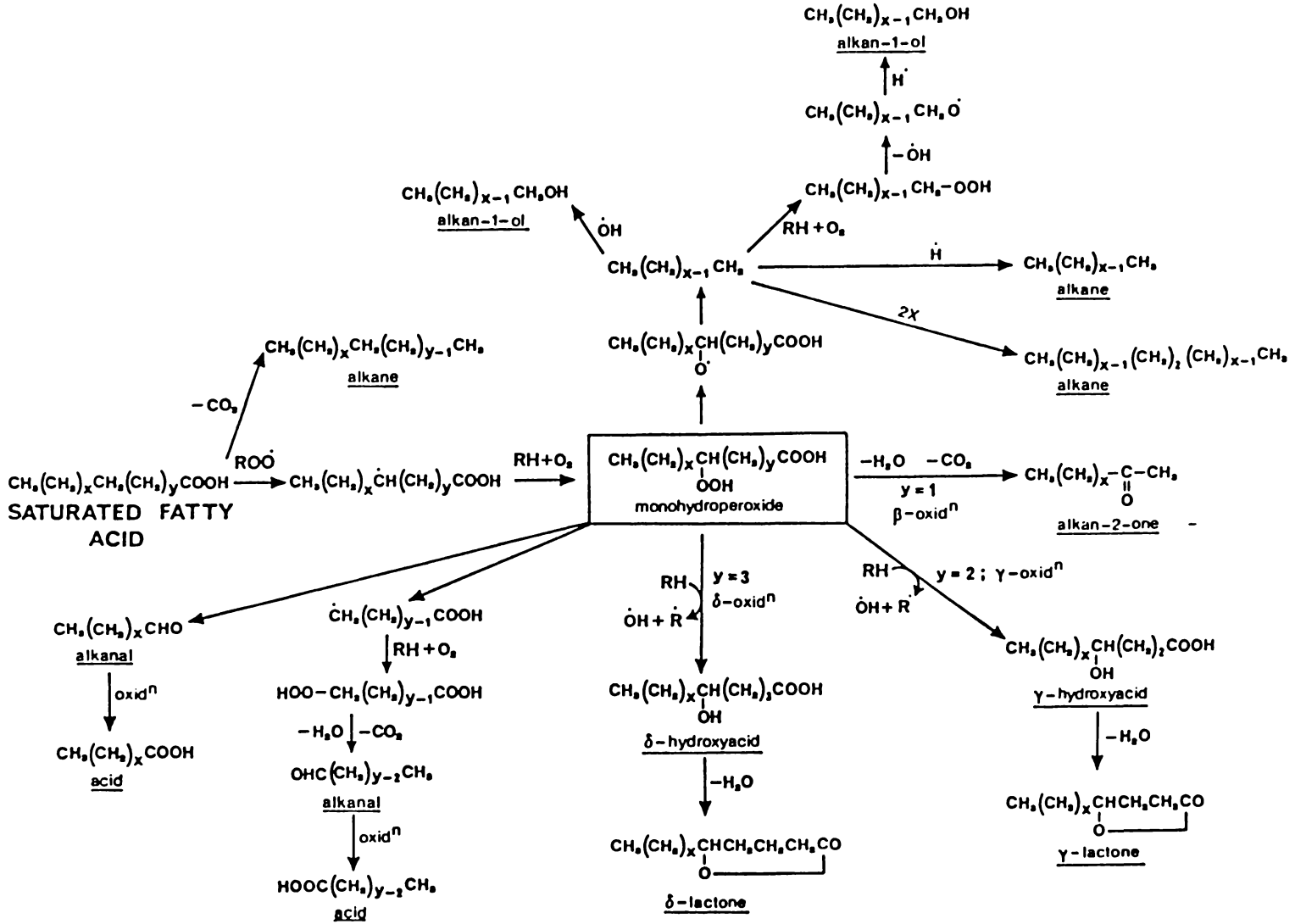


Figure 2-5.

Possible reaction pathways in the thermal oxidation of saturated fatty acids. (MacLeod and Ames, 1988).

Maillard reactions and oxidation of lipids do not occur in isolation when meat is cooked. Carbonyl compounds generated by lipid oxidation can participate in Maillard-type reactions. Fatty aldehydes are implicated in production of pyrazine, heterocyclic and sulphur-containing heterocyclic compounds (Zang *et al.,* 1994). Conversely, Maillard reaction products have been shown to exhibit antioxidant activity towards lipids (Lingnert and Eriksson, 1981).

Recent research (Farmer *et al.,* 1989; Farmer and Mottram, 1990b, 1992) showed the importance that phospholipids may have on the formation of volatile aromas through the interaction with the Maillard reaction. Removal of polyunsaturated rich phospholipids from meat removed the "meaty" character of the aroma and markedly changed the pattern of flavour volatiles produced. By contrast, removal of the triacylglycerols alone did not affect the aroma profile (Mottram and Edwards, 1983).

Obviously temperature also plays an important part in determining the amount and type of compounds produced from cooked meat. For example, lipid oxidation products dominate the profile of meat aroma volatiles in lightly grilled or boiled meat. Conversely heterocyclics could only be found in well-done pork when a range of cooking conditions was investigated (Mottram, 1985).

2.3 Concluding Remarks

In summary, the odour and flavour of cooked meat are governed by many interrelated factors. These include the nutritional status, species, and stress condition of the animal before slaughter - some of which are influenced in a particular manner by New Zealand farming and preslaughter handling practices. In addition, the composition and biochemical status of the meat at time of cooking are important factors. Relative amounts and composition of flavour precursors, influenced by storage condition such as time and temperature, and cooking conditions will also influence the perceived aroma and flavour quality of the final product.

This thesis pays special attention to the influence meat ultimate pH and storage conditions have on the flavour quality and volatile aroma profile of cooked sheepmeat.

Chapter 3.

Collection and Analysis of Volatile Compounds

3.1 Comparison of Solvent Elution, Thermal Desorption And Supercritical-CO_2 Elution of Tenax Traps

Introduction

Methods to analyse volatile compounds from foods, fats and oils include steam distillation, simultaneous distillation and extraction (SDE) (Likens and Nickerson, 1964), solvent extraction, direct thermal desorption (Dupuy, 1973), static headspace sampling (Macku *et al.*, 1988) or dynamic headspace sampling with a porous polymer (e.g. Tenax) trapping (MacLeod and Ames, 1986b). Identification and quantisation are usually done by gas chromatography (GC) combined with either mass spectrometry (MS), flame ionization detection (FID) and/or odour port analysis (OPA).

Each of these sampling techniques have advantages and disadvantages when compared with each other. For example, during SDE and solvent extraction, contaminants of the solvent may be concentrated to unacceptable levels when the extract is reduced in volume before GC analysis. Further, there is potential to lose highly volatile compounds during the concentration step when nitrogen gas is blown over the solvent surface. Steam distillation requires high temperatures for distillation which may generate compounds not associated with the original odour or flavour of the material under study. Solvent extraction can produce extra peaks (e.g. fatty acids and acylglycerols) in a GC chromatogram that confuse the chromatographer, making identification difficult. The results may not reflect the true odour and flavour profile of the original material.

SDE, a combination of both distillation and solvent extraction, has the potential of contamination from solvent impurities (Jeon *et al.*,1976) and thermally induced changes to arise (Sugisawa, 1984). To get as close as possible to the true odour profile of a food product, most researchers rely on either direct headspace sampling or dynamic headspace purge-and-trap sampling.

In dynamic headspace purge-and-trap analysis, a sample is purged with a flow of gas in a semi-closed environment. The volatiles stripped from the headspace above the sample are then trapped either cryogenically in a small tube, or on GC column packings such as Porapak Q and Chromosorb 105 or charcoal. A popular trapping material is a porous polymer based on 2,6-diphenyl-*para*-phenyleneoxide (Tenax-GC or TA). Tenax-TA has been the porous polymer of choice because of its good trapping efficiency of a wide range of food-derived organic compounds at ambient temperature, high thermal stability (up to 450°C), relative low water retention during trapping and low bleed (breakdown) during thermal desorption (Macleod and Ames, 1986a).

A number of studies have been done with Tenax to characterize retention data and breakthrough volumes for several organic compounds (Brown and Purcell, 1979; Maier and Fieber, 1988), to optimise sampling conditions (Wyllie *et al*, 1978; Contraini and Leardi, 1994), to compare with other adsorbents (Bishop and Valis, 1990), and to evaluate its quantitative performance (Olafsdottir *et al*, 1985).

There have been concerns about possible thermal conversion of labile compounds during thermal desorption of food volatiles from Tenax traps (Lewis and Williams, 1980; Snyder and Mounts, 1990). Although volatile compounds are trapped at relatively low temperatures (around ambient and below), thermal desorption takes place at temperatures up to 260°C for periods as long as 20 minutes (Mottram *et al.*, 1982). For this reason, some researchers prefer elution of volatile compounds from absorbent traps with a solvent such as diethyl ether (Olafsdottir *et al.*, 1985; Buttery *et al.*, 1987; Vercellotti *et al.*, 1992). This avoids possible thermal effects on compounds and allows replicate analysis of the volatiles from the same trap. In addition, only one GC run is possible with each desorbed trap.

Solvent elution not only has the potential to introduce solvent borne contaminants, but also very volatile compounds can be lost during the volume reduction step before GC analysis. Recently Hawthorne *et al.* (1986) and Wong *et al.* (1991) investigated supercritical carbon dioxide as an alternative eluent for Tenax traps.

When gases are compressed isothermally to pressures above their critical pressure, their densities increase to near that of a liquid (figure 3-1). In the vicinity of their critical temperature, a supercritical fluid shows enhanced solvent power yet exhibit viscosities lower than gases and high diffusivity. Their ability to dissolve solutes can be characterized by the Hildebrand solubility parameter. This parameter, δ, can be related to the density of a gas as given by

$$\delta = 1.25\, P_c^{0.5}\, [\rho/\rho_{liq}]$$

where P_c is the critical pressure, ρ is the gas density and ρ_{liq} is the density of the liquid under standard conditions. On pressurization of the gas, the density increases rapidly as the critical pressure is approached (figure 3-2). It is the density rather than pressure or temperature that is the parameter crucial to solubility (Giddings *et al.*,1969) as the equation makes clear. This characteristic makes it a powerful extraction technique. Relatively small changes in temperature and pressure around the critical point can dramatically change the solvating power of the fluid.

Supercritical fluid extraction (SFE) is now extensively used commercially as an extractant for the fractionation of a variety of products. Numerous reports cover applications such as food flavours (Ondarza and Sanchez, 1990; Sinha *et al.*,1992; Polesello *et al.*, 1993), fractionation of lipids (Singh and Rizivi, 1994) and deodorization of edible oils (Ziegler and Liaw, 1993). In the last 10 years there has also been a dramatic increase in the application of SFE as an analytical tool. For a landmark paper refer to Hawthorne (1990).

Supercritical carbon dioxide (pressure > 72 atm, temperature > 31 °C) is an excellent solvent for the extraction of volatile compounds from food products. It can extract compounds of a reasonably wide polarity range from different matrices in relatively short times and low temperatures, compared to classic solvent extractions. Carbon dioxide also has the advantage that on a depressurization, it vaporizes and leaves no residue in the extract. Carbon dioxide is also nonflammable, nontoxic and relatively inexpensive.

There are two main types of analytical SCO_2 extraction methods in use: off-line SFE and on-line SFE. Both methods use the same principal of sample extraction but differ in the manner in which the solutes are collected (figure 3-3). In Off-line SFE, the dissolved solutes exit the extraction vessel via a capillary restrictor and are precipitated and subsequently dissolved in a trapping solvent as the CO_2 vaporizes on depressurization at ambient pressure. Blocking of the capillary restrictor can sometimes occur and various methods of prevention have been investigated (Burford *et al.*,1992). The trapping solvent is then volatilized to concentrate the solutes and an aliquot analysed, on a gas chromatograph, for example. Alternatively the solutes can be collected on solid phase traps before elution and chromatography (Mulcahey, 1992).

In on-line SFE the outlet capillary restrictor is part of the analytical flow line. In the case of a gas chromatograph, solutes are deposited directly into the split/splitless injector or the head of the column of an on-column injector (Nielen *et al*, 1989; Hawthorne *et al*, 1990; Raymer *et al,* 1991). Capillary restrictor blocking is less of a problem as the restrictors is kept at a high temperature by the heated GC injector.

Compared with thermal desorption, supercritical carbon dioxide elution has an advantage of eluting volatile compounds at relatively low temperatures thus avoiding possible thermal degradation effects. Because of this advantage, it was decided to evaluate SCO_2 as an alternative elution procedure to thermal desorption and organic solvent elution of food flavour volatile compounds from Tenax traps.

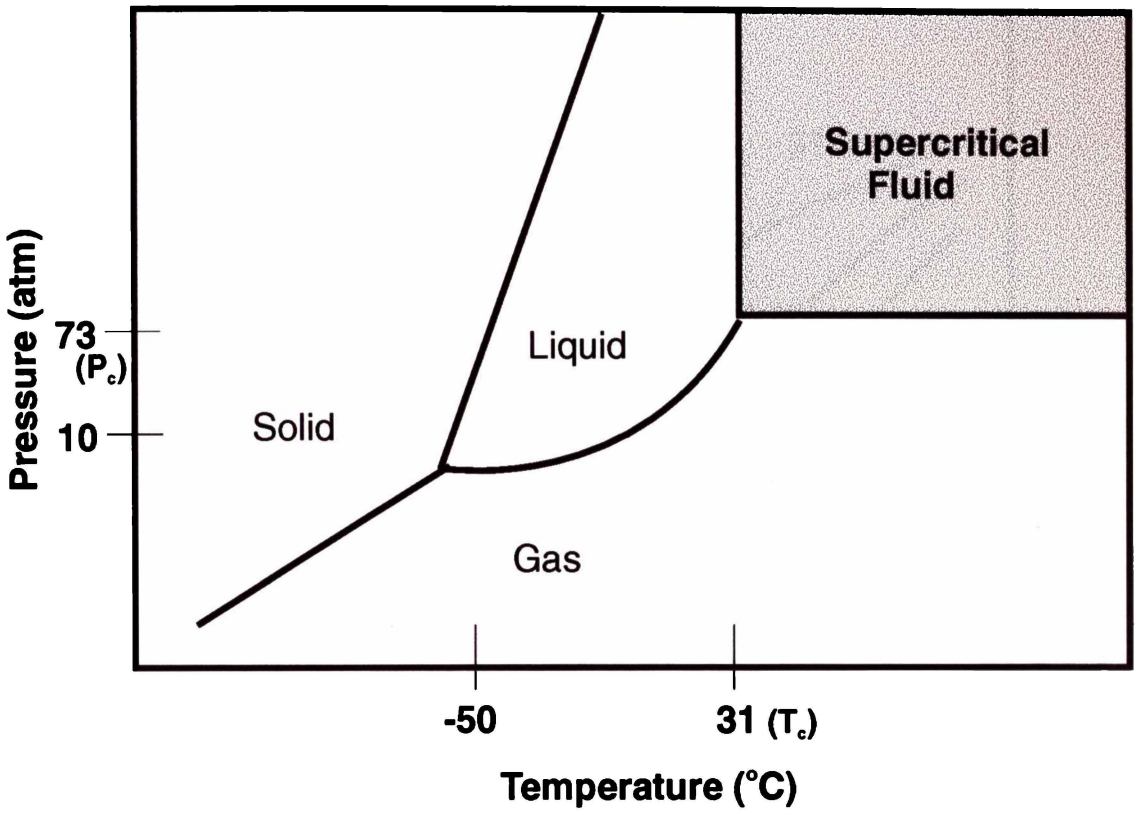


Figure 3-1.
Phase diagram for carbon dioxide. CO_2 becomes supercritical at 73 atm and 31 °C.
At any pressure above 73 atm and temperature above 31 °C,
CO_2 behaves as a supercritical fluid.

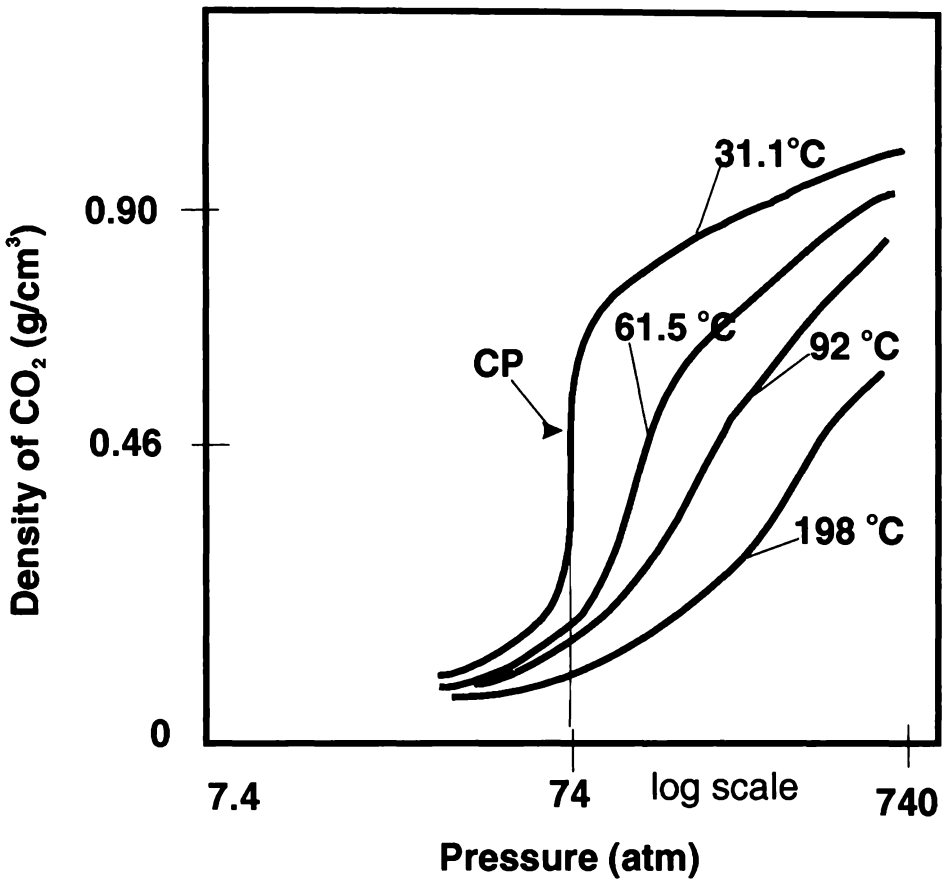


Figure 3-2.
Pressure and density relationships of CO_2 at different temperatures.
CP = Critical Pressure.

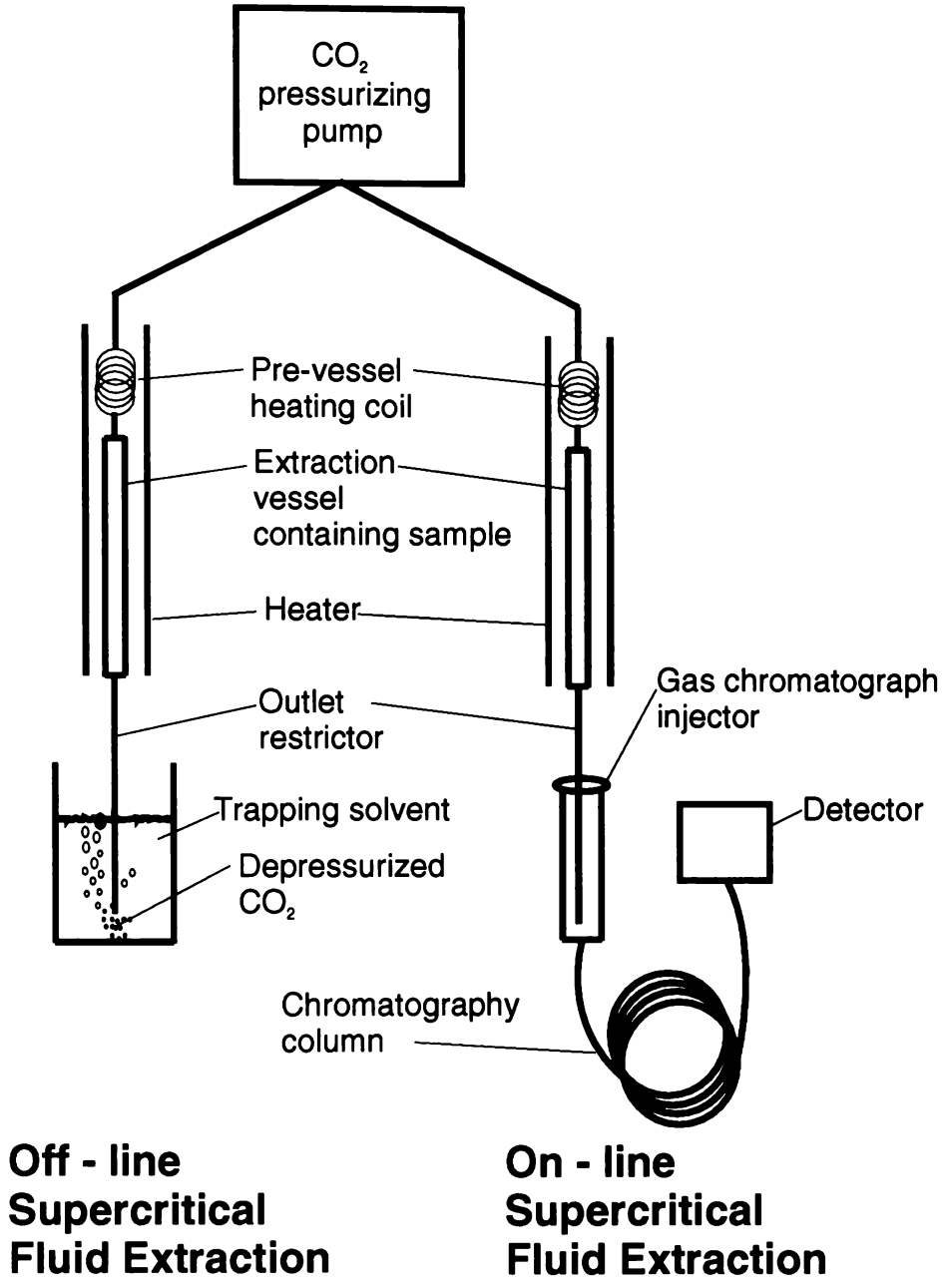


Figure 3-3.
Schematic of on-line and off-line supercritical fluid extraction setup.

Purge-and trap-apparatus construction

The vessel used to trap volatile compounds from whole ground minced meat consisted of a 1 L Quickfit three-port, round-bottomed flask (RBF). A four-port manifold, constructed from 6 mm internal diameter (ID) glass tubing was inserted in the middle port of the RBF through a Quickfit adaptor (ST/52/13). Up to four Tenax Traps could be connected in parallel to the manifold at anyone time with Teflon unions. The second RBF port was used as the entry point for the purge gas. A glass thermometer was positioned in the third port (figure 3-4). Before each trapping experiment glass components were washed in laboratory detergent, rinsed in distilled water, methanol, acetone then hexane before being dried for one hour in an oven set at 100°C.

Tenax traps

Traps were constructed from 17cm x 0.4cm ID glass-lined stainless steel tubes (GLTTM, SGE Scientific Pty Ltd., Australia) fitter with Vespel ferrules (VFF/4, SGE) and stainless steel reverse ferrules (SCF/4, SGE). These were secured with long coned stainless steel nuts (SSNE-4, SGE) to ¼" zero-volume, stainless steel, Swagelok column-end fittings (SS-400-1FGC, Swagelok Co., Solon, OH, USA). Glass-lined stainless steel tubes were used to provide inert and pressure resistant holders for the Tenax. Thus, the same tube could be used for both thermal desorption and SCO_2 elution. In some cases, one method of removing trapped volatile compounds from the Tenax was immediately followed by the second method (or vise versa) to check the elution efficiency of the first method. Because of the high pressures (≥ 600 atm) used during SCO_2 elution, standard glass Tenax tubes could not be used.

All traps were washed in analytical grade methanol, acetone then diethyl ether before flushing with carbon dioxide at 600 atm and 40°C for 45 minutes. The clean traps were then loaded with 200 mg of conditioned Tenax-TA (60/80 mesh, Alltech). Conditioning of Tenax-TA was done by raising the temperature of a bulk amount of Tenax, packed in a large conditioning tube (10 mm x 100 mm), from ambient to 320°C under a stream of high purity nitrogen. This temperature was maintained for two hours then reduced to 110°C overnight while maintaining the flow of N_2.

Desorber heaters

The desorber heaters for both thermal desorption and supercritical carbon dioxide (SCO_2) elutions were constructed from thin steel tubing, (9 x 120 mm) and (25 x 350 mm) respectively, wound with nichrome heating wire, insulated with fibreglass tape and housed in a heat stable outer casing (Turfnol). Both heater units were fitted with three type T thermocouples positioned equidistantly along the length of the heating tubes and controlled by a three channel Eurotherm 808 PID controller and power supply. For thermal desorption, the tube temperature was raised from ambient to 250 (±0.5)°C within two minutes and maintained at that temperature for 10 minutes. For SCO_2 elution the heater was heated to the desired temperature before insertion of the Tenax trap into the heater barrel. These heater designs allowed portability between different gas chromatographs with little modification to the instruments.

Gas chromatograph modifications for thermal desorption and SCO_2 elution

A stainless steel 3-way valve (# 41XS2, Whitey/Swagelok) was placed in the principal carrier gas flow line, between the total flow controller and before the injector inlet of a Hewlett Packard 5890 Series II Gas Chromatograph (figure 3-5). In the primary position of the 3-way valve the normal carrier gas flow path was maintained to the split/splitless injector. For thermal desorption the valve was switched to reroute the carrier gas flow to the inlet of the Tenax trap positioned in the desorber heater. The Swagelok reducing union (SS-400-1FGC), at the outlet of the Tenax trap, was fitted with a 0.5 mm OD x 0.2 mm ID stainless steel side-hole injection needle (No. ASH-5, SGE). During thermal desorption this injection needle was inserted through the septa of the split/splitless injection port, thus maintaining the carrier gas flow to the column of the gas chromatograph (GC) (figure 3-5a).

For SCO_2 elution experiments the split/splitless injector unit was fitted with a septumless injector (No. SLI-M, SGE) that was inserted into a 1/8" hole drilled through the septum nut.

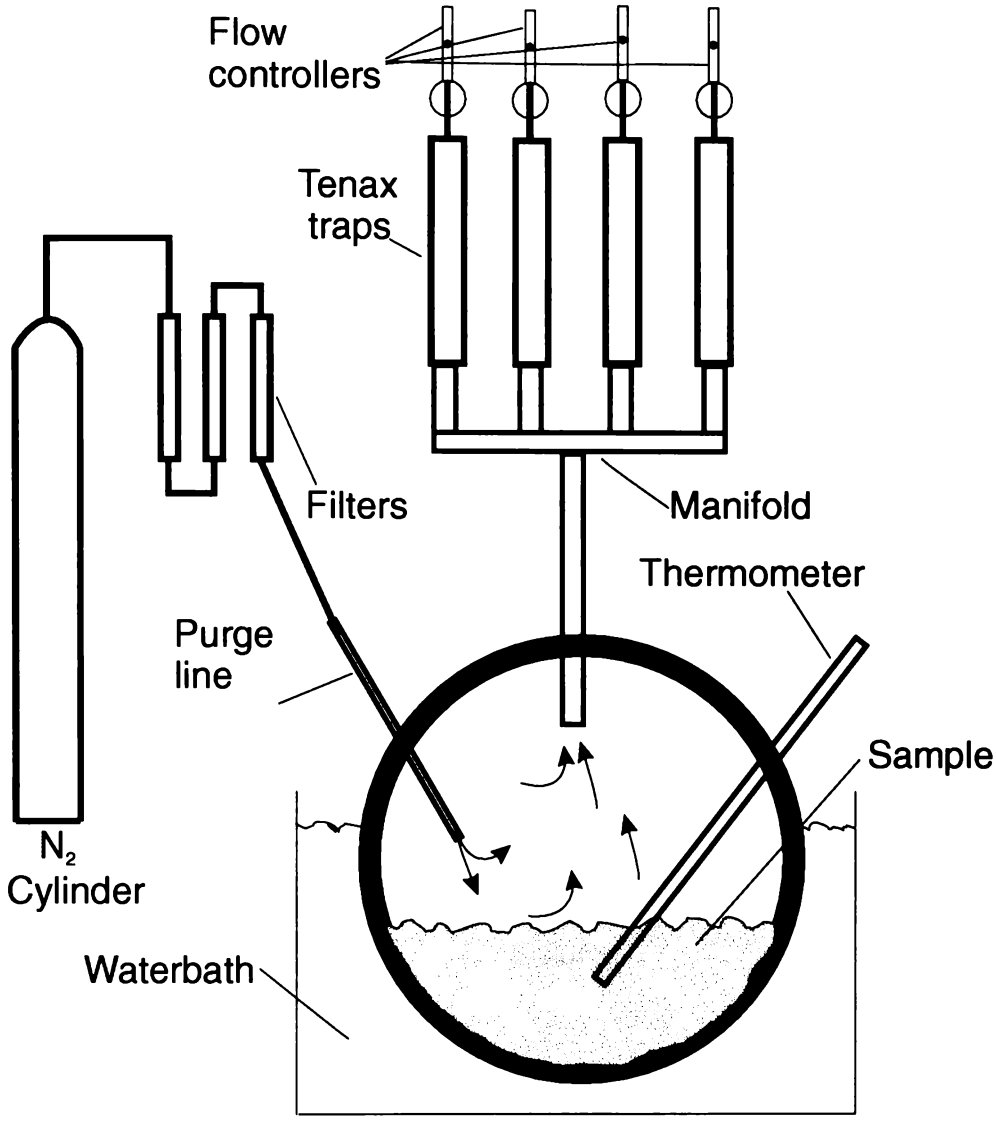


Figure 3-4.
Purge-and-trap apparatus. The three-port round bottom flask was immersed in a temperature controlled waterbath.

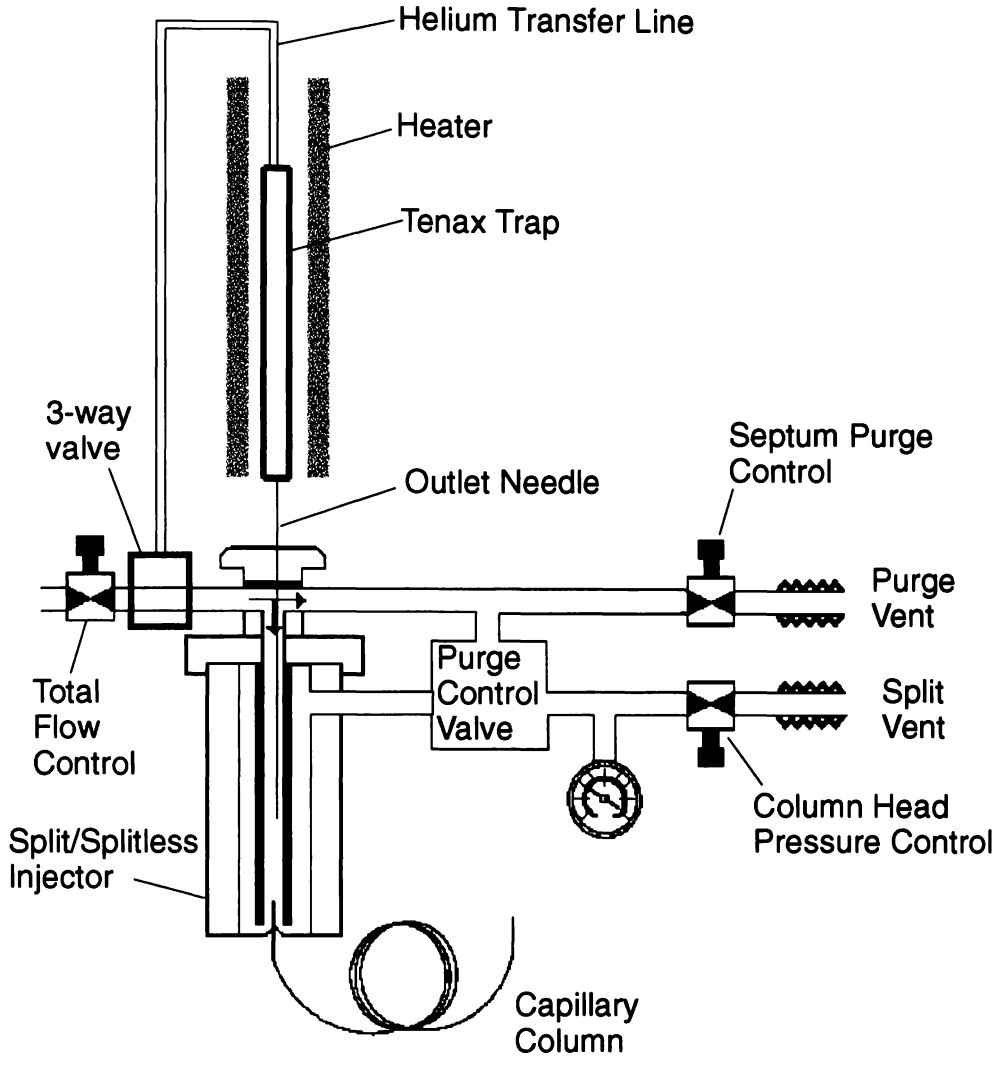


Figure 3-5a.
Schematic of the thermal desorption heater unit and Hewlett-Packard split/splitless injector.

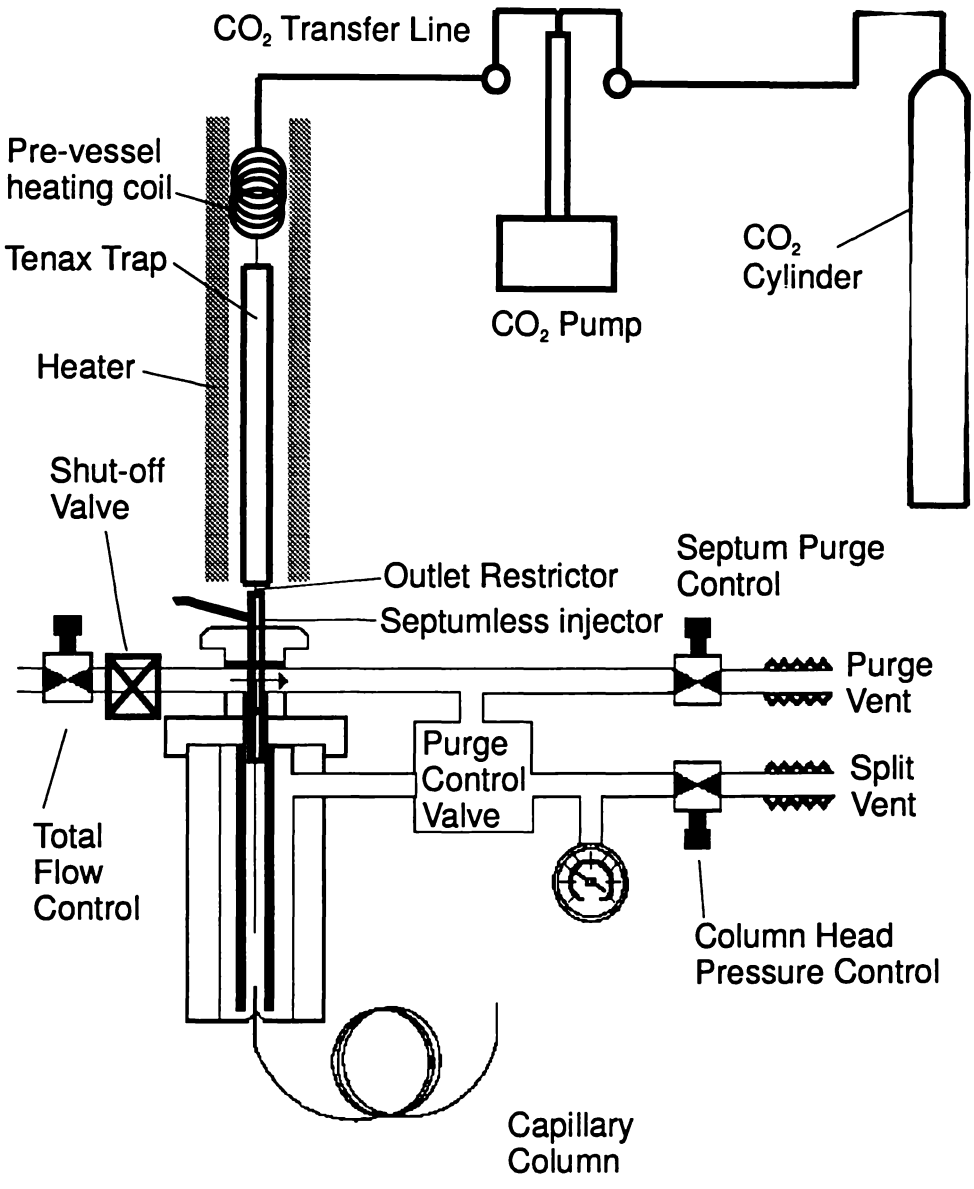


Figure 3-5b.
Schematic of the supercritical fluid elution apparatus and Hewlett-Packard split/splitless injector fitted with an SGE septumless injector.

Gas chromatography and detection

Mass spectral analysis was done on an HP 5890GC/5988A Mass Spectrometer (GC/MS) fitted with a 30 m x 0.32 mm, 1.0 μm film DB5 column (J and W Scientific). For some method development an HP 5890 Series II GC fitted with a 30 m x 0.53 mm 1.0 μm film DB5 column (J and W Scientific) was also used. Column effluent was split at a ratio of 2.5:1 between an olfactory port (SGE) and flame ionization detector (FID).

Sample preparation

Ground mutton was prepared in a bowl chopper by blending the lean meat and adipose tissue of a mutton carcass to produce a mince with a 25% (w/w) fat content (calculated on the basis of an estimated lean fat content of 5% (w/w) and added weight of adipose). Lots of ground meat (200 g) were vacuum-packed in polypropylene bags (with a stated O_2 transmission rate of 30 mL m^{-2} day^{-1} at 1 atmosphere and 75% relative humidity) and stored at - 35°C until use.

Model mixture preparation

Model mixtures of selected chemical standards were prepared in 100% soy bean oil (Wesson) known not to contribute background headspace volatile compounds.

Benzothiophene (10 mg) (Aldrich Chem., Co., Wisconsin), and 2,3-dichloropyrazine (Pyrazine Specialists, Atlanta) were weighed into separate vials each containing Wesson oil (5 g). Each vial was warmed and sonicated to aid solubilization. One gram of each solution was added to 8 g of Wesson oil to give a 0.2 mg of each analyte per g solution. After thorough mixing, 2 g of this stock were added to 18 g of oil to give a 20 μg g^{-1} working solution (Stock C).

For liquid standards, one μL of each of the following compounds was added to 50g of Wesson oil to give a 0.02μL g^{-1} working solution (Stock L_1): heptanal (Polyscience); 2-pentyl-furan (Aldrich); nonanal (Polyscience); 2-undecanone (Aldrich); and *trans*, *trans*-2,4-heptadienal (Bedoukian Research Inc., Danbury, CT) Later, a second Stock (L_2) was prepared by incorporating all the above standards plus pentanal, hexanal and octanal

(Polyscience).The final concentrations expressed in $\mu g\ mL^{-1}$ are presented in Table 3-1. All standard solutions were flushed with nitrogen and stored at -25°C.

Table 3-1.

Concentrations of standards in Wesson oil.

Standard	Molecular weight ($g\ mole^{-1}$)	Density ($g.\ cm^{-3}$)	Working standard ($\mu g\ g^{-1}$)	Solution name	Weights in purge flask (μg)
benzothiophene	188	-	20	C	20
2,3-dichloropyrazine	149	-	20	C	20
pentanal	86	0.810	16.2	L_1	16.2
hexanal	100	0.834	16.7	L_1	16.7
heptanal	114	0.816	16.3	L_1	16.3
2-pentylfuran	138	0.886	17.7	L_1	17.7
octanal	128	0.821	16.4	L_1	16.4
2,4-heptadienal	110	0.881	17.6	L_1	17.6
nonanal	142	0.825	16.5	L_1	16.5
2-undecanone	170	0.830	16.6	L_1	16.6

An external standard solution, used to check the repeatability of Tenax trap elution, was prepared by weighing 21.5mg of 2-chloropyrazine (Pyrazine Specialists) into 5 mL of methylene chloride. One microlitre of this solution was placed the glasswool at the outlet of each Tenax trap.

For analysis, 1 g each of Stocks C and L (or L_1) were added to 8 g of Wesson oil in the 3-port RBF.

Collection of volatile compounds on Tenax-TA traps

Up to four separate Tenax traps were fitted to the manifold. Nitrogen purge gas was purified by passage through a Teklab filter-drier (FD2000), a Supelpure (HD-2-2446) and

a 10ft x 1/8" stainless steal column filled with Tenax-GC. A 7 cm x 4 mm ID glass tube filled with Tenax-TA and preconditioned for ten minutes at 260°C just before use, was also connected immediately before the purge inlet to the RBF.

Samples of meat (200 g) or Wesson oil (10 g) was placed into the RBF that was then flushed with nitrogen to remove air from the headspace, before connecting the manifold fitted with freshly conditioned Tenax sampling traps. A magnetic stirring bar was placed in the RBF, to ensure adequate mixing during purging of Wesson oil. The outlet of each trap was connected to a ball-flow meter/needle valve regulator and the nitrogen flows adjusted to 25 mL $min.^{-1}$ per trap. The RBF was then immersed into a water bath set at the desired temperature. All flow rates were monitored and adjusted to maintain the intended flow for the required cooking period.

At the end of the trapping period, each trap was removed from the apparatus and, for meat samples only, dry-purged with nitrogen at 100 mL per minute for 20 minutes to remove moisture. The ends of each trap were sealed with Teflon tape and stored at ambient temperature until desorption in an airtight glass screw-cap tube purged with nitrogen. All traps were eluted or desorbed within 6 hours of sampling.

Thermal desorption of Tenax-TA traps

Each trap was fitted with a needle assembly and placed in the desorber heater before attaching the carrier gas bypass line to the top of the trap. The gas chromatography oven was cryofocused to the desired temperature before inserting the trap needle into the split/splitless injector. By turning the 3-way valve to the secondary position, carrier gas flow was redirected from the split/splitless injector port to the top of the trap (see figure 3-5). The trap was then heated to heat the trap to 250°C for 10 minutes. The 3-way valve was returned to its primary position to redirect carrier gas to its original flow path and the trap removed from the injection port. An extra two minutes was allowed for the mass spectrometer vacuum pump to recover from the high column flows before the column temperature was increased for the set cryofocusing temperature to +35°C at a rate of 70°C per minute. The column was maintained at that temperature for 5 minutes before

being raised, at 5°C minute^{-1}, to a final temperature of 260°C. After 10 minutes at the final temperature the column was equilibrated to the starting temperature. The split/splitless injector port was set at 250°C and the FID to 300°C. The helium carrier gas was set to the desired head pressure and split ratio depending on experimental conditions. Nitrogen make-up flow was 30 mL min.$^{-1}$ and FID gas flows were 30 mL min. $^{-1}$for helium and 400 mL min.$^{-1}$ for air. For the GC/MS runs the spectrometer transfer line and source temperatures were 280 and 200°C respectively.

Supercritical carbon dioxide elution of Tenax-TA traps

The inlet of each trap was attached to a 1/16″ stainless steel tube, incorporating a preheating coil, connected to an ISCO 100D Supercritical Fluid Pump (ISCO Inc., Lincoln, NE, USA). The preheating coil was essential to ensure adequate heating of the CO_2 by the time it entered the Tenax trap. The outlet was fitted with 10 cm of 25 μm (ID) fused silica restrictor (Polymicro Technologies Inc. Phonex, AZ., USA). After the trap assembly was placed into the SFE column heater and allowed to equilibrate to the desired temperature, the gas chromatograph oven was cryofocused to the desired temperature. Next, the three-way carrier-gas switching valve was turned midway between the primary and secondary flow positions. This stopped carrier gas flow to the split/splitless injector. The restrictor was inserted into the septumless injector and the carbon dioxide flow started by opening master valve on the fluid pump.

At the end of the ten minute desorption period, the carbon dioxide flow was stopped, the trap's restrictor removed from the septumless injector and the carrier gas flow reinstated by turning the 3-way valve to the primary position. GC conditions were the same as those for thermal desorption. Carbon dioxide flow rates were monitored at the SFE pump (mL liquid min.$^{-1}$) and at the split vent outlet (mL gas min. $^{-1}$) during each run.

Solvent elution of Tenax traps

Two 6 mL volumes of freshly distilled diethyl ether (Gunston, 1992) were slowly forced through the Tenax trap with a glass syringe. The eluent was collected in a glass vial and the contents concentrated to about 40μL under a stream of nitrogen that had previously

been passed through a Tenax conditioning trap. Concentrated samples were transferred to glass autosampler vials fitted with low volume inserts, sealed with Teflon coated crimp caps and stored at -25°C until gas chromatographic analysis (usually within 24 hours of sampling).

Method development for supercritical carbon dioxide elution

In preliminary experiments a range of temperatures and pressures were selected to give a range of supercritical CO_2 densities (Table 2).

Table 3-2.

Densities (g cm^{-3}) of supercritical CO_2 at various temperatures and pressures.

Pressure (atm.)	Temperature (°C)		
	33	50	70
100	0.76	0.38	0.25
132	0.84	0.65	0.42
300	0.94	0.87	0.79

Other variables investigated were SCO_2 elution times and gas chromatography capillary column cryofocusing temperatures.

Repeatability

One microlitre of external standard solution (21.5 mg of 2-chloropyrazine in 5 mL of methylene chloride) was added to the glasswool at the outlet of 8 Tenax traps. Half the number of traps was thermally desorbed while the remainder were eluted with SCO_2. This was done to check the repeatability of each method to remove the external standard from the traps.

After optimization of the SCO_2 method, repeatability of the purge-and-trap, elution of the Tenax traps and chromatography was investigated. Triplicate Tenax traps, connected in parallel to the purge vessel, collected volatile compounds from the standard Wesson oil mixture for three hours at 80°C. Purge gas flow rates were kept at 25 mL $min.^{-1}$ for each

trap. This was repeated on three separate occasions. Each trap was eluted with SCO_2 directly into the GC/MS to investigate method variability.

In a separate set of 6 experiments, triplicate Tenax traps, containing volatile compounds collected under the same conditions, were eluted with either SCO_2, diethyl ether or thermally desorbed.

Statistical analysis

Repeatability data were examined by standard analysis of variance (ANOVA) (Genstat).

Results and discussion

Performance of the supercritical carbon dioxide elution heating system

The aim of this investigation was to develop a supercritical fluid desorption method that would operate effectively at the lowest possible temperature so preventing possible production of thermal artifacts. However, below 31 °C, CO_2 becomes subcritical and forms a liquid with diminished diffusivity. Therefore, ensuring that the heater could maintain a low as possible set temperature during SCO_2 operation was essential.

Three thermocouples, attached to the exterior of the vessel assembly, monitored the temperature at the pre-trap heating coil, the top and centre of the trap (figure 3-6). The trap was equilibrated to 33 °C, pressurized to 132 atm and later depressurized to ambient.

Immediately after the valve controlling CO_2 flow into the trap was opened, the temperature at the pre-trap heating coil decreased by 3 °C with a concomitant similar increase, at the centre of the trap. The temperature at the top of the trap decreased by 0.5 °C (figure 3-7). These changes are well known thermodynamic effects that occur when a compressed gas expands on entry to a larger vessel. The heater was slow to respond to the rapid temperature changes at such a low, near ambient, set temperature. With this type of convection heater design, addition of a cooling coil would be required to reduce the observed temperature rise in the trap. Alternatively a forced air oven, like those used for gas chromatography, would improve temperature control. Unfortunately

such facilities were not available at the time of this study. In any event the system maintained the trap temperature above the critical temperature (31°C) of CO_2. A 3°C change in SCO_2 temperature would not greatly affect the fluid density at the pressures used in this study and would not be expected to increase thermal artifacts significantly.

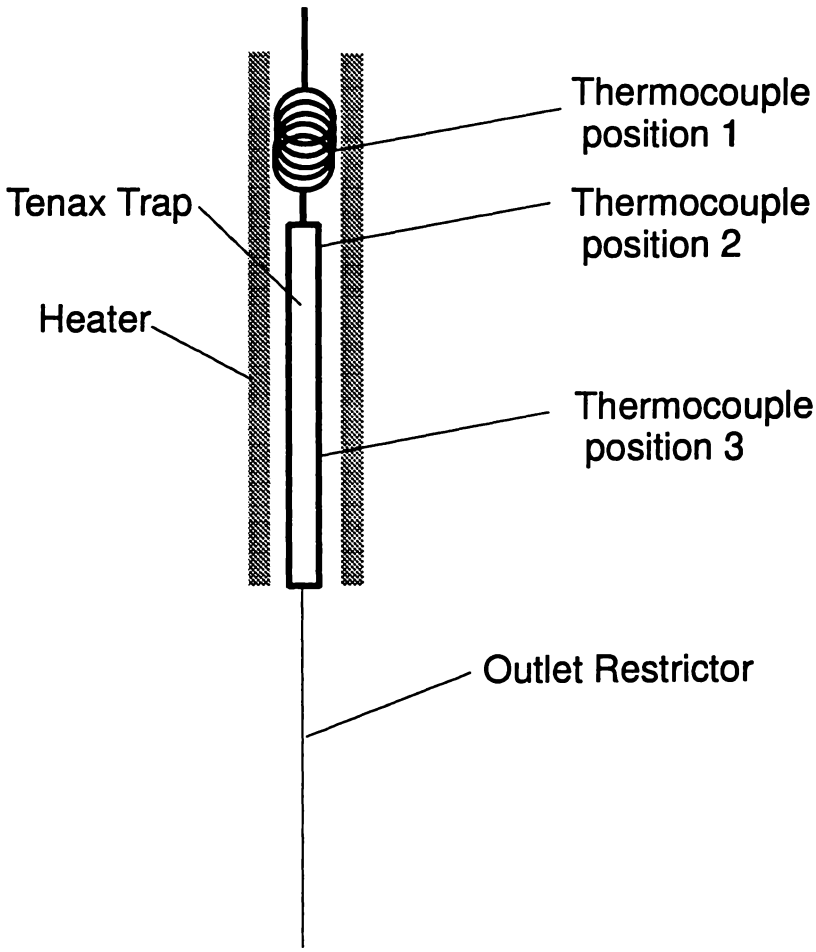


Figure 3-6.
Positioning of thermocouples to monitor temperature changes during SCO_2 elution of Tenax traps

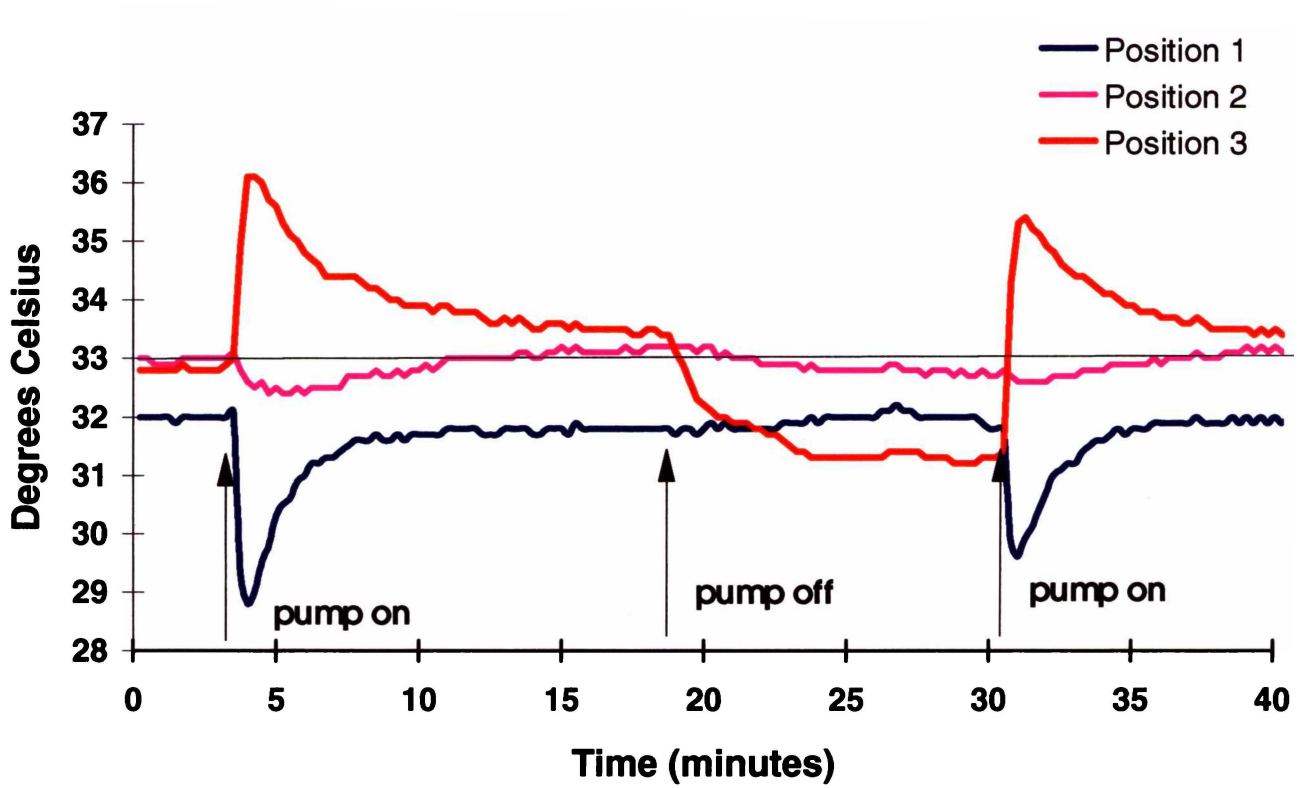


Figure 3-7.
Plot of temperature recordings at 3 positions, as indicated in figure 3-6, on the Tenax trap during SCO_2 elution.

Effects of SCO_2 pressure on the elution of volatiles from Tenax traps

Four replicate traps were connected to the manifold and the volatiles from 200 g of meat collected for 3 hours. Three of the Tenax traps were then eluted with SCO_2 at 33°C and 100, 132 and 300 atm respectively onto the 0.53 mm i.d capillary column connected to a FID detector. No adjustments were made to the split vent flow controls for this experiment. The eluted volatiles were cryofocused at +1°C for 10 minutes before the temperature ramp was started. After SCO_2 elution, the three traps and the fourth (unextracted) trap in the series was thermally desorbed at 250°C for 10 minutes onto the same column using the same chromatography conditions. The series of chromatograms in figure 3-8 show that SCO_2 is effective, particularly at the two lower pressures, at eluting trapped meat volatiles from the Tenax traps at 33°C. Apart from a peak at 49 minutes, few volatile compounds remained on the traps, as shown by subsequent thermal desorption.

Reduced loading of the amount of the sample on the column is noticeable at 300 atm compared with the two lower elution pressures. Also evident is a significant reduction in the column loading of all three SCO_2 traps compared with the fourth trap that was thermally desorbed (figure 3-8d). Each SCO_2 chromatogram showed poor resolution during the first 20 minutes and was investigated in full at a later stage in this study.

The extra trace in figure 3-8d represents a Tenax trap fitted to a purge flask without a sample added and purged for 3 hours at 80°C to show the level of contamination from the purge system and blank trap. The chromatogram shows little contribution from compounds derived from the purge gas, cooking vessel and thermally desorbed Tenax trap.

The observed reduction in column loading during SCO_2 was further investigated.

Flow dynamics of the HP 5890/II gas chromatograph during thermal desorption and supercritical carbon dioxide elution

Figure 3-8 showed low levels of loading of cooked meat volatile compounds onto the GC column when SCO_2 was used as the Tenax eluent compared with thermal desorption. Thus, an investigation into the apparent loss of volatile compounds during SCO_2 elution was undertaken.

Table 3-3 records the flow rates and estimated split ratios of carrier gas during thermal desorption and SCO_2 at various CO_2 pressures.

Table 3-3.

Flow rates recorded at the SF pump and the split vent of the GC during SCO_2 elution and thermal desorption on an HP 5890/II fitted with a 30 m x 0.53 mm i.d DB5 column. Column temperature was -40°C, column flow was 11.8 ml/min, and column head pressure = 5 p.s.i.

Elution type	Pressure (atm)	Liquid CO_2 flow (mL min^{-1})	Initial split flow (mL min^{-1})	Run split flow (mL min^{-1})	Split ratio
Thermal	-------	-------	17	14	1.2 : 1
SCO_2	100	0.47	20	68	5.8 : 1
SCO_2	132	0.59	20	95	8.1 : 1
SCO_2	300	1.69	20	280	23.7 : 1

Flow of expanded gas recorded at the split vent outlet increased 20 times when the pressure of the CO_2 was set at 300 atm compared with thermal desorption. This was also reflected in the split ratio (split vent flow: column flow) at a constant column head pressure, assuming no increase in column flow.

The data in Table 3-3 suggested that the reduced loading on the column at higher pressures was due to an increased split ratio. Further evidence of this was shown when, in a separate experiment, a trap was eluted with SCO_2 with an unaltered GC split vent

(figure 3-9a) and compared to a trap eluted with SCO_2 when the split vent was blocked off with a plug (figure 3-9b) and with a trap desorbed thermally (figure 3-9c).

In this experiment the thermally desorbed trap was also operated with the split vent blocked and column head pressure rose from 34.5 to 55.2 kPa.

Comparison of peak shapes of chromatograms after 30 minutes retention time showed loading of the SCO_2 trap increased to about the level of the thermal desorption when the split was blocked (figure 3-9b). However, a significant difference in the chromatograms was evident between 0 and 30 minutes. When, as in this trial, the split vent was blocked during SCO_2 elution, the column head pressure increased from 34.5 to 179 kPa. This increased the column flow from about 9 to 76 mL min^{-1}. At this high CO_2 flow rate through the 0.53mm column, the oven cryofocusing temperature of +1 °C was not low enough to prevent distortion of the early eluting peaks. This effect is discussed in detail below.

However, it was still possible that the difference was due to incomplete elution of the Tenax trap by SCO_2 while the trap was connected to the gas chromatograph. This is because at the end of the elution period the trap was removed from the gas chromatograph and allowed to depressurize to ambient pressure off-line. During this time further elution of trapped volatile compounds could still occur but go undetected. Miller *et al.* (1993) found that rapid depressurization (< 30 seconds) of a supercritical fluid extraction vessel held in a static state (ie, no flow out the restrictor) at 400 atm and 50 °C for 15 minutes resulted in good recoveries of a series of alkanes spiked onto sand. Thus, the possibility of rapid elution of retained residues on the Tenax trap was investigated by using SCO_2 to elute trapped meat volatiles from one trap onto a freshly cleaned secondary trap.

The outlet restrictor of the first SCO_2 trap was connected to the inlet of the secondary trap kept on a bed of dry-ice. SCO_2 elution was for 10 minutes at 33 °C and 132 atm. After elution of the volatiles from the first trap onto the second, this second trap was allowed to come to room temperature. The contents of the second trap were then

thermally desorbed at 250°C for 10 minutes onto the GC capillary column (figure 3-10a) and compared with a replicate primary trap similarly thermally desorbed (figure 3-10c). Similar peak shapes and peak areas were observed in each of the profiles.

Subsequent thermal desorption of the first trapeluted with SCO_2 onto the secondary trap showed negligible material remained on the trap after SCO_2 elution at 33°C and 132 atm. for 10 minutes (figure 3-10b).

These results confirm that the main cause of loss in sensitivity during SCO_2 elution compared with thermal desorption was due to the increased split ratio rather than the inability of SCO_2 to effectively elute trapped volatile compounds from Tenax-TA.

This can be explained by the flow characteristics of the Hewlett Packard gas chromatograph. The flow characteristics of the HP 5890/II split/splitless injector are shown in figure 3-11. In Split Mode (Purge control valve "open"), under normal liquid injection conditions, the flow of most of the carrier gas, which is set by a combination of the total flow controller and the back pressure regulator, is down the injector insert. A small amount (~1 mL min^{-1}) of helium carrier gas is bled off at the top of the insert for the septum purge. Any liquid sample injected by syringe into the injector insert is vaporized and swept along by most of the carrier gas (~50 mL min^{-1}). When the flow reaches the bottom of the injector insert, the split occurs. About 1 mL min^{-1} (for a 30 m x 0.32 mm i.d column) of vaporized sample/carrier gas mix enters the column and the remainder travels through the open purge valve and exits the GC at the split vent (figure 3-11a). The flow set by the total flow controller determines the split flow.

By contrast, in the splitless mode the purge valve is maintained in the closed position. Although the total flow remains the same, only about 1 mL/min of carrier gas enters the injector insert. The remainder of the carrier flow does not enter the injector insert but is instead directed by the purge valve to the split vent (figure 3-11b). Under normal liquid injection conditions all of the sample in the injector insert is swept onto the column after it is vaporised.

Preliminary experiments with both elution systems used in this study showed that the flow path is interestingly in the split mode when the purge control valve is either open or closed (i.e. split or splitless). When the purge control valve is closed (normally splitless) and the needle from the thermal desorber (or the restrictor from the SCO_2 elution trap) is inserted into the injector port, the carrier gas (or the carbon dioxide) carries the desorbed solutes from the Tenax trap to near the bottom of the injector insert. About 1 mL $min.^{-1}$ of the sample/carrier gas mix enters the column. The amount is regulated by the column head pressure controller. The remainder of the flow passes back up the injector insert and out the split vent - along the same flow path as the excess vaporised sample in a normal liquid injection in the splitless mode. This is because the total flow of carrier gas enters at the tip of the needle deep in the injector insert and not via the total flow inlet at the top of the insert. Therefore the sample is mixed with all the carrier gas (or SCO_2) before the split takes place, whereas with a normal liquid injection the split occurs before the carrier mixes with the sample. Thus, if all the carrier flow cannot enter the column much of the sample is lost out the split vent.

When the purge valve is open (i.e. split), the flow characteristics are the same as for a split mode of operation during a normal liquid injection.

Increasing the sample volume entering the column during thermal desorption and SCO_2 Elution can be accomplished by three possible methods:

(i) Increase the column head pressure, which reduces the flow out the split vent, increasing the back pressure thus increasing carrier gas flow into the column.

(ii) Decrease the total flow to the injector insert so a greater portion of the total flow from the Tenax trap enters the column - assuming the column head pressure remains unchanged.

(iii) On-column injection where all the flow from the trap must enter the column.

On-column injection was not possible with the 0.32 mm i.d column used in this study because the mass spectrometer vacuum pump could not cope with the high CO_2 gas flows (up to 600 $min.^{-1}$) during on-line SCO_2 elution.

Manually decreasing the total helium flow during the desorption period then returning to normal flows after desorption was difficult to control reproducibly between runs for thermal desorption. Hewlett Packard has recently produced an electronic pressure/flow controller that would now make this possible. Manual flow reduction was also ineffective during SCO_2 elution because the carrier flow was shut off and replaced by the Supercritical CO_2 that entered the GC after the total flow control valve. Thus, the valve can not regulate the CO_2 flow - that is dependent on the CO_2 pressure and the length and diameter of the capillary restrictor.

The first option, increasing the column head pressure only for the duration of the desorption period, was chosen.

A needle flow valve was connected to the split vent of the Gas chromatograph to increase the loading onto the column. During thermal desorption and SCO_2 elution, the column head pressure was increased from 34.5 to 307 kPa by adjusting a combination of the column head pressure valve and the needle control valve at the split vent. This procedure forced increased carrier-flow onto the column. At the end of the desorption or elution periods the column head pressure and the split vent out flows were returned to their original settings.

Once the problem of insufficient column loading was resolved, the cause of poor resolution of early eluting peaks was investigated.

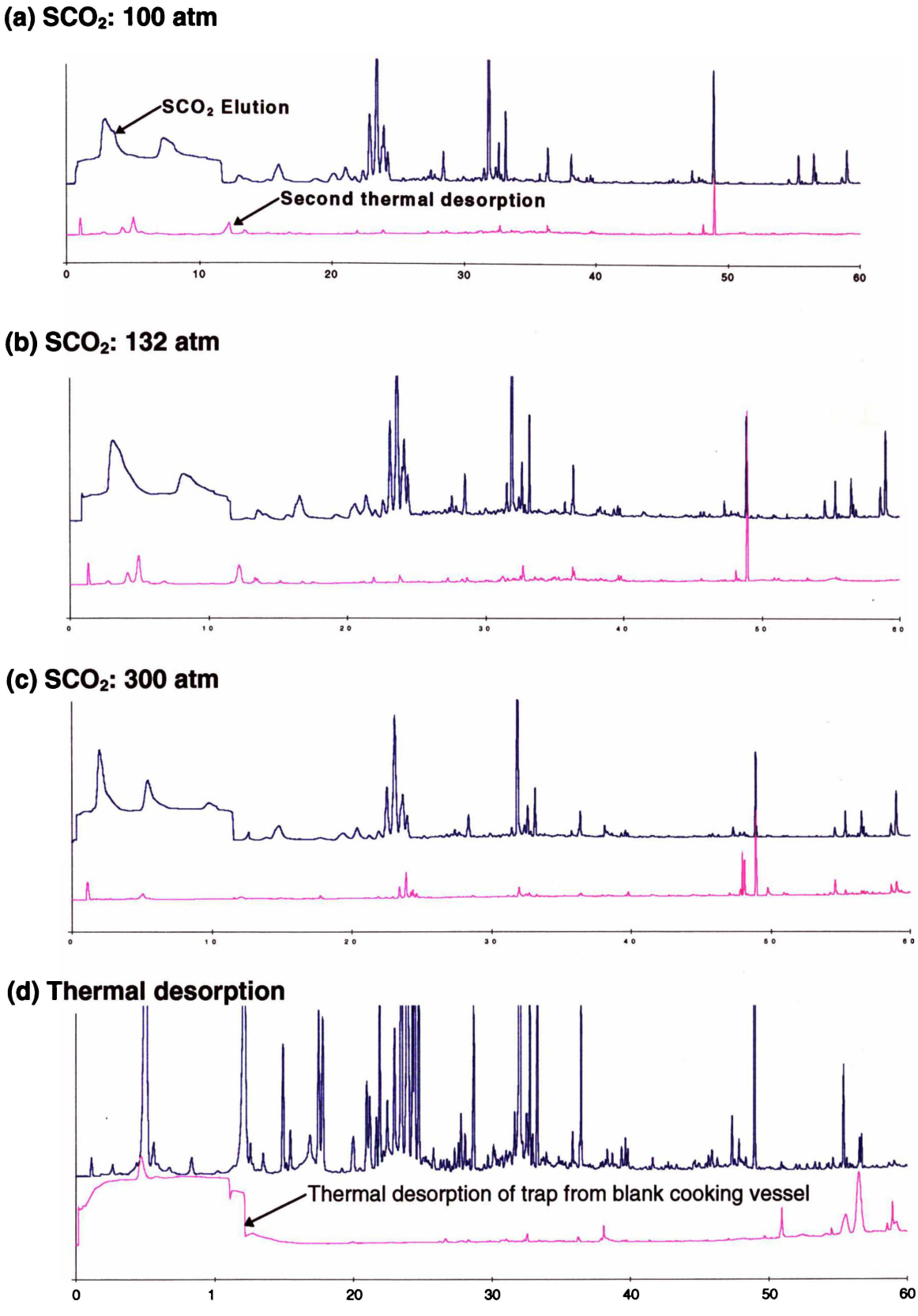


Figure 3-8.

Effects of SCO_2 pressure on elution of volatile compounds from Tenax traps.

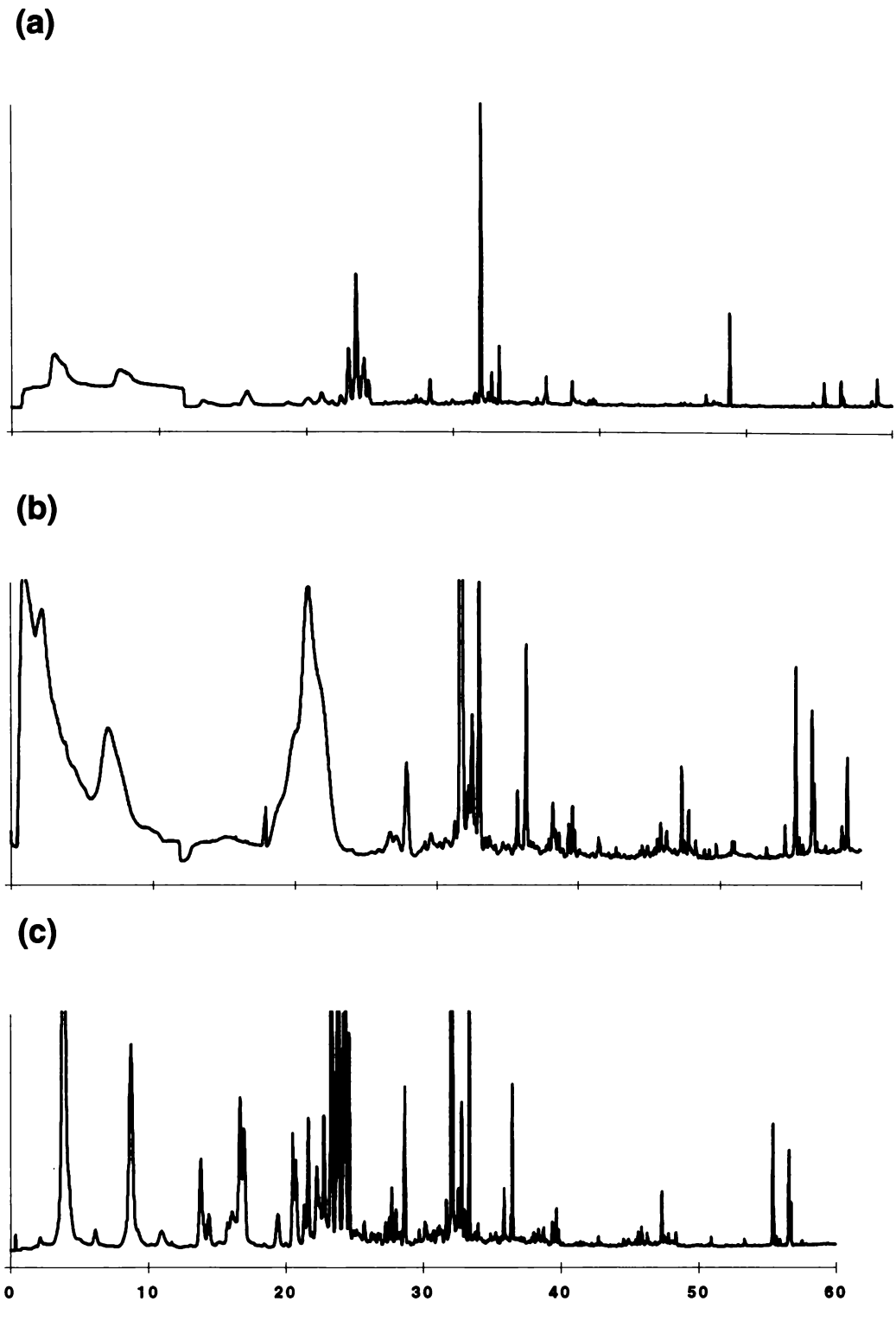


Figure 3-9.
Comparison of SCO_2 eluted traps (a) with GC split vent unaltered and (b) GC split vent blocked off with (c) a similar trap thermally desorbed.

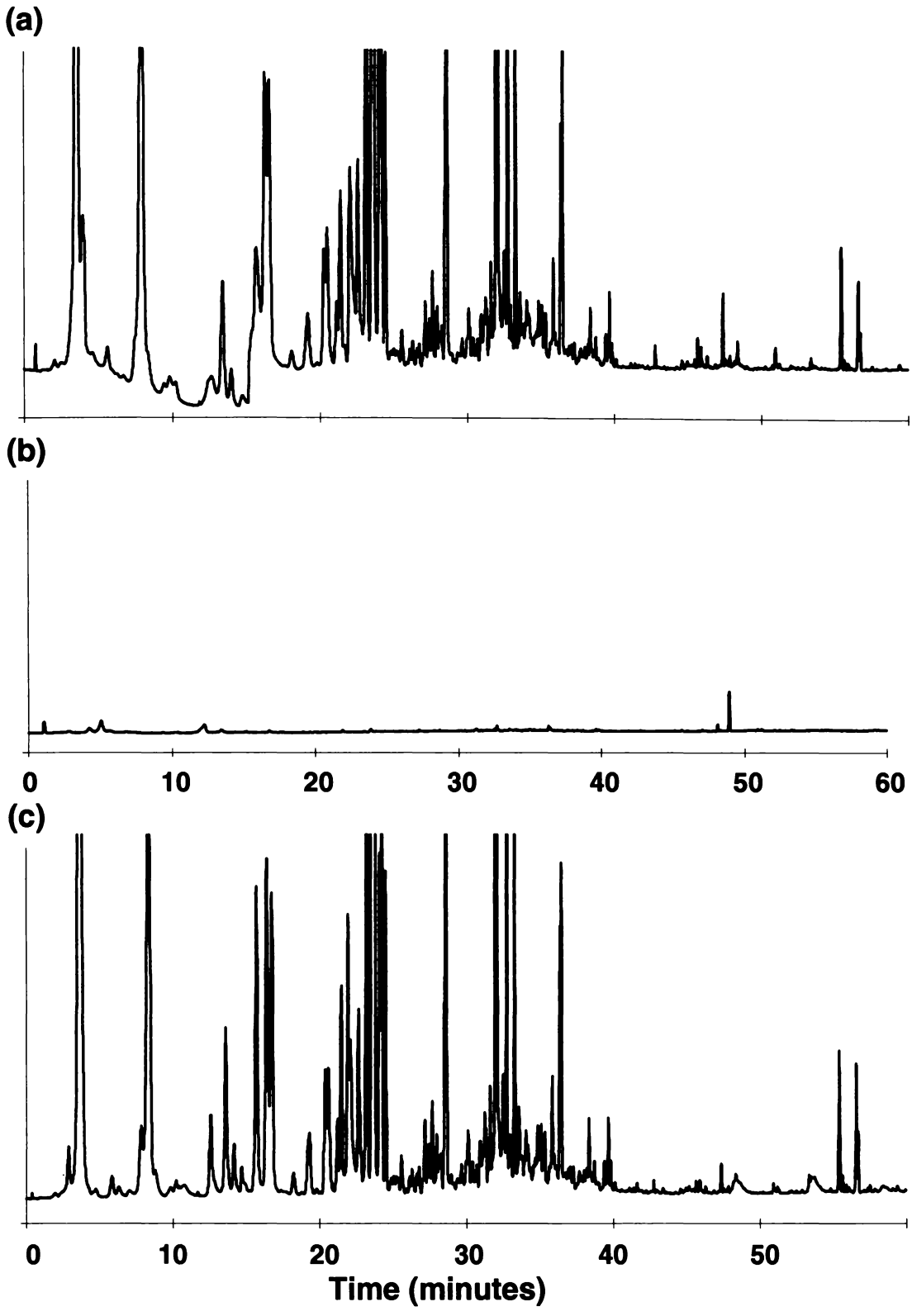
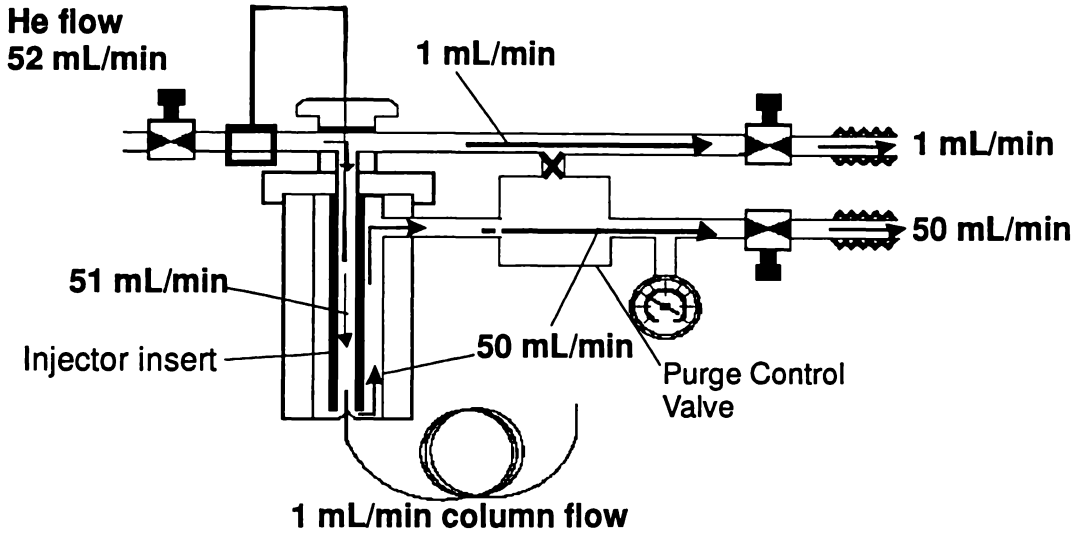


Figure 3-10.

Chromatogram of FID responses of (a) thermally-desorbed secondary Tenax trap used to trap meat volatiles eluted from a primary trap with SCO_2. (b) the thermally desorbed primary trap after SCO_2 elution and (c) a replicate primary trap only thermally desorbed.

(a) Split mode



(b) Splitless mode

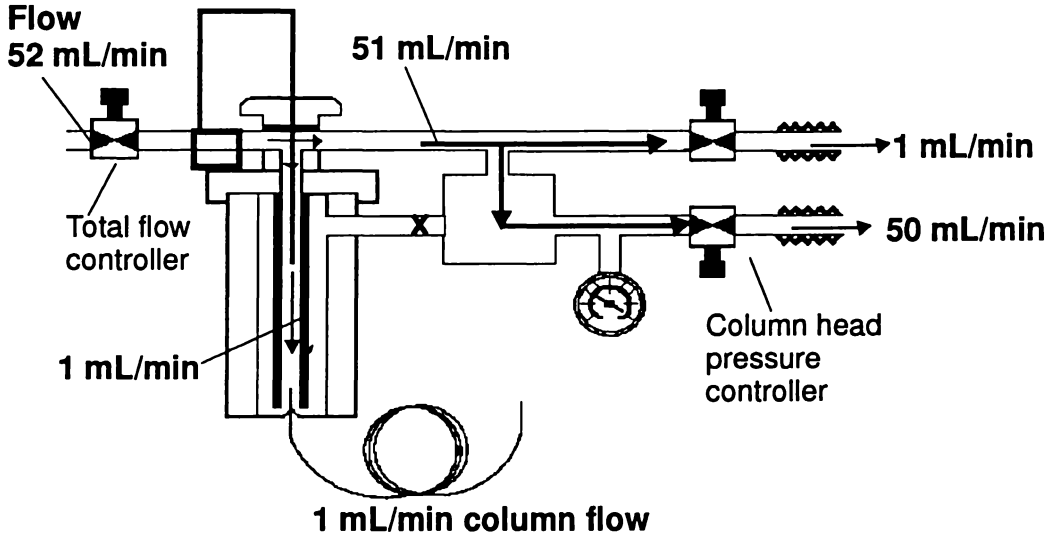


Figure 3-11.
Schematic diagram of flow paths of a Hewlett Packard split/splitless capillary injector in (a) split mode and (b) splitless mode. X, indicates a closed port.

Column flow rates and analyte peak shapes during thermal desorption, SCO_2 elution and ether elution

Effective trapping of highly volatile compounds at the head of the column during an extended desorption period requires cooling of the capillary column to. A cryofocusing accessory (Hewlett-Packard) that regulates the flow of liquid CO_2 (very cold) into the GC oven, allows accurate and reproducible control of the GC oven below ambient temperatures. Figure 3-12 shows the relationship of column flow rate and column temperature for both column diameters used in this part of the study. Flow of helium through a capillary column increases due to decreasing viscosity of the gas when the temperature of the column is lowered. Thus, cooling the oven has two potentially beneficial effects during SCO_2 elution and thermal desorption. First, low column temperatures would help retain highly volatile compounds at the head of the column and so help maintain peak resolution. Second, increasing column flow would enhance loading of volatile compounds from the Tenax trap onto the capillary column because a decrease in the split ratio.

To test the effect the initial column temperature has on peak resolution, replicate traps from the same purge and trap of a sample of cooked meat were eluted and cryofocused at + 5, - 20 and - 40°C. This was repeated for each trap elution method. Figure 13 represents the first 30 minutes of GC/MS chromatograms for each elution method.

All three methods show improved analyte loading and peak shapes of early eluting compounds as the cryofocusing temperature was decreased to - 40°C. The effect was most dramatic for SCO_2 elution, very little retainment or resolution of peaks eluting before 28 minutes was observed at +5°C because of the higher column flow rates during SCO_2 elution compared with the other two elution methods. Recently Burford *et al.*, (1994) found similar effects when optimizing chromatographic parameters for SFE-GC of a BTEX mixture. They also found improved retention of low molecular weight compounds when using a thicker (5μm) capillary column stationary phase.

In subsequent experiments a column cryofocusing temperature of - 40°C was used.

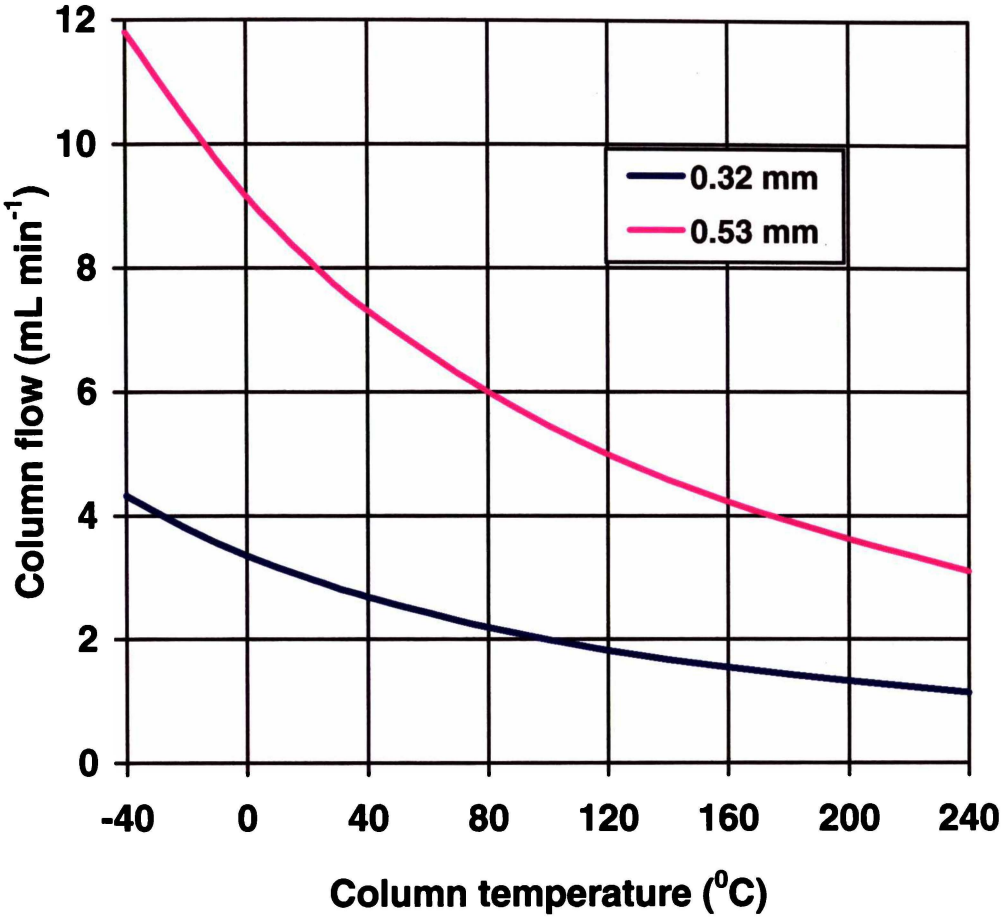


Figure 3-12.

Relationship between gas chromatography capillary column flow rate and column temperature for 30 metre by 0.32 mm or 0.53 mm i.d columns. Note the higher flow rates at the Tenax desorption/elution temperature of -40 ^{0}C.

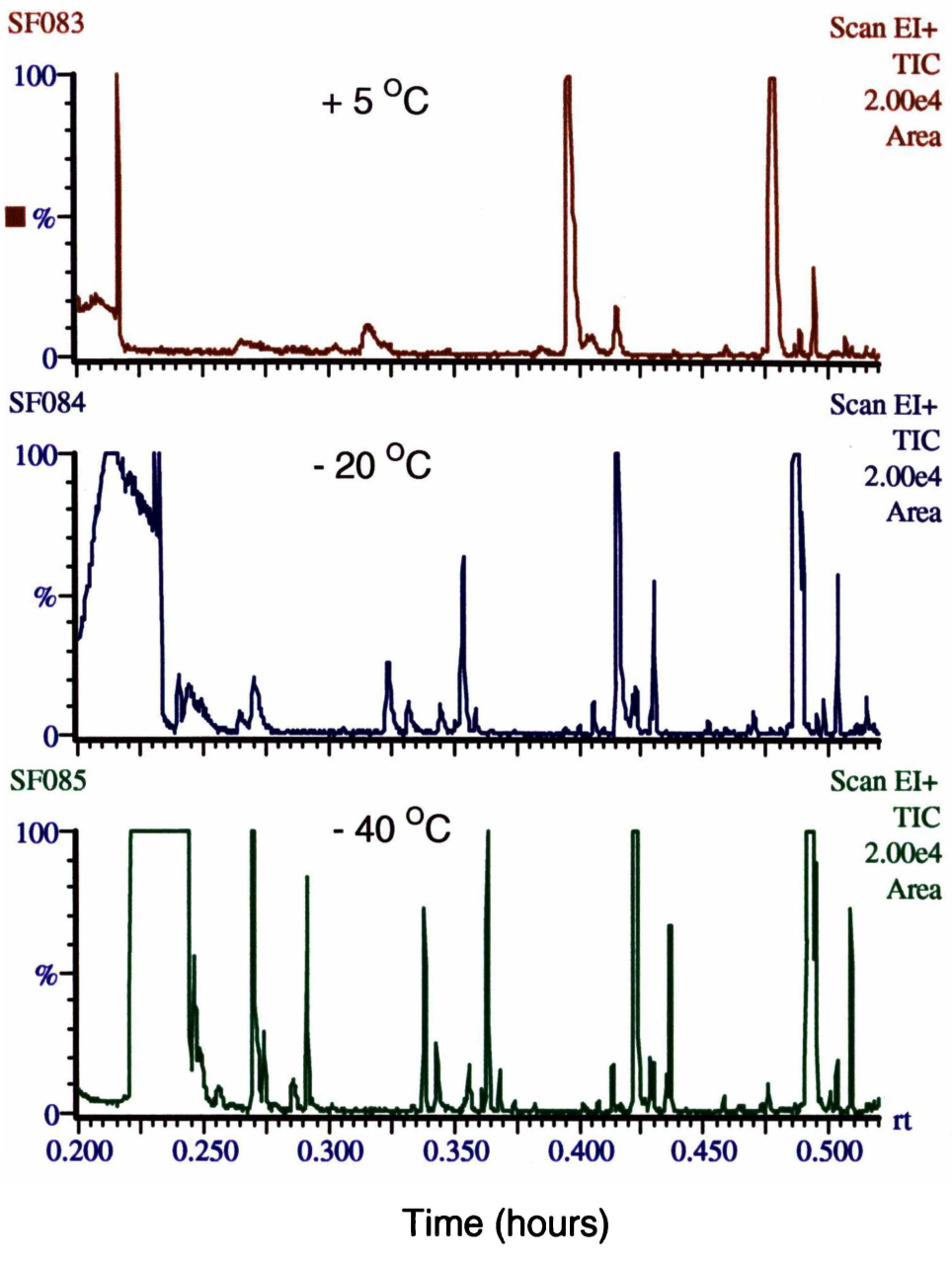


Figure 3-13a.

The effect of capillary column cryofocusing temperature on the elution profile of cooked meat volatile compounds eluted by SCO_2 from Tenax traps. Peaks are displaced because of early elution at higher cryofocusing temperatures.

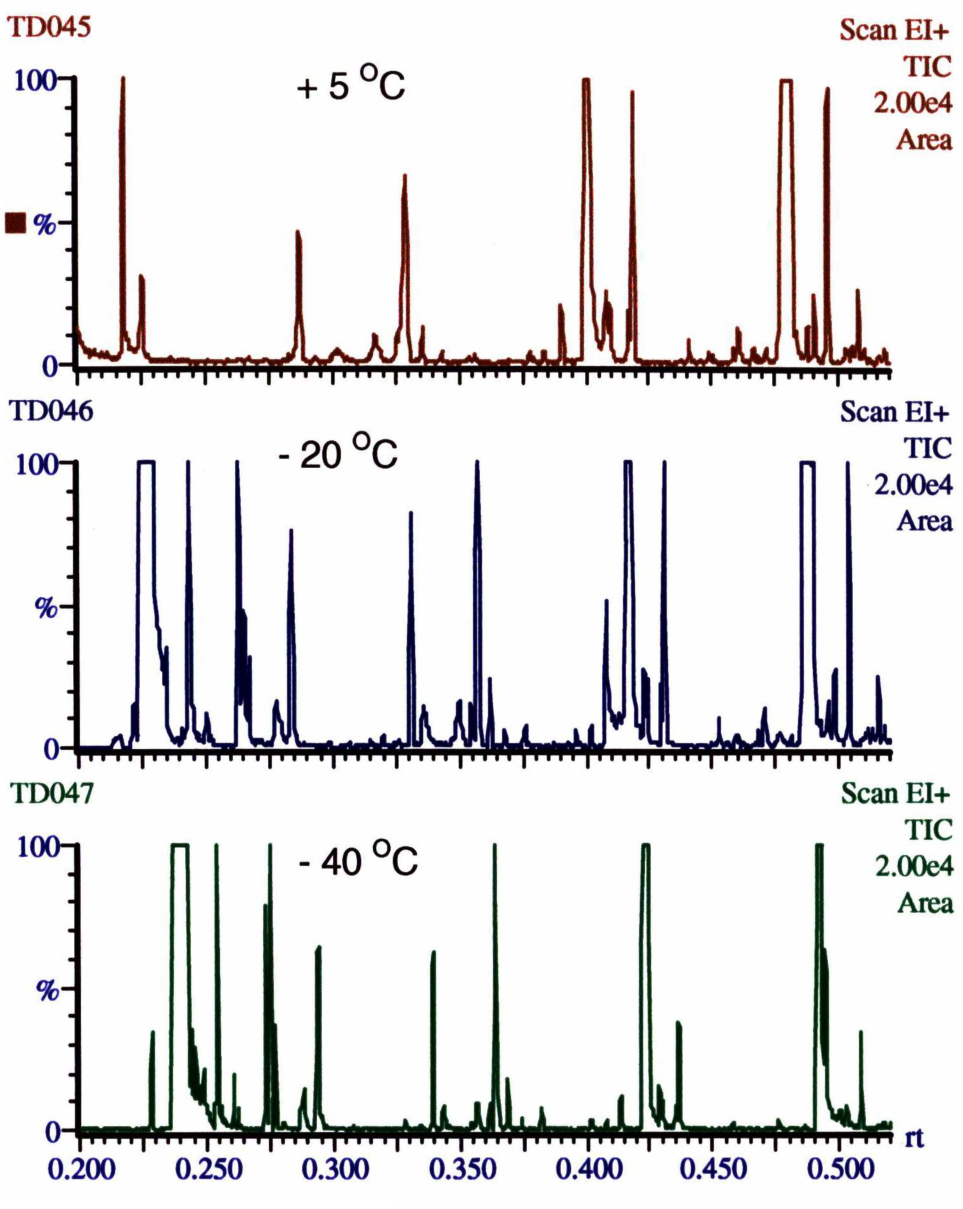


Figure 3-13b.

The effect of capillary column cryofocusing temperature on the elution profile of cooked meat volatile compounds thermally desorbed from Tenax traps. Peaks are displaced because of early elution at higher cryofocusing temperatures.

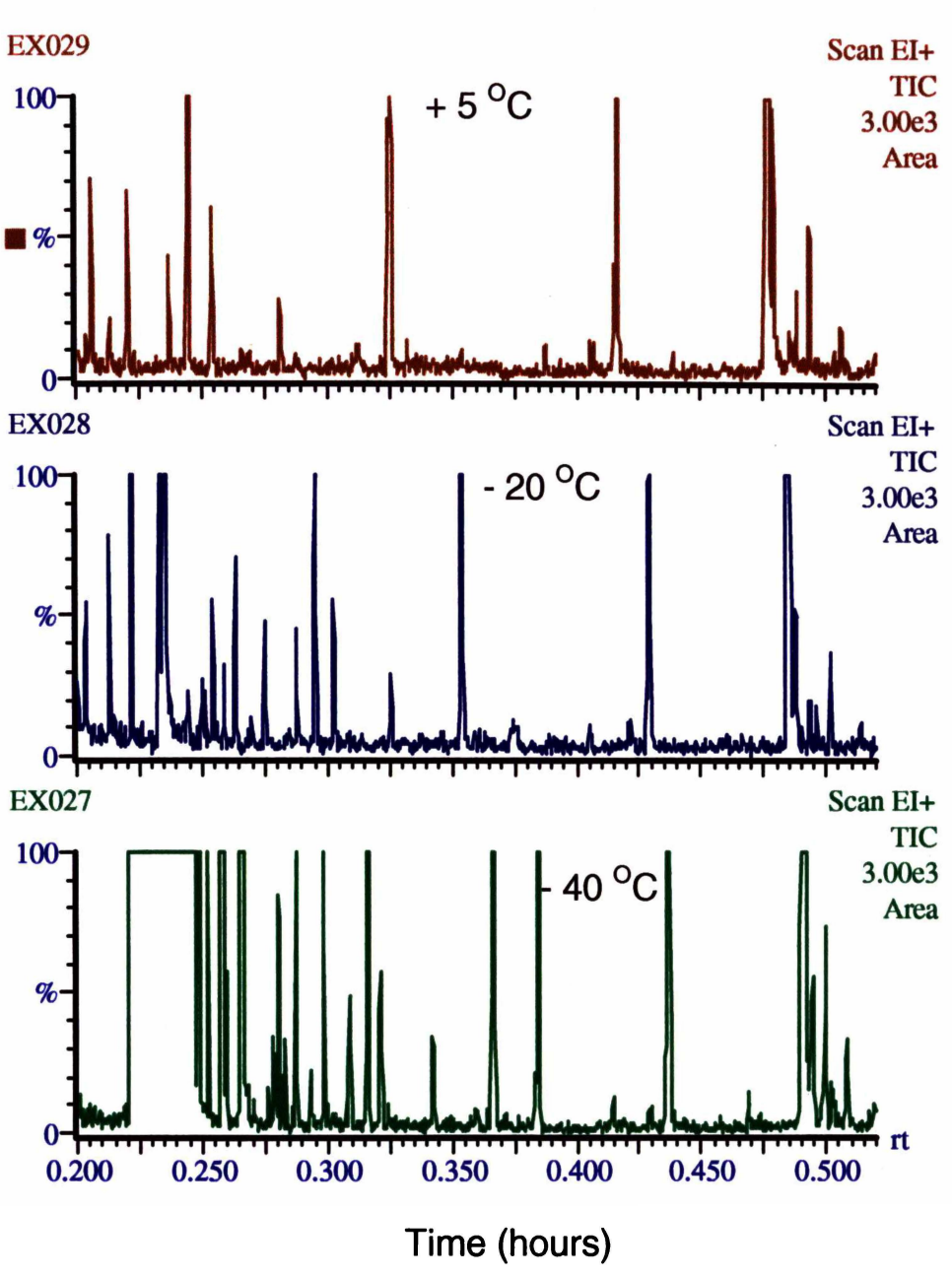


Figure 3-13c.

The effect of capillary column cryofocusing temperature on the elution profile of cooked meat volatile compounds eluted by diethyl ether from Tenax traps. Peaks are displaced because of early elution at higher cryofocusing temperatures.

Effects of SCO_2 temperature, at constant pressure, on the elution of volatiles from Tenax traps

Three traps simultaneously collected volatiles purged for 3 hours from the model mixture (Table 3-1) containing heptanal, 2-pentylfuran, 2,4-hepadienal, 2,3-dichloropyrazine, nonanal, benzothiophene, and 2-undecanone. Each trap was eluted for 10 minutes with SCO_2 at 132 atm at either 33, 50 or 70°C.

Initial inspection of raw area counts from the three temperatures suggested that a SCO_2 elution temperature of 33°C was most effective at eluting the volatile compounds from the Tenax traps (figure 3-14a). However, when each compound was normalized to the external standard, 2-chloropyrazine (figure 3-14b), there was a trend of increasing quantities eluted as elution temperature increased. This was most probably because of experimental variation, because a repeat desorption of the 33°C trap (at the same temperature) showed no retained compounds that would have been expected if the first elution were incomplete.

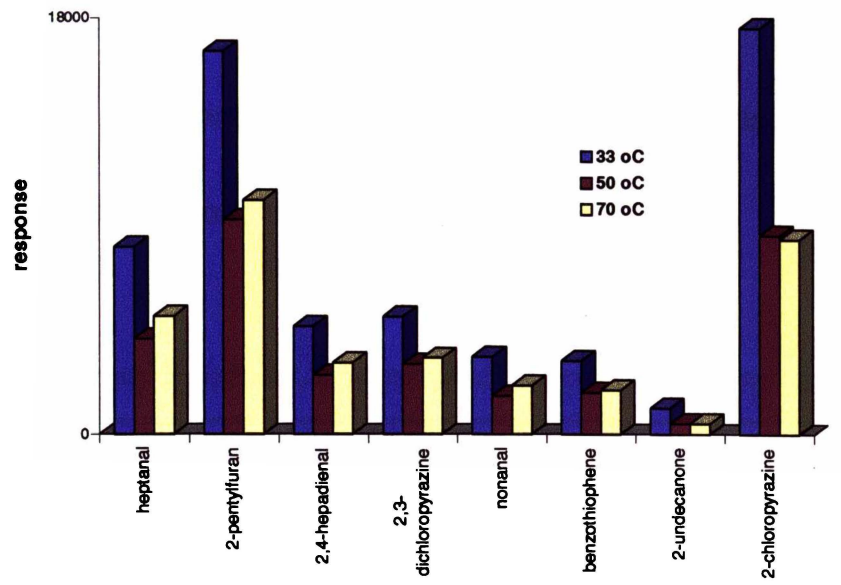
Thus, elution at 33°C, the lowest possible temperature of the supercritical carbon dioxide, was considered appropriate for future experiments.

Varying SCO_2 elution times

Three replicate traps were eluted at 33°C and 132 atm for 2, 5 or 10 minutes to optimize the SCO_2 elution times. Each trap was then re-eluted, omitting the external standard 2-chloropyrazine, with SCO_2 at 33°C and 132 atm for 10 minutes to check for compounds retained after the initial elution.

The results show (figure 3-15) that 2 and 5 minute initial elution times were inadequate to elute all volatile compounds from the traps. The second elution of the 10 minute elution replicate, showed that 10 minutes was the best elution time at 33°C and 132 atm.

(a) Data expressed as raw area counts



(b) Data normalized on 2-chloropyrazine

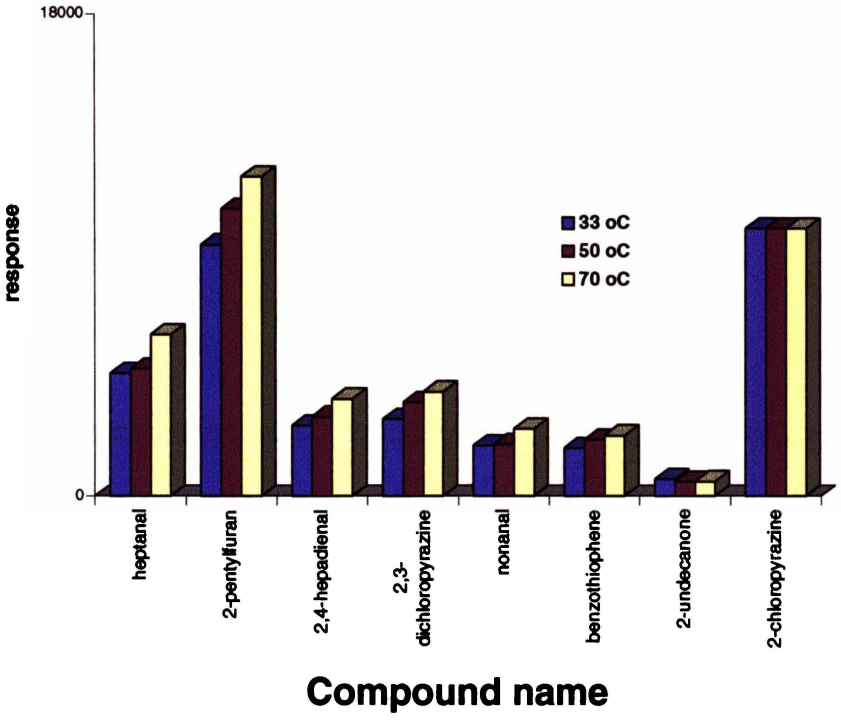


Figure 3-14.

The effect of varying temperature at constant pressure (132 Atm) on elution of volatile compounds from Tenax traps. Standard amounts of each compound was spiked into Wesson oil, heated to 80^{0}C, purged with N_2 and trapped onto Tenax for 3 hours. The external standard, 2-chloropyrazine, was added to the outlet of each trap immediately before elution with SCO_2.

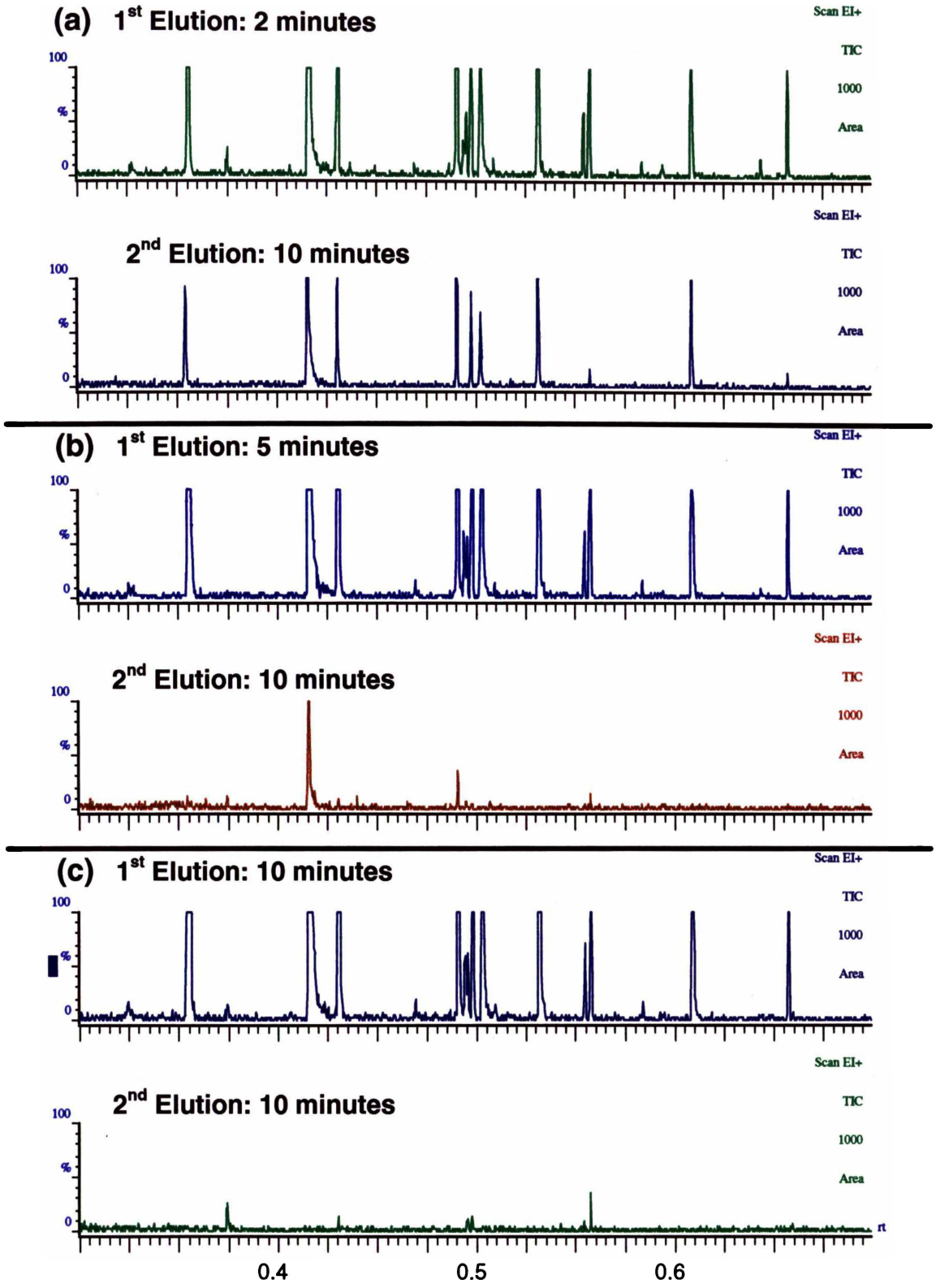


Figure 3-15.

Varying SCO_2 elution time. A separate Tenax trap containing volatile compounds from spiked Wesson oil was initially eluted for either (a) 2, (b) 5, and (c) 10 minutes at 33°C and 132 atm. Each trap was then eluted for a further 10 minutes (33°C, 132 atm) to check for volatiles retained.

Method repeatability

The coefficients of variation of 1 μL of 2-chloropyrazine added to the outlet of four separate blank Tenax traps thermally desorbed or eluted with SCO_2 was 11 and 13% respectively.

The coefficients of variation of raw area counts for each compound trapped from the Wesson oil (Table 3-1) on triplicate traps on three separate occasions and desorbed by SCO_2 (Table 3-4) show a range from 10 to 47%. Area counts were then normalized to the external standard, 2-chloropyrazine, to reduce variation due to the SCO_2 elution step. Variation of heterocyclic compounds reduced noticeably with a concomitant increase in variation of aliphatic compounds. Conversely, when data was normalized to heptanal, representative of an aliphatic compound, variability of this chemical class decreased while the variability of heterocyclics increased.

Table 3-4.

Coefficients of variation for each compound added to Wesson oil and the internal standard, 2-chloropyrazine, added to the outlet of each Tenax trap and eluted with SCO_2. Data represents three replicate traps from each of three separate trapping experiments.

Compound	Peak #	Raw data	Normalization on: 2-chloropyrazine	Normalization on: heptanal
Heteorcyclics				
2,3-dichloropyrazine	8	11	9	10
2-chloropyrazine	3	16	-	16
2-pentylfuran	5	10	9	9
benzothiophene	10	13	10	12
Aliphatics				
heptanal	4	10	15	-
pentanal	1	22	27	18
hexanal	2	14	20	9
octanal	6	14	22	11
2,4-heptadienal	7	16	14	12
nonanal	9	47	58	48
2-undecanone	11	35	38	38

This was further investigated by analysing data from a separate experiment that compared each of the elution methods. On six separate occasions, three traps were connected to the RBF to collect volatile compounds liberated from the Wesson oil model mixture heated at 80°C for 3 hours. One trap was desorbed by one method on each occasion so generating a 6 x 3 x 3 factorial. Data for each desorption/elution method was combined and expressed as:

(i) raw area counts

(ii) area counts normalized on the external standard, 2-chloropyrazine

(iii) area counts normalized on heptanal, an analyte representative of aliphatic compounds

(iv) area counts normalized on 2,3-dichloropyrazine, an interal analyte representative of heterocyclic compounds

Coefficients of variation for each method of data expression were then calculated and are presented in figure 3-16.

The figure 3-16a clearly shows similar variability between elution techniques and between heterocyclics and aliphatics when expressed as raw area counts. An unexplained high variability was observed for 2-undecanone when thermally desorbed compared to ether and SCO_2 elution.

When normalized on the external standard, 2-chloropyrazine, variability again decreased - more so for the heterocyclics than the aliphatics (figure 3-16b). This compound class effect was even more evident when the data were normalized on heptanal (figure 3-16c) and 2,3-dichloropyrazine (figure 3-16d). Normalization on either of the latter two compounds simulates internal standardisation and should account for overall variability of the trapping, elution and chromatography steps combined. Thus, the variability for all compounds is further reduced. However, variability of heterocyclic compounds was lower when normalized on one chemical of the same class (2,3-dichloropyrazine). A similar trend was observed for the aliphatics. This effect was common to the three methods of elution.

One possible reason for this interesting effect is that some chemical class-related variation in purge and trapping efficiency occurred. Alternatively, a similar chemical class related effect may be evident during calculation of the total ion area counts by the mass spectrometer.

Whatever the reason for the chemical class-related difference, using an appropriate internal standard for each chemical class is desirable. Table 3-5 lists the coefficients of variation for each compound using heptanal and 2,3-dichloropyrazine as the internal standard for aliphatic and heterocyclics, respectively.

Table 3-5.

Coefficients of variation, expressed as a percentage of mean area counts after normalization on heptanal for aliphatic compounds, and 2,3-dichloropyrazine for heterocyclic compounds, for each of the Tenax elution methods.

Peak #	ID	Boiling point (°C)	Elution/Desorption method		
			SCO_2	Thermal	Diethyl Ether
1	pentanal	102	6	6	5
2	hexanal	130	3	3	4
6	octanal	171	5	13	6
7	2,4-heptadienal	84	14	46	14
9	nonanal	191	24	25	23
11	2-undecanone	231	18	116	35
5	2-pentylfuran	163	3	8	8
10	benzothiophene	221	7	6	5

All compounds, except 2,4-heptadienal, nonanal and 2-undecanone, gave acceptable variability for each of the three elution/desorption methods. This suggests that the high variability, that tended to increase with increasing compound chain length, for the three aliphatics is independent of the elution/desorption method and probably due to variations in trapping for these compounds from the Wesson oil. This is quite possible since the purge temperature was 80°C and at this temperature these compounds would not be as volatile as the lower boiling point compounds.

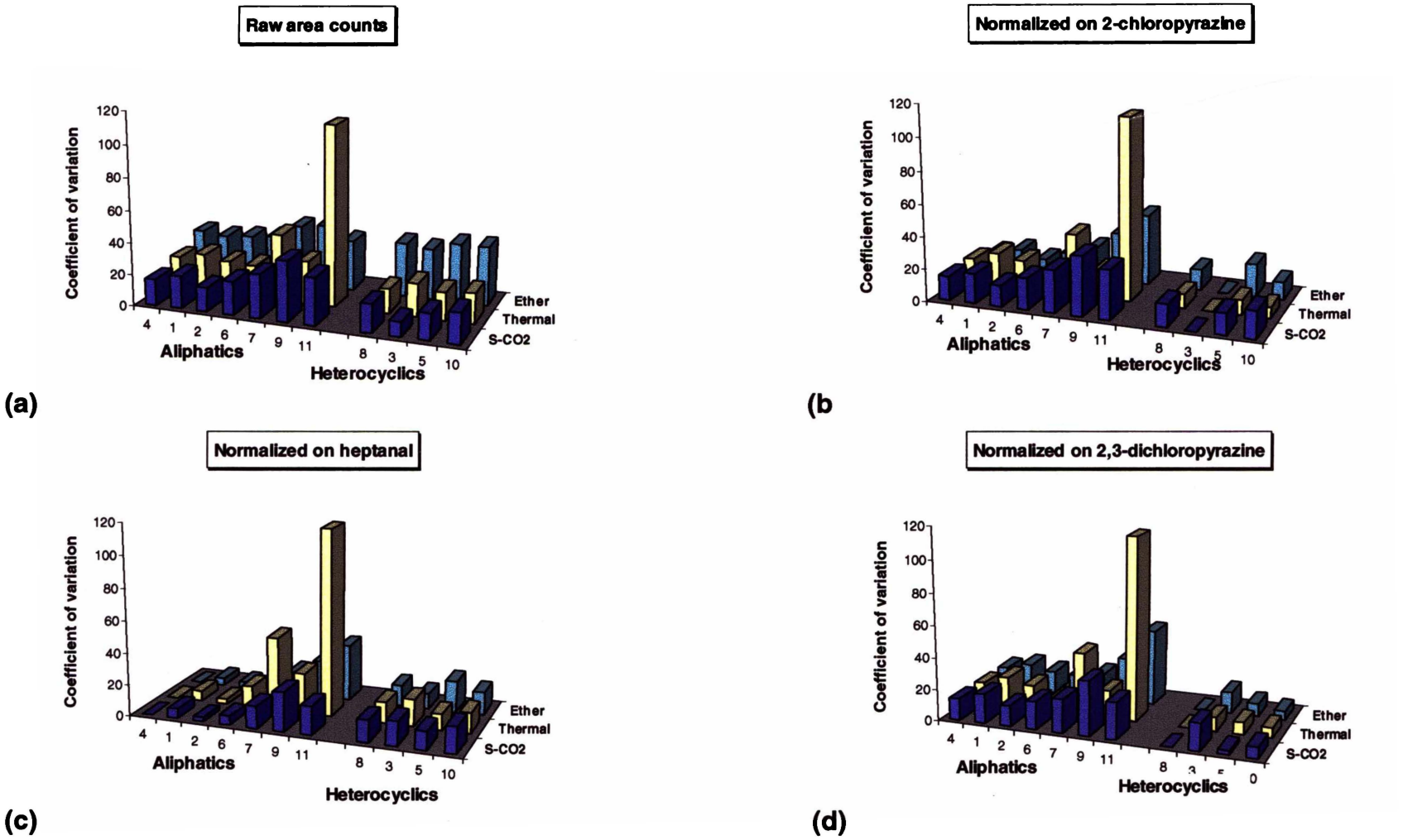


Figure 3-16.

Coefficients of variation for heterocyclic and aliphatic compounds for each of the three Tenax trap elution methods. Compound identification by number are listed in Table 3-4.

The greatest variability was observed for 2-undecanone during thermal desorption. This high variability could be due to a combination of purge-and-trapping variation and incomplete elution during the thermal desorption. Figure 3-17 shows chromatograms of Tenax traps of Wesson oil volatiles:

(a) initially desorbed by SCO_2, then thermally and

(b) initially thermally desorbed, then with SCO_2.

Supercritical CO_2 effectively eluted all volatile compounds from the trap as evidenced by the relative absence of peaks in the subsequent thermal desorption of the same trap. When a trap was initially desorbed thermally, only a small amount of 2-undecanone was eluted compared with SCO_2 elution. However, a second elution of the same trap with SCO_2 eluted residual amounts of 2-undecanone, 2,4-heptadienal and nonanal. It was later found that a cold spot was inadvertently created at the outlet of the trap during thermal desorption causing condensation of some compounds. This was because of a fault in the heater design. The thermal desorber heating jacket did not extend far enough to incorporate the base of the trap that connected into the Swagelok reducing union. The design was then modified to include more effective heating at the outlet. No evidence of thermal degradation products was evident in each chromatogram.

The SCO_2 elution method was considered optimized enough to concentrate on comparing all three elution/desorption methods to effectively remove cooked meat volatiles from Tenax traps.

Figure 3-18 shows chromatograms for Tenax traps that contained meat volatile compounds collected at 100°C for 3 hours and compared by the same reverse order contrast method as in figure 3-17.

Thermal desorption of the trap initially eluted with SCO_2 (figure 3-18a) showed some residual peaks. Four of these were from column bleed (characteristic *m/z* 207, 281 and 283 siloxane ions). A residual amount of 2,3-octanedione, the most abundant compound in the initial trap, was also present. In addition, many late eluting compounds (between 50 and 54 minutes), were also present in the second thermally desorbed trap.

Only residual amounts of 2,3-octanedione (the most abundant compound in the first thermal desorption of this trap) and an unidentified peak at 34 minutes was detected in the second SCO_2 elution of the trap initially thermally desorbed (figure 3-18b). No thermal degradation products were observed other than one peak attributed to capillary column bleed (*m/z* 207 ion).

Close inspection of the chromatograms (figure 3-19) of each initial trap revealed no differences in the number or relative intensity of eluted compounds. The main difference in the chromatograms is due to greater column loading of the thermally desorbed trap. Differences at the very start of the chromatogram are attributable to lack of retention and poor resolution of highly volatile compounds during SCO_2 elution (as discussed earlier). For example, hexane and 2-butanone were unresolved in the SCO_2 elution chromatogram whereas they resolved quite well in the thermal desorption chromatogram. Only two compounds, methylene chloride and 2-methylpropanal were present after thermal desorption but absent after SCO_2 elution. It is not likely that methylene chloride is a result of thermal degradation of volatile compounds found in cooked meat and is most probably a contaminant. The origin of 2-methylpropanal is uncertain. Its apparent absence after SCO_2 elution is difficult to confirm because of such poor resolution at the very start of the chromatogram and low column loading during SCO_2 elution. Lower cryofocusing temperatures could be employed to give better resolution but at temperatures below -60°C, the column stationary phase behaves as a solid (Grob and Grob, 1979).

These results contrast those of a recent study by Snyder and King (1994). These authors showed that elution of Tenax with SCO_2 (at 50°C and 2,000 psi for 1 minute) was more effective at desorbing less volatile compounds deposited on traps than thermal desorption at 150°C for 1 minute. They also stated that thermally generated compounds were formed during thermal desorption and SCO_2 elution at elevated temperatures (150°C). A possible reason for the difference in findings might relate to the way Snyder and King prepared their traps. They extracted corn oil with supercritical CO_2 and trapped directly on Tenax. It would be expected that more semivolatile compounds would be extracted from corn oil by SCO_2 and directly deposited on to Tenax traps, than trapping volatiles purged from oil

heated at 80°C. These semivolatiles would act as a pool of potential precursors for thermal degradation. Comparisons of SCO_2 and thermal chromatograms in this study (figures 3-18 a and c, respectively) show similar elution patterns. No evidence of greater elution of less volatile, high molecular weight compounds by SCO_2 of traps containing volatiles from cooked meat was observed.

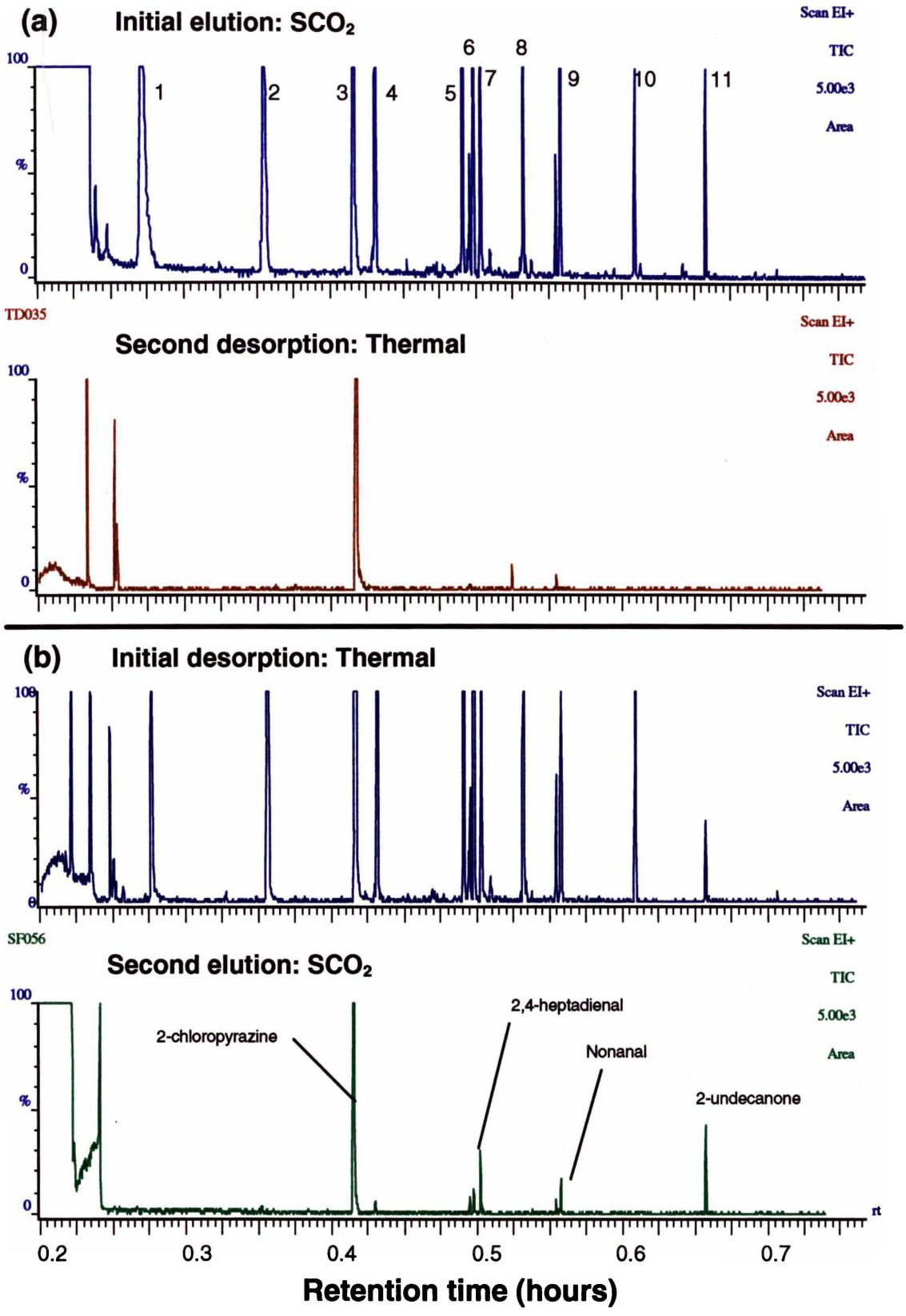


Figure 3-17.

Chromatograms of Wesson oil volatile standards

(a) initially eluted with SCO_2, then thermally desorbed

(b) initially desorbed thermally, then eluted with SCO_2

Peak identifications are listed in Table 3-4.

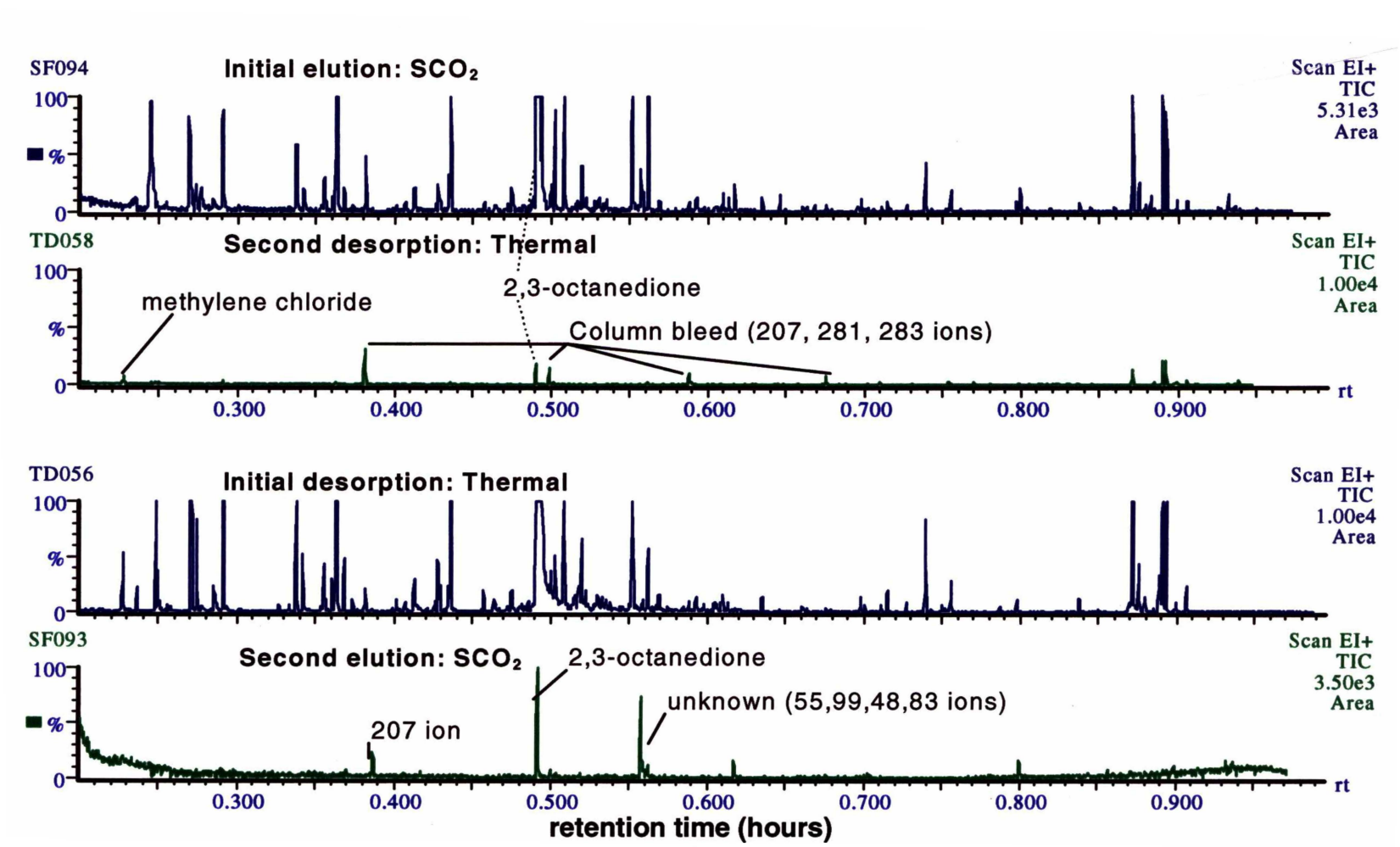


Figure 3-18.

Comparison of Tenax traps containing volatiles from cooked meat (a) first thermally desorbed, then SCO_2 eluted and (b) first SCO_2 eluted, then thermally desorbed. Intensity scales were adjusted to allow for lower column loadings during SCO_2 elution.

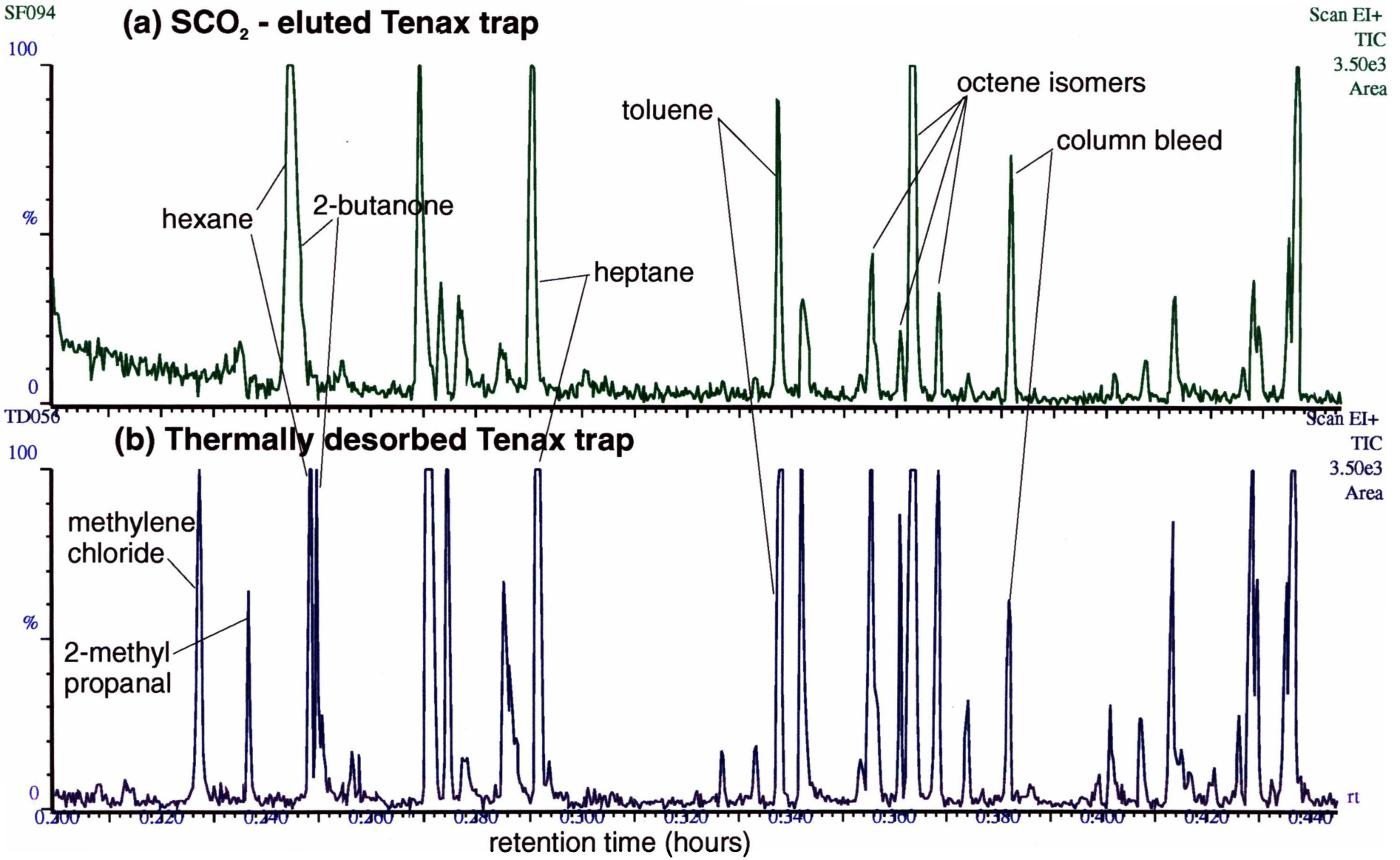


Figure 3-19.
Comparison between SCO_2 eluted and thermally desorbed Tenax traps of early eluting peaks from volatiles generated from cooked meat.

Selection of the appropriate method for elution of volatile compounds from Tenax traps
The results show that elution of volatile compounds from Tenax traps by SCO_2 offers no major advantage over thermal desorption under the conditions used in this study. Once optimized, SCO_2 elution, thermal desorption and diethyl ether elution showed similar variability.

Solvent elution of Tenax traps with diethyl ether showed reduced sensitivity mainly because only a small portion of dried-down eluent could be injected onto the column. For example, if the 12 mL of eluent is reduced in volume to 40 μL and 1 μL injected into a splitless injector, only 1/40 of the total volatile compounds is loaded onto the capillary column. Improvement in sensitivity could be achieved by further reduction in the volume of the eluent and on-column injection. However, the extra step of reducing solvent volume is time consuming and potential of accidental evaporation of all solvent and volatiles increases. These potential problems can be avoided by SCO_2 elution and thermal desorption. (However the latter two elution methods do have the disadvantage of only a single analysis per trap whereas repeated injection of eluted solvent is possible).

Close inspection of chromatograms in figure 3-19 shows that even at relatively low capillary column cryofocusing temperatures (-40°C), poor resolution of early eluting peaks during SCO_2 elution was evident. This has also been shown by Burford *et al.*, (1994) to be associated with high volume flow rates through the capillary column. These authors found that a combination of lower column temperature (-50°C), reduced column flow (increasing the split ratio) and a thicker film column restored peak shape for compounds as volatile as *n*-butane. Unfortunately, increasing the split ratio would lower the amount of volatile compounds loaded on to the column thus limiting the sensitivity of the technique.

After modifications are made to the Hewlett-Packard gas chromatograph, to accommodate high CO_2 flow rates during SCO_2 elution, and optimization of chromatographic conditions such as cryofocusing temperature, split ratio and column film thickness is carried out, SCO_2 elution of Tenax traps is as effective as thermal desorption.

Some loss of sensitivity is possible with SCO_2 elution particularly when very high CO_2 flow rates and high split flows are used.

No evidence of thermally produced artifacts was observed during thermal desorption under conditions used in this study. Incomplete thermal desorption of 2-undecanone from Tenax was indicated in figure 3-17. However, this observation may be clouded by the high variability of purging 2-undecanone from oil at relatively low temperatures (80°C).

Thermal desorption of volatile compounds from Tenax traps was selected as the method of choice because it gave better resolution of early eluting peaks, was more sensitive, did not require restriction of split vent flows during desorption or require expensive, high purity liquid CO_2 supply. In addition, future work was carried out on a Fisons MD800 gas chromatograph/mass spectrometer with a vacuum pump (60-L min^{-1}) that was incapable of handling high column flow rates encounted with SCO_2 elution.

SCO_2 elution, while effective at eluting volatile compounds from Tenax traps at low temperatures, did not offer any advantage over the more classical thermal desorption methodology.

Selection of appropriate sampling conditions for future experiments

Comparison of volatile compounds trapped from cooked meat, cooked meat fat and cooked meat broth

Proposed studies on cooked meat odour and flavour required development of a Tenax trapping technique that would test the same cooked mince meat sample as used by a trained sensory evaluation panel.

Constraints imposed by the experimental design for the meat odour and flavour experiment required individual sheep *m. semimembranosus* muscles to be used. However, they were too small to allow both sensory and instrumental analysis on the one minced muscle. Thus, trapping of volatile compounds from fat rendered during cooking of minced meat was investigated as an alternative to trapping volatile compounds from whole mince.

One Tenax trap collected volatile compounds purged with nitrogen (60 mL min^{-1}) from the headspace of 200 g of whole minced meat (lean + fat) for 3 hours in a waterbath set at 100°C. At the end of the cooking period, the rendered fat and aqueous broth were separated from the cooked meat, comprising mainly lean tissue by this time. Each separated component was subsequently purged, under the same conditions used for the whole cooked mince, and trapped volatiles thermally desorbed onto the DB5 capillary column coupled to the HP 5988A mass spectrometer.

Example chromatograms for each of the three matrices tested are shown in figure 3-20. Of particular interest are two compounds present in relatively higher concentrations in the broth compared with the meat and rendered fat. One compound that eluted at a retention time of 0.35 was consistently present only in broth samples. It is yet to be identified but has a molecular weight of 84 daltons and significant fragment ions of *m/z* 84, 83 and 55. The other compound was benzaldehyde (K_I = 960, confirmed with authentic standard) (figure 3-20b). Its presence in the aqueous extract only is surprising since it is only slightly soluble in water. However, it is volatile with steam which may explain its liberation from the heated broth but still does not explain its presence in broth at relatively high concentrations compared with meat and rendered fat. Macleod and Ames (1987) noted a seven-fold increase in the concentration of benzaldehyde after beef was freeze-dried, defatted and rehydrated then cooked, compared with untreated cooked beef. Removal of triglycerides and phospholipids enhances this effect (Mottram and Edwards, 1983) suggesting protein origin and/or quenching by further reaction with lipids in normal beef.

Little difference is observed between the chromatograms of the original cooked meat and rendered fat, whereas the meat broth showed far fewer compounds. Therefore purge-and-trap of rendered fat gives a good representation of the volatiles generated from cooked meat as is understood in a culinary sense.

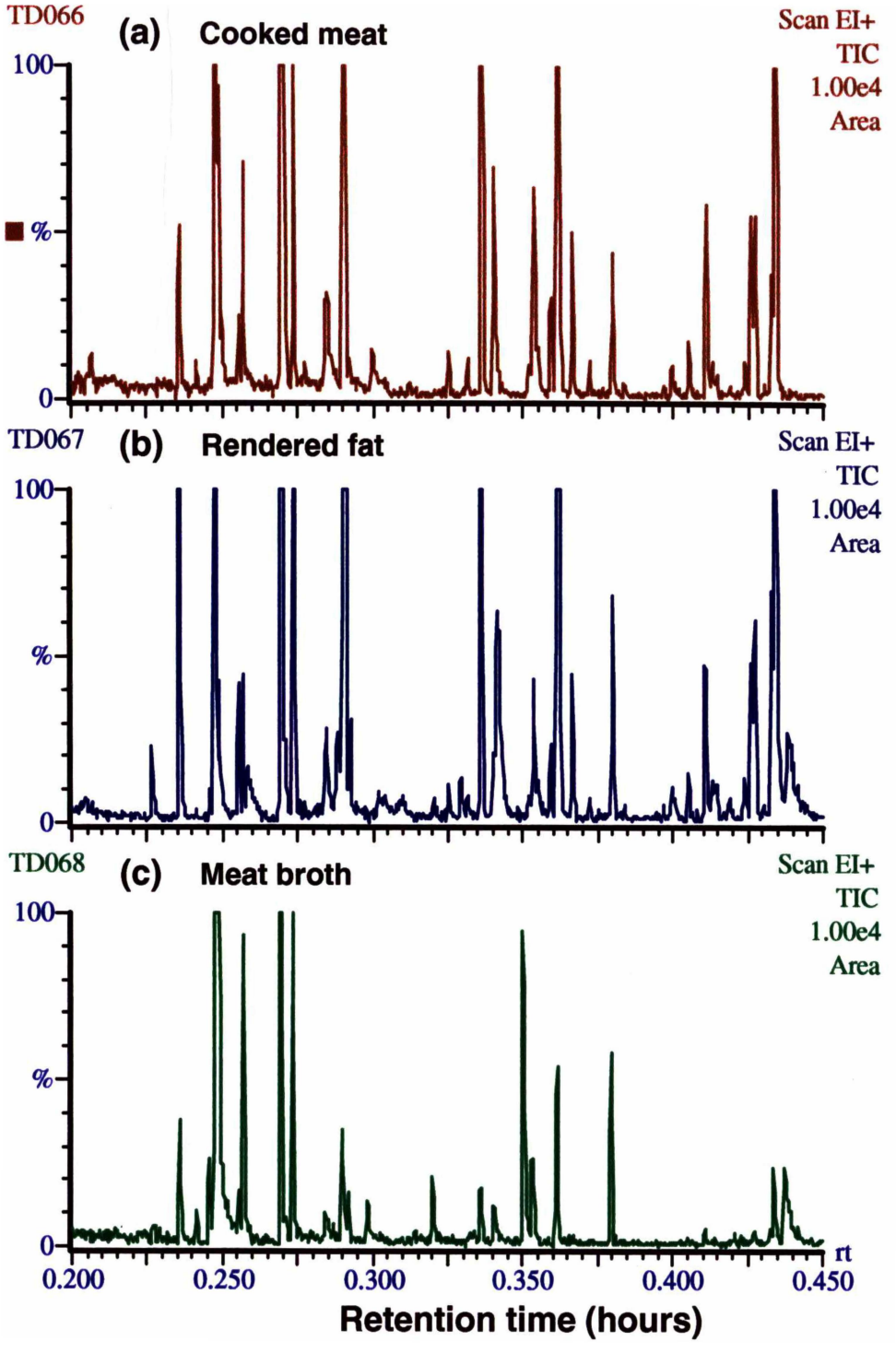


Figure 3-20a.

Chromatograms (from 12 to 27 minutes) of volatile compounds trapped from the headspace above (a) cooked mince meat (b) rendered fat and (c) meat broth.

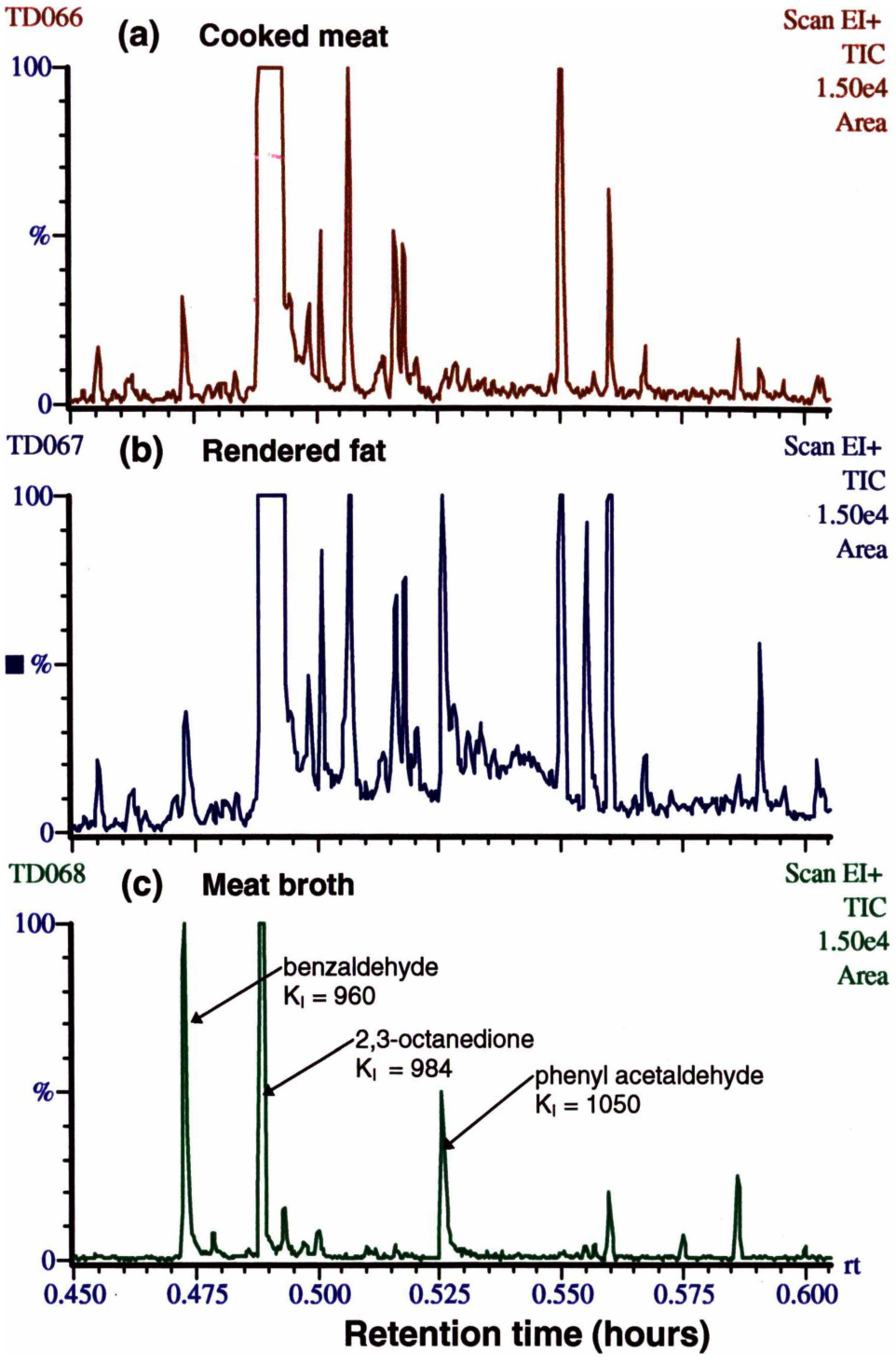


Figure 3-20b.

Chromatograms (from 27 to 36 minutes) of volatile compounds trapped from the headspace above (a) cooked mince meat (b) rendered fat and (c) meat broth. K_I = Kovats' index number.

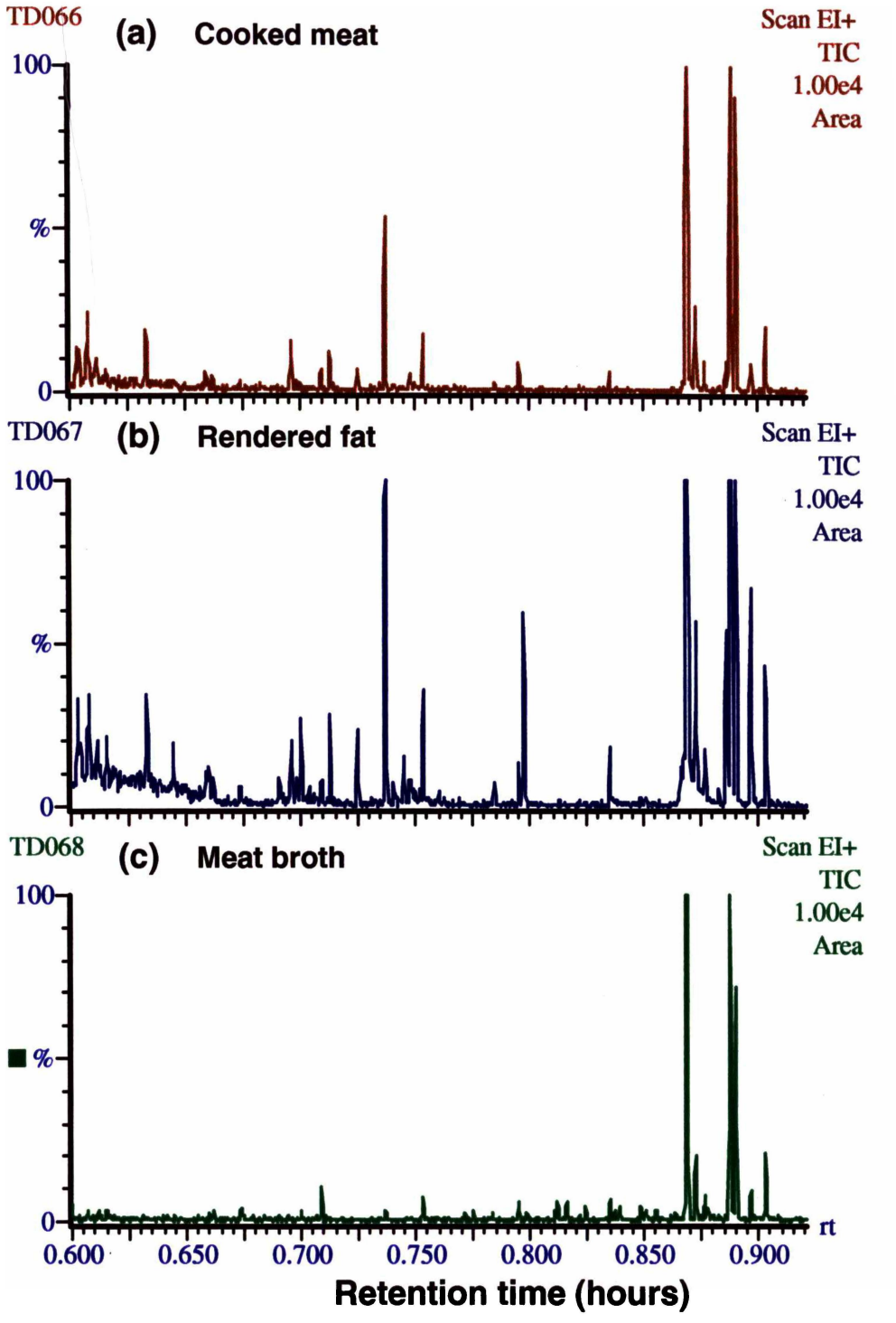


Figure 3-20c.

Chromatograms (from 36 to 54 minutes) of volatile compounds trapped from the headspace above (a) cooked mince meat (b) rendered fat and (c) meat broth.

Modifications to the rendered fat sample weight, purge vessel design and trapping time

Three hours trapping of volatile compounds from the headspace of about 20 g of rendered fat onto Tenax traps and analysis using the HP 5988A mass spectrometer gave a reasonable number of chromatographic peaks. However, it was anticipated that the small amount of rendered fat generated from the cooked *semimembranosus* muscle would be insufficient to allow replicate GC/MS analysis if about 20 g were used. In addition, three hours of trapping volatiles from rendered fat, derived from cooked meat immediately before assessment by sensory panellists, may not truly represent the volatile compounds panellists would smell. Thus, reduction of the sample weight, trapping time and subsequent purge vessel design was investigated.

Figure 3-21 shows a comparison of (a) volatiles from the headspace of 20 g of rendered fat trapped for 3 hours from the RBF vessel and thermally desorbed from a Tenax trap onto a 30 m x 0.32 mm i.d DB5 column fitted to a HP 5988A mass spectrometer and (b) volatiles from the headspace of 2 g of rendered fat trapped for 30 minutes from a new purge vessel (figure 3-22) and thermally desorbed from a Tenax trap onto a 30 m x 0.25 mm i.d DB5 column fitted to a Fisons MD800 mass spectrometer. Generally the chromatogram profiles are similar. However, some differences are evident. Differing retention times are because of dissimilar column diameters and a slight difference in column temperature ramp. Chromatogram (a) was generated from a separate meat sample from a different carcass some months before installation of the Fisons MD800 used to generate chromatogram (b). Relative intensities and qualitative differences could quite conceivably be accounted for by animal variation. For example, 2,3-octanedione, the largest peak in chromatogram (a) is a well known indicator of a forage diet (Suzuki and Bailey, 1985).

The most noticeable difference was in overall peak intensity. Volatiles generated from the headspace of 2 g of rendered fat trapped for 30 minutes from the new purge vessel and analysed with the Fisons MD800 gave about 100 fold increase in peak intensities. Most of this enhanced sensitivity is attributed to the superior sensitivity of the MD800 as the same desorption apparatus and similar column split ratios were used. Some of the

difference could be due to the new purge vessel design, as greater velocity of purge gas across the surface of the fat could conceivably improve the partitioning of volatiles into the dynamic headspace. This argument is weakened somewhat by the fact that the volatiles were purged from the RBF for six times longer than those purged from the new vessel.

These results showed that the redesigned purge vessel, reduced sample weight and trapping time and alternative mass spectrometer generated a similar chromatographic profile of greater intensity to that of the original system. At this stage the assumption was made that this volatile profile would adequately portray odours smelt by the sensory panellists.

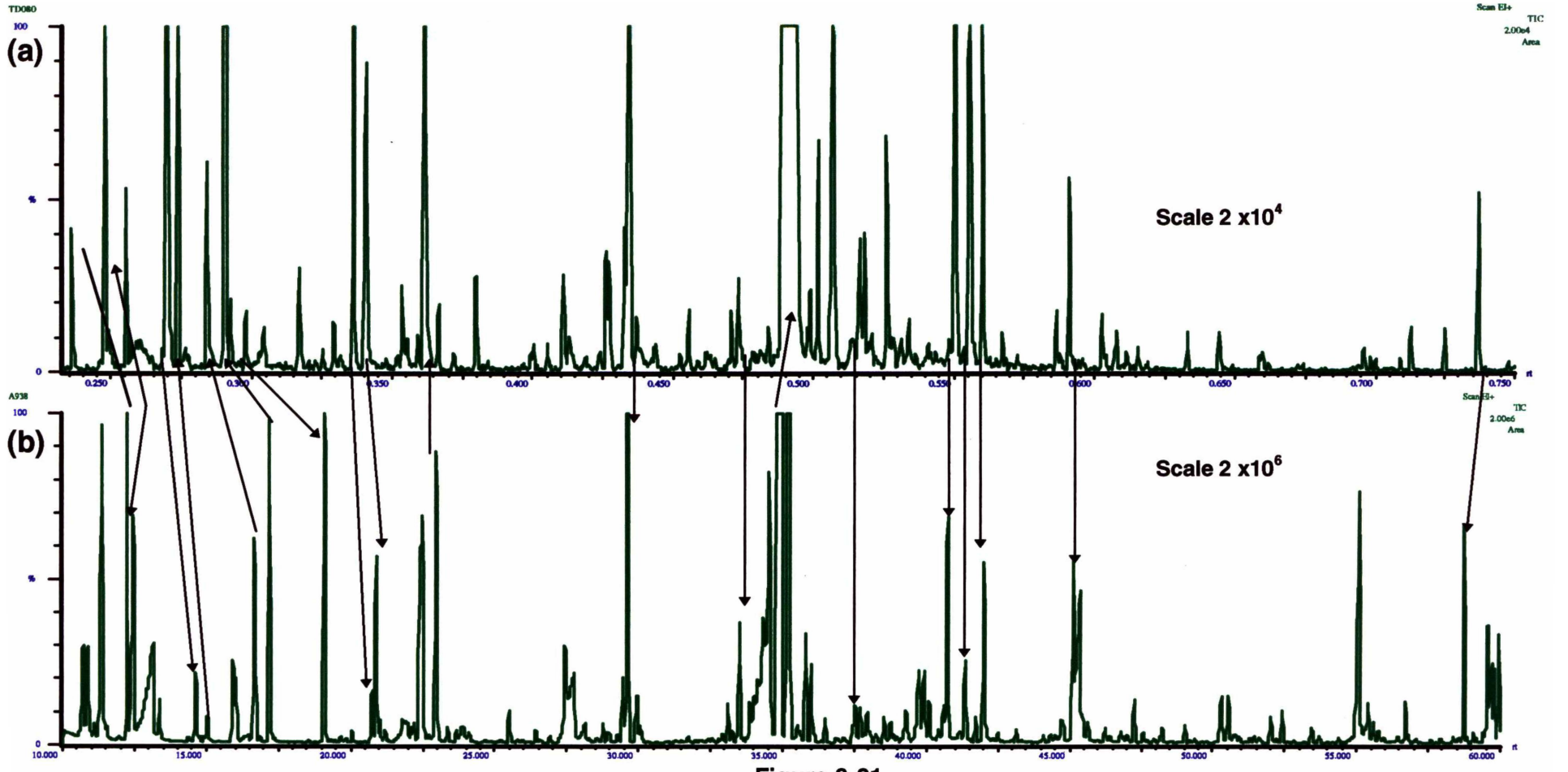


Figure 3-21.

Comparison of total ion chromatograms of volatiles generated from (a) 20 g of rendered fat heated for 3 hours and analysed using a HP5988A GC/MS and (b) 2 g rendered fat heated for 30 minutes and analysed using a Fisons MD800 GC/MS. Scales adjusted to give relative peak intensities. ⟶ indicate some similar compounds.

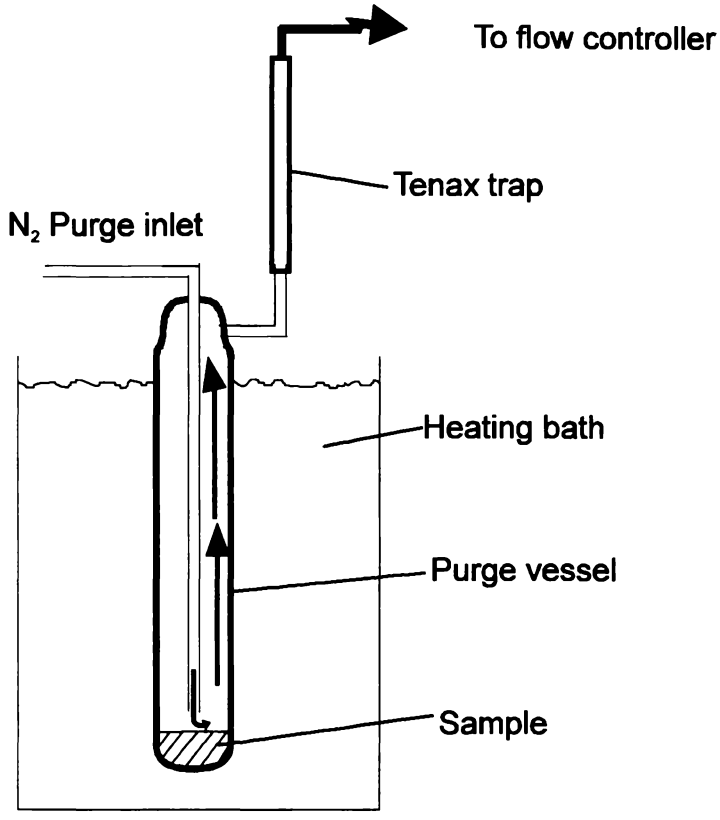


Figure 3-22.
Purge-and-trap vessel design used for trapping volatile compounds from rendered fat samples.

Repeatability of the revised purge-and-trap system

Coefficients of variation of selected compounds covering a wide range of retention times during the chromatograms of volatiles trapped from the headspace of 5 replicate 2 g rendered fat samples are listed in Table 5. The data was expressed as raw area counts and normalized to an internal standard, 2-octanone, added to each sample immediately before trapping.

Table 3-6.

Coefficients of variation, expressed as a percentage of mean area counts before and after normalization on 2-octanone (added to each sample as an internal standard).

		% coefficients of variation	
Retention time (minutes)	Compound Name	Raw area counts	Normalized on 2-octanone
10.77	methylene chloride	9	10
15.14	unknown (56,41,45,55)	13	13
19.26	2-hexanone	4	2
29.8	hexanal	10	7
35.16	2,3-octanedione	12	10
35.43	2-octanone (IS)	3	-
42.2n	nonanal	13	10
58.9	a methyl alkane (71,57,43)	7	4
68.0	phytol	9	11

Numbers in parentheses refer to principle ions observed, in decreasing order of intensity, for unidentified compounds. IS, 2.05 μg 2-octanone in 5 μL pentane added as internal standard.

The coefficients of variation are similar whether expressed as raw area counts or normalized to the internal standard suggesting consistency over all facets of the trapping technique. The results a comparable, if not better than, those obtained for the Wessol oil mixture (Table 3-5) and are consistent with results of others (e.g. Vercellotti *et al.*, 1992).

Chapter 4.

Effect of Meat Ultimate pH on Cooked Odour And Flavour

4.1 Introduction

The ultimate pH of meat (pH at rigor) is governed by the animal's preslaughter reserves of muscle glycogen. After death, the muscle breaks down glycogen via the anaerobic glycolytic pathway to produce lactic acid. Increased lactic acid is responsible for lowering muscle pH. If the animal's glycogen reserves were depleted preslaughter, for example by stress or exercise, insufficient lactic acid is produced to lower the pH of the muscle to its normal value, around pH 5.6 (Devine and Chrystall, 1989). Elevated pH affects several meat characteristics, including appearance and water-holding capacity. In its extreme, high ultimate pH meat is called DFD because of its dark, firm, and dry appearance.

As mentioned in Chapter 2, at pH values above 5.8 the keeping quality of fresh chilled meat is adversely affected because of altered bacterial growth arising from the lower content of glucose. Because lactate is the final electron donor, the lactate anion and H^+ are also inhibitory toward bacterial growth (Gill and Newton, 1981). Elevated ultimate pH can also affect eating quality, particularly tenderness (Howard and Lawrie, 1956; Hedrick *et al.*, 1961).

Moreover, high ultimate pH affects cooked beef flavour (Lawrie, 1985; Purchas *et al.*, 1986). Other sensory studies show that high-pH beef is less flavourful, disliked by panellists (Dransfield, 1981; Purchas *et al.*, 1986), and has more off-flavours than normal, pH beef (Fjelkner-Modig and Ruderus, 1983).

Studies to date have concentrated only on sensory effects of high ultimate meat pH on cooked meat flavour. Instrumental measurement of quantitative and qualitative chemical changes in cooking odour caused by high ultimate pH have been overlooked by most flavour researchers. In a brief report, Park and Murray (1975) noted large differences in the semiqualitative composition of the steam-volatile fraction from normal- and high-pH meat. No mention was made of the species tested or types of compounds involved.

In this part of the investigation, preslaughter injections of adrenaline (epinephrine) were administered to sheep to produce carcasses of low, medium and high meat ultimate pH. The effect of this chemically-induced preslaughter stress on the cooking odour and flavour of sheepmeat was assessed by a trained sensory panel, and cooking odour was evaluated by gas chromatography/mass spectrometry (GC/MS) and, later, gas chromatography/olfactometry (GC/O). Data sets were statistically compared to help identify volatile compounds that contributed to the changes in cooked meat odour and flavour as influenced by meat ultimate pH.

4.2 Materials and Methods

Animals

Fifty Coopworth female lambs from one flock, raised on a predominantly ryegrass and clover pasture, and weighing on average 35 kg (range 29 to 44 kg), were held in a separate paddock near an abattoir with free access to pasture and water for 3 days before treatment. On each of 5 consecutive days, 10 randomly selected animals were removed from pasture and placed in an indoor holding pen. These animals received two subcutaneous doses of adrenaline at 17 and 3 hours before slaughter totalling between 0 and 0.3 mg kg^{-1} of live weight.

Ethical approval for this study was given by the Ruakura Agricultural Centre Ethical Committee.

Animals were conventionally slaughtered by throat cut after a head-only stun. Carcasses, none of which were electrically stimulated, were held above 6°C for 6 hours followed by 22 hours at 3°C. Carcasses were then removed from cold storage, and the *m. semimembranosus* from the left leg of each animal was excised and stored in a permeable plastic bag (Cryovac, W.R.Grace Ltd.) with an oxygen transmission rate (OTR) of 3500 mL^{-1} m^{-2} 24 hr^{-1} at 1 atm, 23°C, and 75% relative humidity. A portion of subcutaneous fat taken from above the *m. longissimus dorsi*, to the depth of the muscle, along the length of the back of the same carcass was included with each leg muscle. Samples were stored at -35°C for 8 weeks until evaluation by a trained sensory panel.

Sample preparation

The meat ultimate pH was measured for each animal 28 hours postslaughter by homogenizing a 1-g sample of *m. longissimus dorsi* in 10 mL of 4 m*M* sodium iodoacetate at pH 7 (Devine and Chrystall, 1989). From these results, 30 of the original 50 *semimembranosus* muscles were selected to give three distinct groups of 10 samples from carcasses with non-overlapping pH values. The mean pH was 5.6 for the low-pH group, 6.0 for the medium-pH group, and 6.6 for the high-pH group. Immediately before sensory analysis, a sample of each minced *m. semimembranosus* was tested in triplicate for pH as described above. This served to confirm the original classification and to pin point the exact pH value of the material tested by sensory panel and gas chromatography/mass spectrometry.

For sensory analysis, the whole *semimembranosus* from each carcass was tempered to -5°C over 2 hours, diced, and passed twice through a 3-mm plate mincer with enough diced backfat from the same animal to produce a mince with a 20% (w/w) fat content [calculated on the basis of an estimated lean fat content of 5% (w/w) and the added weight of backfat]. To minimize lipid oxidation, samples were prepared less than 1 hour before cooking and kept at 4°C in sealed low O_2 permeability plastic bags (Cryovac) with a stated OTR of 30 mL^{-1} m^{-2} 24 hrs^{-1} at 1 atm, 23°C, and 75% relative humidity. Samples were cooked to an internal temperature of 75°C (measured by a temperature probe) by placing the minced meat into stainless steel beakers that were positioned in a water bath at 100°C. Minces were stirred regularly, with individual spoons, to ensure even cooking.

After cooking, the rendered fat and broth from each sample were carefully poured into glass beakers. The separated fat was removed for instrumental analysis and the remaining broth was quickly returned to the cooked mince. The mixture was then reheated for about 1 minute before presentation to panellists.

The fat samples were held at 60°C and centrifuged for 2 minutes at 2000 rpm to separate any remaining lean tissue and water from the fat. The clear supernatants were transferred

to glass vials with Teflon-coated screw caps and flushed with high-purity nitrogen. After the screw caps had been firmly tightened, vials were placed in gas-impermeable foil-laminated bags (Borden NZ Ltd., Auckland, New Zealand), which were vacuum packed, placed in a second foil-laminated bag and vacuum packed again, and then stored at -35°C until instrumental analysis.

Sensory analysis

Selection and training

Selection and training of panellists were done principally as described by Winger and Pope (1981) for the sensory evaluation of meat flavours. Selected panellists were further trained for the present study on two occasions (3 and 4 days) before the first of 5 consecutive daily evaluation sessions. Training samples for overall and foreign flavour and odour intensity were prepared from a low-pH (5.6) and a high-pH (6.8) meat sample as described below for the evaluation samples.

Training samples for sheepmeat flavour and odour intensity were derived by mixing various proportions of mince meat known to vary in sheepmeat intensity. Intensity differences were discussed and defined among the group of panellists. Foreign odour and flavour were identified by panellists as an attribute considered not normally present in sheepmeat of acceptable eating quality. Individual self-generated odour and flavour descriptors were also discussed, and a consensus of significant descriptors was made.

Sample evaluation

In any one daily session, hot samples (about 20 g) of cooked mince, from two randomly selected samples from each of the three pH groups, were transferred from the stainless steel beakers to 50 mL screw-capped glass jars placed in a waterbath at 70°C. The samples, coded with three-digit random numbers, were immediately served, fully randomized to each booth, one at a time. The order of panellists for each booth was changed each session. A reference sample of low-pH (5.60) *semimembranosus* meat, obtained from the carcass of an animal from the original flock but not part of the treatment groups, was also included at each session. Apple juice, flat Coca-Cola, and dry crackers

were presented between samples to clean the palate. Evaluations were made in individual positive air-pressured sensory booths at 22°C and under subdued red lighting to mask any possible variation in meat colour.

The 12 panellists were asked to score for overall odour, sheepmeat odour and foreign odour, immediately after the screw cap was removed from the jar. They were then asked to remove samples from each jar with individual clean spoons and score for overall flavour, sheepmeat flavour, and foreign flavour. All attributes were scored on a scale of 0 to 100: 0 signified no odour or flavour and 100, extreme. Panellists were also asked to record self-generated descriptors of the odours and flavours.

Instrumental analysis of volatile compounds

Gas chromatography/mass spectrometry

Fat samples were melted by placing vials in 60°C water. Two grams (±0.005 g) of melted fat was placed into the bottom of a 50-mL (25 x 150 mm) clean glass-purge tube fitted with a ground-glass stopper joint. An internal standard consisting of 2.05 μg of 2-octanone in 5 μL of pentane was rapidly injected deep into the fat, and the tube was stoppered, gently agitated, and left to equilibrate at room temperature for 5 minutes. A glass nitrogen gas purge tube was then positioned 5 mm above the surface of the fat, and a Tenax TA collection trap (200 mg in a 150 mm x 4 mm i.d. glass tube plugged at each end with silylated glass wool) was attached to the outlet of the purge tube. The Tenax trap had been previously conditioned for 10 minutes at 260°C with a helium flow of 20 mL min^{-1}. The purge vessel was immersed in a glycerol bath maintained at 100 (±0.01) °C. Instrument grade nitrogen was passed through molecular sieve and activated charcoal filters (Alltech) and a tube filled with Tenax TA (200 mg) before finally passing over the fat surface at the bottom of the heated tube.

Volatile compounds generated from the heated fat were dynamically purged from the headspace above the sample by a flow (60 mL min^{-1}) of this high-purity nitrogen gas for 30 minutes and collected on the Tenax TA.

The glass purge and trap assembly was checked for contamination by purging without a fat sample, using the sampling conditions just described. Also, no volatile compounds, as detected by smell and GC/MS analysis of a second trap attached in series, broke through the collection trap when fat samples were analysed.

The volatile compounds were then thermally desorbed at 250°C for 10 minutes with a flow (20 mL min^{-1}) of redirected gas chromatograph helium carrier gas onto the head of a cryogenically cooled (-10°C) DB5-MS capillary column (30 m x 0.25 i.d mm, 1.0-μm film thickness) housed in a Fisons 8000 GC. The chromatography conditions were as follows: injector temperature, 260°C; split flow, 64 mL min^{-1}; column head pressure, 82.7 kPa; split ratio of 32:1 and column flow of 2.0 mL min^{-1} (measured at -10°C); temperature program, -10°C for 10 min, raised to 40°C at a rate of 50°C min^{-1}, held for 5 min, raised to 150°C at 3°C min^{-1} and then to 280°C at 6°C min^{-1} with a final hold time of 5 minutes.

The capillary column was connected to a Fisons MD 800 mass spectrometer with a transfer line temperature of 280°C and source temperature of 200°C. Mass spectra were generated at 70 eV and a detector setting of 350 V. Data were recorded from 40 to 350 mass range by MASSLAB integration software (Fisons) in the total ion monitoring mode. Spectra were compared with an NIST mass spectral data base supplied with MASSLAB. In nearly all cases samples were analysed in duplicate.

Gas chromatography/mass spectrometry

For each pH group, three grams of fat rendered from each of six cooked minces was pooled to produce one composite for each group. samples were stored at -35°C until analysis. Not all 10 animals from each pH group were sampled because four samples from one pH group were compromised due to accidental procedural loss at this stage of the experiment. To ensure balanced sampling between each pH group, six randomly selected samples from each of the remaining two pH groups were selected for pooling. Tenax TA traps containing volatile compounds from duplicate 2 ± 0.005 g aliquots of these pooled samples, prepared without internal standard, were thermally desorbed onto a 30 m x

0.53 mm i.d, 1.0-μm film DB5 capillary column. The chromatographic conditions were similar to those for the GC/MS analysis, except that the initial temperature was -40°C to aid retention of low boiling point compounds on the larger diameter (0.53 mm i.d as opposed to the GC/MS 0.25 mm) column, and the initial ramp to 40°C was at 70°C min^{-1}. The column head pressure was 34.5 kPa and the split flow was 28 mL min^{-1} to give a split ratio of 2.4:1 and column flow of 11.8 mL min^{-1} measured at -40°C. The effluent from the end of the capillary column was split 1:1 between a flame ionization detector (FID) and the olfactometer (SGE, Australia).

The odours emitting from the column were sniffed by me after being combined with a flow of humidified air (near 100% relative humidity at 22°C). Retention times of the odours were recorded by push button that sent an electronic signal to a Maxima integration software package. The button remained depressed for as long as I could detect the specific odour. Odour port evaluation was carried out for the first 60 minutes of the run. The author also recorded a descriptor for the odour and scored its intensity on a 9-point hedonic scale: where 0 was no odour and 9 was extreme.

An alkane series (C_5 to C_{22}) was run on each chromatography column and Kovats' indicies calculated by fitting an equation to the curve of a plot of carbon number vs retention time using Table Curve (version 3, Jandel, AISN Software.) software.

Data analysis

For GC/MS data, all peaks were integrated and peak areas were normalized to the internal standard, 2-octanone, to remove analytical variation of the purge-and-trap and GC/MS steps, and expressed as total ion peak areas. All samples were analyzed at least once and in most cases in duplicate. Eight of the 30 samples were analyzed only once either due to insufficient sample or because of MS malfunction.

Compounds were identified by comparison of peak mass spectra with those in the NIST MS data base (supplied with MASSLAB), Kovats' retention indices (Kovats and Keulemans, 1964), and, when available, authentic standards. Peak identification of GC/O

and GC/FID was done by overlaying FID and olfactometer signal traces and using Kovats' indices. Comparison between GC/MS data and GC/FID/O data was done using Kovats' retention indices. Olfactometer signals were interpreted as the area under the step/impulse response "curve" using Maxima integration software. Corresponding "area counts" represented a combination of nonlinear odour intensity and odour duration and are hereinafter referred to as "odour responses". These values give only an approximate relative intensity of each odour.

The residual maximum likelihood (REML) routine in the GENSTAT (U.K.) statistical software was used to interpret sensory panel data and $\log_{10}$-transformed GC/MS chromatography peak area counts normalized to the internal standard. Log_{10} transformation of GC/MS data was preferred, as preliminary data analysis indicated a skewed data set. The REML routine adjusts for possible imbalance of data, caused by missing data, across multiple levels of variation (Patterson and Thompson, 1971). In this study REML accounted for variation among carcasses, replicate thermal desorptions, pH and sensory panellists and session. Mean $\log_{10}$ area counts for each of the pH groups were then back-transformed to geometric means and converted to concentrations (nanograms per gram of fat) by using the known concentration of the added 2-octanone internal standard (see Table 4-4).

An unsupervised pattern recognition multivariate analysis (principal component analysis, Unistat, Ltd., U.K.) was also done to seek to explain the maximum proportion of variance of the collective relationship of all volatile compounds as affected by meat ultimate pH (data from Tables 4-4 and 4-5). For an in-depth explanation of principal component analysis, the reader should refer to Zervos and Albert (1992) or Manly (1994).

4.3 Results and Discussion

Ultimate pH and sampling

The wide range of ultimate pH values generated by adrenaline injection (figure 4-1) is consistent with results of others (Hedrick *et al.*, 1961; Watanabe *et al.*, 1996) who have attempted to generate a range of pH values.

For the three groups of 10 carcasses with non-overlapping ultimate pH values (selected using the *m. longissimus dorsi* pH values) subsequent analysis of *semimembranosus* muscles gave mean pH values of 5.66 for the low group, 6.26 for the medium group, and 6.81 for the high group (Table 4-1).

Table 4-1.

pH values for *Longissimus Dorsi* (LD) and *Semimembranosus* (SM) muscles for selected carcass groups (n = 10 for each group).

	low pH		medium pH		high pH	
	LD	SM	LD	SM	LD	SM
mean	5.56	5.66	6.07	6.26	6.69	6.81
max	5.68	5.71	6.40	6.36	7.07	6.98
min	5.44	5.60	5.75	6.13	6.30	6.45
SD	0.08	0.03	0.21	0.07	0.22	0.15

The mean pH values for the *semimembranosus* muscles were about 0.2 unit higher than for the corresponding *longissimus dorsi*, a difference that represents normal intermuscle pH variation (Talmant *et al.*, 1986). Raw *m. semimembranosus* weights for each pH group covered a wide range, and although mean weights decreased with increasing pH, the differences were not significant ($P > 0.2$). The amount of rendered fat recovered after cooking also covered a wide weight range since each sample was prepared to a constant percent fat concentration. No difference ($P > 0.9$) was observed in rendered fat recovered among the pH groups (Table 4-2), nor was there any correlation between pH and *semimembranosus* weights in the original 50 animals treated with adrenaline.

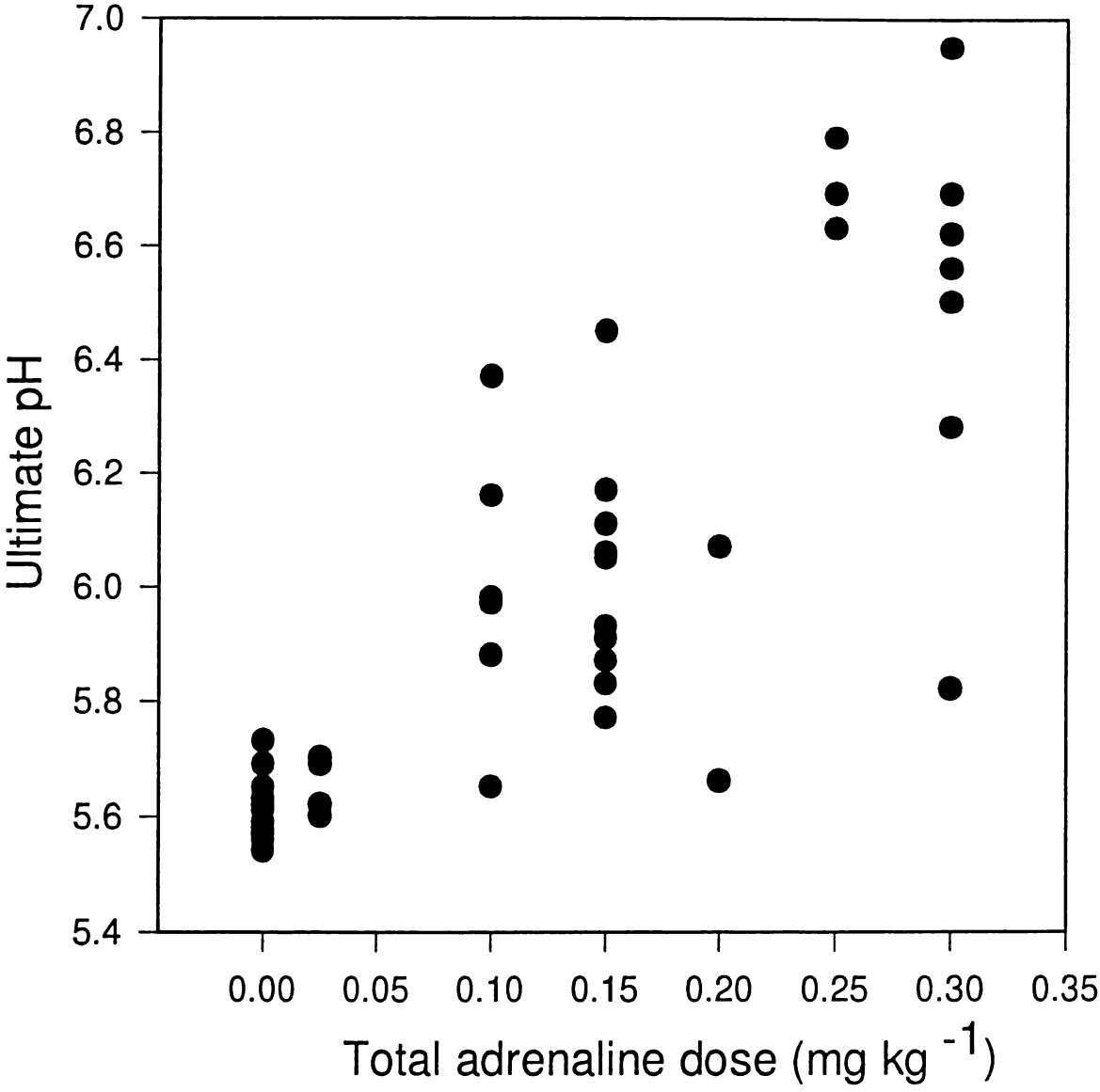


Figure 4-1.
Effect of total adrenaline dose on meat ultimate pH. Each point represents one animal.

Table 4-2.

Summary of *Semimembranosus* weight and rendered fat weight of selected subgroups (n = 10 for each group).

	low pH		medium pH		high pH	
	muscle (g)	rendered fat (g)	muscle (g)	rendered fat (g)	muscle (g)	rendered fat (g)
mean	359	12.4	347	12.4	327	11.5
max	467	20.4	443	23.9	383	19.3
min	292	5.6	302	7.0	283	6.6
SD	48	5.5	43	5.5	26	4.2

On average, 12.1 g of rendered fat was recovered from the gently strained mince. This represented about 20 % (w/w) of the fat content of the raw mince, after allowing for the moisture content (25 %) of the added backfat. Samples of mince presented to sensory panellists therefore contained close to 16% (w/w) fat — sufficient to maintain adequate mouth feel and carry a significant fraction of the fat-derived flavour and odour compounds.

Sensory analysis

Table 4-3 shows the intensity score means and levels of statistical significance among the pH groups. The panellists found that overall odour and flavour decreased significantly as pH increased. The change was significant among three pH groups ($P < 0.05$) but was most obvious between the low and high pH groups ($P < 0.001$). Panellists could not detect a change in sheepmeat odour between any of the groups, but did detect a decrease in sheepmeat flavour between the low and high and the medium and high groups ($P < 0.01$). A smaller decrease ($P < 0.05$) in foreign odour was observed between the low- and high-pH groups only. There was no significant difference in foreign flavour among any of the groups.

The observation by the sensory panel that overall flavour decreased with increasing pH agrees with similar work on beef (Dransfield, 1981; Fjelkner-Modig and Ruderus, 1983)

and pork (Buscailhon *et al.*, 1994). The most frequent descriptors from panellists' comments on odours and flavours are shown graphically, by frequency of occurrence, in "radar" plots of odours and flavours (figure 4-2). These plots show a general movement of emphasis from desirable descriptors to undesirable descriptors as pH increased. For example, the frequency of bland/flat/low and stale/musty flavour descriptors, considered undesirable attributes of cooked meat, increased as pH increased. Others (Dutson *et al.*, 1981; Fjelkner-Modig and Ruderus, 1983) have also found an increase in negative or off-flavours in high-pH beef. At the same time, strong, beefy, meaty, and sweet flavour descriptors, considered desirable attributes, decreased.

Table 4-3.

Mean sensory panel intensity scores for cooked *Semimembranosus* mince from animals in three pH groups.

	low (pH 5.66)	medium (pH 6.26)	high (pH 6.81)	low vs medium	low vs high	medium vs high
overall odour	51.1	44.8	39.8	*	***	*
overall flavour	50.3	41.7	27.0	*	***	**
sheepmeat odour	28.5	26.9	25.9	NS	NS	NS
sheepmeat flavour	38.1	34.8	21.7	NS	**	**
foreign odour	21.9	19.6	16.6	NS	*	NS
foreign flavour	13.4	14.8	16.0	NS	NS	NS

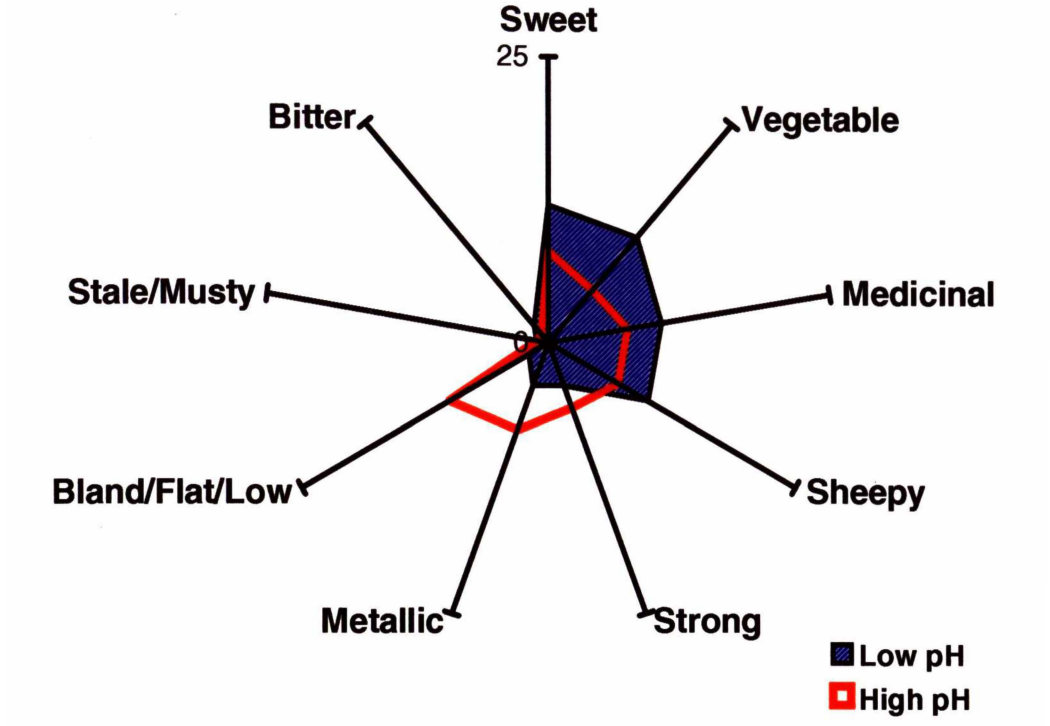
samples were scored on a scale of 0-100: 0 signified none and 100, extreme.

NS, not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$

As pH increased, foreign odour scores decreased ($P < 0.05$), suggesting that whatever compounds were responsible, they were not the same as those responsible for undesirable odour notes such as metallic and stale/musty. Although panellists comments have not been subjected to rigorous statistical analysis, these descriptor plots show a general movement of emphasis from desirable descriptors to undesirable descriptors as pH increased. These results should not go unreported as they do represent the perception of

a number of panellists. In retrospect, it might have been useful for the panellists to have also scored for changes in all descriptors. However, giving panellists too many attributes to consider can confuse and jeopardize the integrity of the sensory session. Young *et al.*, (1993) compared odour and flavour differences between Coopworth and Merino lambs grazed on similar pasture and noted that panellists' descriptors for Coopworth samples (mean pH 5.77) were also dominated by beefy, sweet, and big-flavoured, whereas bland, fishy/stale/rancid, grassy, and bloody descriptors dominated in Merino samples (mean pH 6.16).

(a)



(b)

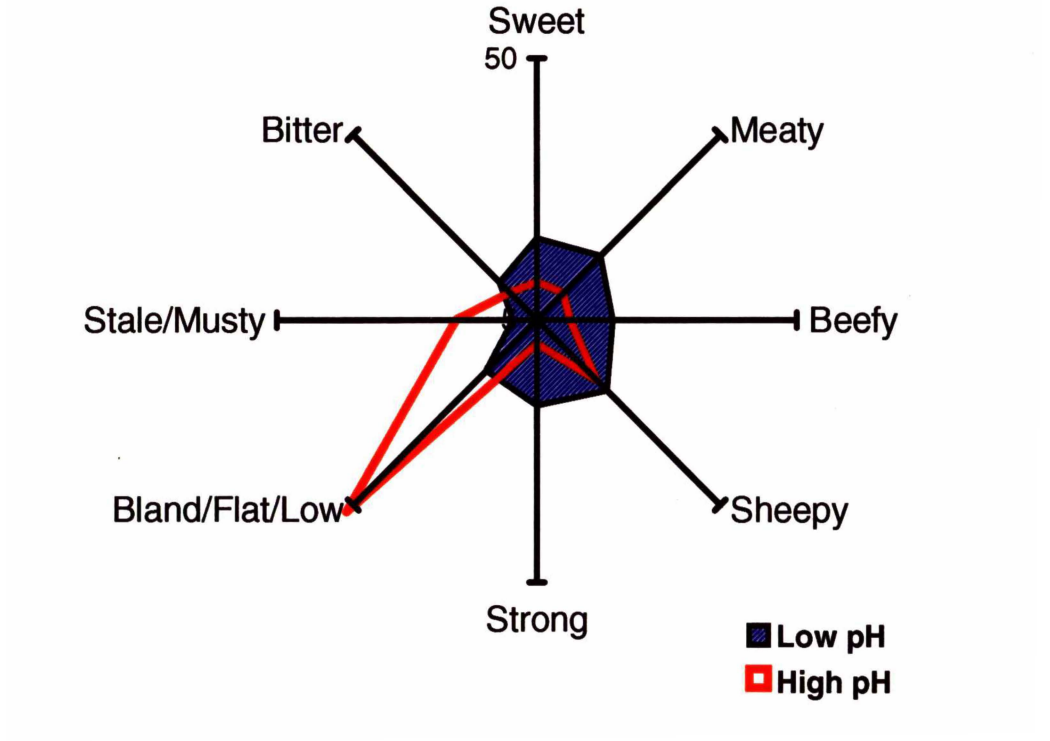


Figure 4-2.

(a) frequency of odour descriptors (top) and (b) frequency of flavour descriptors (bottom) for low (shaded area) and high (red line) ultimate pH sheepmeat.

GC/MS analysis

In this dynamic headspace analysis, thermal desorption of the Tenax TA traps eluted more than 300 compounds. These were recorded as total ion chromatogram peaks by GC/MS for each sample of rendered fat. Generally, total volatile compounds concentration decreased with increasing pH. Summation of total mean area counts for each sample showed a relative reduction from 100 for the low-pH group to 80 and 75 for the medium- and high-pH groups, respectively.

Twenty-nine compounds decreased ($P < 0.05$, most cases $P < 0.001$) among all sample groups as pH increased (Table 4-4). Of this group of compounds, twelve were identified as aldehydes, eight were alcohols, one was an alkane, one was a fatty acid, and six remain unidentified.

In addition, nine compounds (one ketone, five alkanes, and three unknowns) decreased in concentration ($P < 0.05$) from the low- to medium-pH group, three compounds (two alcohols and one fatty acid) decreased ($P < 0.05$) from the medium- to high-group, and five compounds (one ketone, one acid, and three unknowns) decreased ($P < 0.05$) from the low- to high-pH group.

Most interestingly, only four compounds (two alkanes and two alkenes) showed an increase ($P < 0.02$) in concentration with increasing pH among any of the treatment groups. Hydrocarbons have high odour thresholds and are not considered significant contributors to meat odour (Shahidi *et al.*, 1986). These compounds therefore do not explain the panellist's observations that the frequency of occurrence of undesirable odour descriptors increased as pH increased. It may be that the purge and trap GC/MS technique as used here lacks the sensitivity to detect odours that possibly are in the parts per billion range but are still above the odour threshold. Alternatively, favourable odour notes present in low-pH meat may mask less desirable notes and only when these masking odours are reduced — due to an increase in meat pH — do the undesirable odours dominate (St. Angelo *et al.*, 1987).

Table 4-4.

Mean approximate concentrations (nanograms per gram of rendered fat) of compounds that changed significantly from low (L) to medium (M), low to high (H) and medium to high pH groups.

peak	Kovats' index	compound	ng g^{-1}			Significance		
			L	M	H	L vs M	L vs H	M vs H
		aldehydes						
28	715	pentanal	293	160	121	***	***	*
155	1103	nonanal	277	112	91	***	***	*
102	963	benzaldehyde	120	48	28	***	***	***
117	1004	octanal	92	39	30	***	***	***
193	1265	(E)-2-decenal,	48	15	9	***	***	**
140	1058	(E)-2-octenal	41	15	9	***	***	***
219	1379	2-undecenal	28	7	4	***	***	***
120	1012	(E,E)-2,4-heptadienal	23	14	9	***	***	***
208	1330	2,4 decadienal	21	5	2.5	***	***	***
69	852	(E)-2-hexenal	13	6.3	4.3	***	***	*
203	1302	(E,E)-2,4 decadienal	11	2.9	1.7	***	***	***
16	672	(E)-2-butenal	7	3.3	2	***	***	*
		alcohols						
109	980	1-octen-3-ol	170	56	25	***	***	***
110	968	heptanol	97	153	50	NS	NS	**
22	702	1-penten-3-ol	95	36	16	***	***	***
144	1068	1-octanol	44	22	16	***	***	**
142	1065	2-octenol	37	12	5	***	***	***
45	772	1-pentanol	142	63	40	***	***	***
46	774	2-penten-1-ol	19	6	2.9	***	***	***
158	1112	2-phenylethanol	8	4.4	2.9	***	***	*
131	1033	2-phenylmethanol	6	3.4	2.3	***	***	*
47	778	3-penten-2-ol	2.9	3.7	1.9	NS	NS	***
		acids						
14	613	acetic acid	278	224	187	NS	*	NS
48	782	butanoic acid	23	29	18	NS	NS	*
141	1063	heptanoic acid	17	7	2.5	***	***	***
		alkanes						
237	1473	2,6,10,14-tetramethylheptadecane	127	96	135	*	NS	(**)
246	1512	pentadecane	39	29	35	*	NS	NS
259	1606	hexadecane	19	14	18	*	NS	(*)
204	1306	tridecane	13	6.3	4.3	***	***	*
229	1414	a methylalkane	12	7.6	8.9	**	NS	NS
239	1484	a methylalkane	5	3.3	4.5	*	NS	NS
		alkenes						
265	1645	unknown (57,97,55,111)	3	2.4	3.7	*	NS	(**)
271	1687	unknown - alkene	3	1.8	2.3	***	NS	NS
285	1783	2,6,10,14 tetramethyl -2-hexadecene	224	161	255	*	NS	(**)

Table 4-4. cont.

peak	Kovats' index	compound	ng g^{-1}			Significance		
			L	M	H	L vs M	L vs H	M vs H
		ketones						
143	1067	1-phenyl ethanone	103	85	70	NS	**	NS
201	1297	2-undecanone	20	14	15	*	NS	NS
		miscellaneous						
258	1599	a phthalate	10	7.6	9	*	NS	NS
55	800	unknown (59, 80)	94	50	25	***	***	***
73	867	unknown (56, 55, 43, 69)	60	27	16	***	***	***
43	762	unknown (55, 83, 84, 41)	16	8.9	5.3	***	***	***
183	1217	unknown (43, 88, 99, 144)	11	7.8	5.8	**	***	*
54	797	unknown (83, 55, 98)	8	5.4	6.8	*	NS	NS
93	923	unknown (57, 41, 59, 81)	8	4.7	3.5	NS	*	NS
146	1077	unknown (68, 81, 119, 134)	7	3.8	2.9	***	***	*
251	1536	unknown (124, 137, 55, 180)	6	4	2.6	*	***	*
175	1189	unknown (43, 58, 70, 83, 97)	3.2	2.6	2	NS	*	NS
27	713	unknown (86, 57)	2.7	2.5	1.8	NS	*	NS
275	1704	unknown (57, 71, 43, 97)	3	2	3	**	NS	NS

NS, not significant ($P > 0.05$); *, $P < 0.05$; **, $P < 0.02$;***, $P < 0.001$.

MS identifications were by comparison with the NIST MS data base and Kovats' retention indices. Numbers in parentheses refer to the principal ions observed, in decreasing order of intensity, for unidentified compounds. Asterisks represent significant decreases (increases) in concentration among pH groups.

Of the identified volatile compounds that decreased significantly as pH increased (Table 4-4), the aldehydes and alcohols were the main contributors (42%), as expressed by compound class, to the significant changes in concentration among all three pH groups (statistically, $P < 0.001$ in at least two and in most cases in all three pH group comparisons). These compounds are generated from the oxidation of lipid-derived fatty acids at cooking temperatures. Many have distinct odours, such as the French fry aroma associated with 2,4-decadienals and the "green" or "grassy" aroma attributed to hexanal. They also play a major role in the Maillard reaction (Reineccius, 1994). The aldehydes (E,E)-2,4-decadienal, 2,4-decadienal, nonanal, and 2-undecenal, which were more prominent in low-pH samples, were identified by Gasser and Grosch (1990), using an aroma dilution technique, as being highly significant contributors to the odour of meat broths.

Changes in the concentration of volatile compounds detected by GC/MS reflect the sensory panellists' perceptions (Table 4-3) and are consistent with biochemical changes expected to occur in postrigor muscle. Proteolysis and lipolysis operate more favourably at lower pH (Buscailhon *et al.*, 1994). These hydrolytic reactions may produce the vital

precursors required for generation of odour and flavour compounds produced during cooking. Alternatively, the greater water-holding capacity of high-pH meat may influence the release of volatile compounds and affect flavour perception (Lawrie, 1985).

Madruga and Mottram (1995) observed increases in a number of volatile heterocyclic compounds in cooked meat that are also thought to contribute to meaty flavour, when they titrated meat from pH 5.6 to 4.0 before cooking. They also found that the total number of volatile compounds increased as the pH decreased.

In this study, 44 heterocyclic compounds [including those observed by Madruga and Mottram, 1995] were specifically targeted using Kovats' indices and quantified using the mass spectral FIND routine within the MASSLAB (Table 4-5).

Table 4-5.

Compounds specifically targeted† with mean approximate concentrations (nanograms per gram of rendered fat) of those compounds that changed significantly from low (L) to medium (M), low to high (L) and medium to high pH groups.

Kovats' index	compound	ng g^{-1}			significance		
		L	M	H	L vs M	L vs H	M vs H
1050	2-methylphenol	1.1	0.73	0.5	***	***	*
1070	4-methylphenol	2.8	1.5	1.3	***	***	NS
1223	unknown (135, 150,121) phenol ?	0.34	0.08	0.04	***	***	***
1019	2-acetylthiazole	0.85	0.42	0.44	*	*	NS
774	2-methylthiophene	0.33	0.24	0.32	NS	NS	NS
991	2-pentylfuran	5.1	1.3	0.61	***	***	***
1089	2-hexylfuran	0.9	0.31	0.26	***	***	NS
751	dimethyl disulfide	0.82	2.4	0.84	NS	NS	*
985	dimethyl trisulfide	0.8	2.4	0.71	(*)	NS	***

NS, not significant ($P > 0.05$); *,$P < 0.05$; **,$P < 0.02$;***,$P < 0.001$.

MS identifications were by comparison with the NIST MS database and Kovats' retention indices. Numbers in parentheses for unknown compounds refer to the principal ions observed, in decreasing order of intensity. Asterisks represent significant decreases (increases) in concentration between pH groups.

† Compounds specifically targeted but not found in any of the samples. (Phenols) thio-, 2-ethyl-, 3,4-dimethyl-, 2,4-dimethyl-, 2-isopropyl-, 2,4,6 -trimethyl-, 4-isopropyl-, 2-methyl-5-(1-methylethyl)-, 5-methyl-2-(1-methylethyl); (Pyrazines) methyl-, 2,5 dimethyl-, 2,6 dimethyl-, 2,3 dimethyl-, 2-ethyl-5-methyl-, 2-ethyl-6-methyl-, 2-ethyl-3,6-dimethyl-, 2,3-diethyl-5-methyl-, ethyl-, ethenyl-, 2-ethyl-3,5-dimethyl-; (Thiazoles) 4,5-dimethyl-, 2,4-dimethyl-, ;(Thiophenes) 2,4-dimethyl-, 3-ethyl-, 5-methyl-2-thiophene-carboxyaldehyde-, 3-methyl-2-thiophene-carboxyaldehyde-, 3-methyl-, 2,3-diformyl-,; (Furanones) 2(5H)-, dihydro-2-methyl-3(2H)-, dihydro-5-methyl-3(2H)-, dihydro-3-methyl-2(3H); (Furans) 2-methyl-, 2-ethyl-, 3-pentyl-, 3-phenyl-, 3-heptyl-, carboxyaldehyde (furfural) 2,5 dihydro-, 2,5 dimethyl-, 2,5-dihydro-3-methyl-, 2,3-dihydro-5-methyl-, 2,3-dihydro-4-methyl-, 2,3-dihydro-3-methyl-, methyl thio-, 2-methylfuranthiol-, 2-furanylmethanethiol sulfide, dimethyl-.

Only 2-pentylfuran, 2-hexylfuran, 2-methylthiophene and 2-acetylthiazole were observed in cooked sheepmeat volatiles. All of these, except 2-methylthiophene, showed a significant decrease with increasing pH.

The low incidence of volatile heterocyclics may be due to the mild cooking conditions employed here. Ground meat was cooked for only 10 ± 1 minutes to a final temperature of 75°C in a beaker immersed in a 100°C waterbath. No significant browning, known to aid the production of Maillard reaction products, occurred. MacLeod and Ames (1986), Spanier *et al.*, (1990) and Drumm and Spanier (1991) found an increase in the production of heterocyclic and sulfur compounds as cooking temperatures and times increased.

Volatile compounds thought to contribute to the species-related odour and flavour of cooked sheepmeat include branched-chain fatty acids (BCFAs) (Wong *et al.*, 1975a) and a number of alkylphenols (Ha and Lindsay, 1991). Two branched-chain fatty acids, 4-methyloctanoic acid and 4-methylnonanoic acid, responsible for sheepmeat odour (Wong *et al.*, 1975b; Ha and Lindsay, 1990; Young *et al.*, 1996) could not be detected in any of the samples under these conditions. The absence of branched-chain fatty acids is probably due a combination of the mild cooking temperature, the short purge and trap time, the lower volatility of these acids, and the fact that they are present in low concentrations (Ha and Lindsay, 1990), particularly in leg muscles (Brennand and Lindsay, 1992a).

In this experiment, mean sensory scores for sheepmeat odour decreased with increasing pH, but the decrease was not statistically significant. In contrast, the decrease in sheepmeat flavour was significant ($P < 0.01$) between low and high-pH and between medium and high-pH groups. These results again can be explained by the mild cooking conditions employed. When comparing volatiles from boiled, fried, or roasted mutton collected during cooking, Brennand and Lindsay (1992b) found that a lesser quantity of volatile free fatty acids (VFFA) was collected from the headspace of boiled and fried mutton than from the headspace of roasted mutton. One explanation given was that the former two methods had shorter cooking times and the surface roasting temperature was much higher. These workers also observed higher concentrations of VFFA in rendered fat from fried samples than in rendered fat from boiled mutton, for which a large portion of the VFFA could dissolve in the aqueous phase. Thus, under the mild cooking conditions used in this experiment, it is probable that the BCFAs were not completely liberated into the fat fraction but mostly were dissolved and retained in the aqueous meat

broth. This would explain the absence of BCFAs in the trapped volatiles and account for the changes in sheepmeat flavour as observed by panellists. Lower pH conditions would favour liberation of the BCFAs from their parent triacylglycerols. The BCFAs would enter the aqueous phase and be detected as flavour.

Only three of the phenols listed in Table 4-5 [2-methylphenol, 3 (or 4)-methylphenol (*m*- or *p*-cresol), and an unknown phenol (M = 150)], were detected, and all showed a decrease in concentration with increasing pH. p-Cresol (2-methylphenol) has been implicated as a contributor to muttony/sheepyard aroma of cooked sheep meat at low (ppb) concentrations (Ha and Lindsay, 1991).

Principal component analysis

PCA was performed on a restricted data set derived from GC/MS data of all compounds that showed a significant decrease in concentration with increasing meat ultimate pH. A plot of the principal components (figure 4-3) shows that 78% of the variability is accounted for in the first two components. Component 1 explains 71% of the variability and shows that the low-pH samples are well discriminated from the medium- and high-pH groups. This shows that increases in pH, from that of the low-pH group (5.6-5.7) to the medium (6.1-6.4) and high (6.5-7.0) groups, had a marked effect on the production of cooked meat volatile compounds, even under mild cooking conditions. Component 2 reveals a degree of variability within the low-pH group that is less evident in the other two groups. Principal component analysis of the same data transformed to $\log_{10}$ (plot not shown) reduced the spread of data points in the low-pH group but had the opposite effect on the high-pH group. It also explained more of the variability in the first two components (75.7 and 4.8% for components 1 and 2, respectively). This suggests that another factor, independent of pH, may affect volatile compound production. No improvement was found for discrimination between the medium- and high-pH groups.

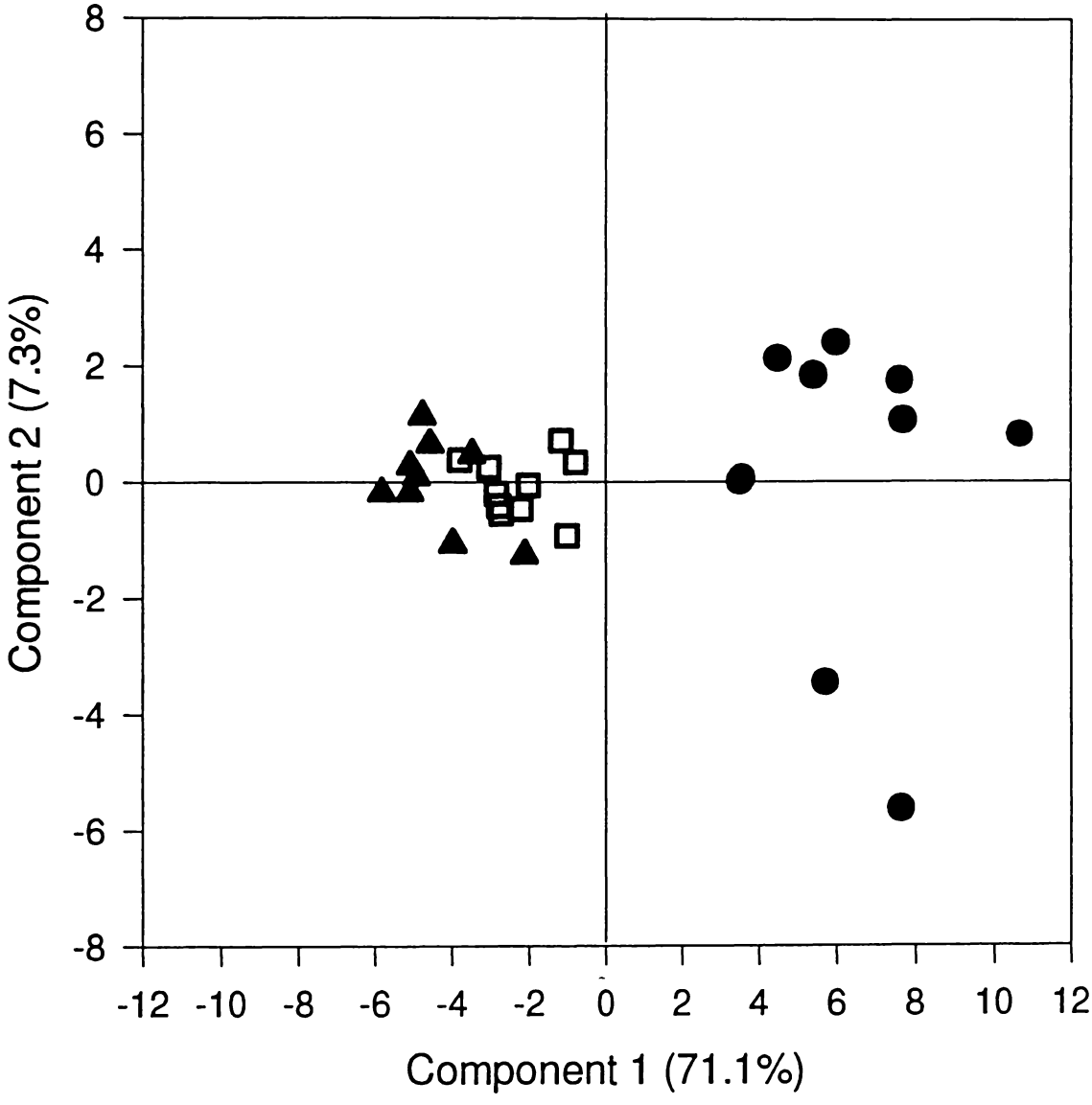


Figure 4-3.

Principal component analysis used to discriminate animals on the basis of volatile compounds. Only compounds that decreased in concentration among all three pH groups were used in the data set: low pH group (●), medium pH group (□), high pH group (▲). Values in parentheses represent the percent of variance explained by each principal component.

Gas chromatography/odour port analysis

No significant difference was observed in odour responses between the pH groups (data not shown), probably because these values were derived by a very subjective method (a single observer and low number of replicates). Relative potencies of each odorous compound would be better estimated by other, more quantitative techniques (Acree *et al.*, 1984; Ullrich and Grosch, 1987). Figure 4-4 shows the odour response for a combined composite sample of rendered fat from the low-pH group. However, qualitatively, 10 of the 54 odourous compounds detected (figure 4-4 and Table 4-6) were also some of the compounds found by GC/MS to decrease with increasing pH. Six of these were aldehydes. This confirms that these compounds contribute to the odour intensity changes due to pH and also confirms proposals (Gasser and Grosch, 1990; Reineccius, 1994) that they play a major role in cooked meat odour.

A number of aldehydes have been shown to be responsible for rancidity changes in cooked meat flavour (St. Angelo *et al.*, 1987). Samples in this experiment were stored in such a way that rancidity onset is not considered a significant contributor to the changes in aldehyde concentrations observed. The results suggest that, at certain concentrations, some aliphatic aldehydes play a major role in the development of a favourable cooked sheepmeat odour and flavour. Similar observations have been reported for cooked chicken (Ho and Chen, 1994).

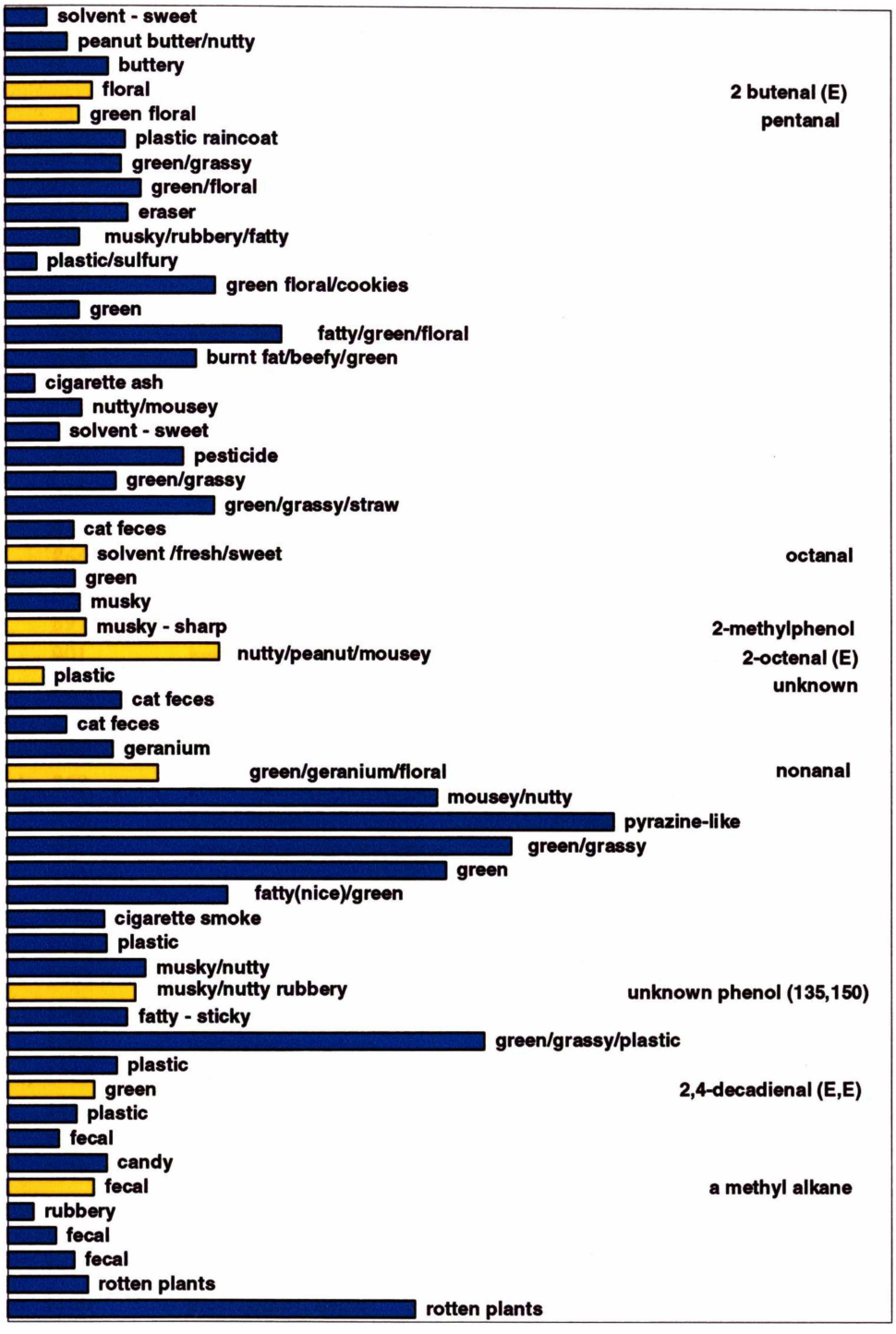


Figure 4-4.

GC/odour responses and their descriptors of odour-active compounds identified in a composite rendered fat sample from the Low pH group. Compounds are listed in order of increasing retention time. Those compounds labelled on the right of the figure were also found to change significantly with meat pH. For full identifications and retention indicies see Table 4-6.

Table 4-6.

List of odour descriptors and tentative MS identification of odourous volatile compounds eluting from the gas chromatograph/olfactometer for a mixed sample of rendered fat (low pH group).

Kovats' index	odour descriptor	tentative identification
513	sweet solvent	cyclopentane
560	peanut butter/nutty	unknown (56, 57, 41)
590	buttery	2,3-butanedione
648	floral	(E)-2-Butenal
682	green floral	pentanal
735	plastic raincoat	unknown (55, 83, 84)
792	green/grassy	hexanal
796	green/floral	hexanal
810	eraser	ND
812	musky/rubbery/fatty	ND
816	plastic/sulfury	1,3-octadiene
866	green floral/cooked cookies	ND
882	green	2- or 3-heptanone
897	fatty/green/floral	4-heptenal
901	burnt fat/beefy/green, lingers	heptanal
913	cigarette ash	3-methylthiopropanal
918	nutty/mousey	dihydro-2(3)-furanone
949	solvent/sweet	propylbenzene
968	pesticide	phenol
978	green grassy	2,3-octanedione
981	green/ketoney/grassy, straw	2,3-octanedione
987	cat feces	ND
1000	solvent like - fresh/sweet	octanal
1011	green	2,4-heptadienal
1033	musky	benzylalcohol
1045	musky, sharp	2-methylphenol
1052	nutty/peanut/mousey	(E)-2-octenal
1069	plastic	ND
1080	cat feces	dimethylbenzyl alcohol
1083	cat feces	ND
1094	geranium	3-hexene-2,5-diol
1097	green/geranium - strong,/floral	nonanal
1100	mousey/nutty	unknown (124)
1115	pyrazine like, lingers	unknown
1136	green/grassy, lingers	2-nonenal (E or Z)
1153	green	ethylbenzaldehyde
1171	slighty fatty (nice) green	unknown (127, 57, 43)
1204	cigarette smoke	unknown
1211	plastic	unknown (43, 88, 99, 144)

Table 4-6. cont.

Kovats' index	odour descriptor	tentative identification
1213	musky/nutty (pyrazine?)	unknown
1226	musky/nutty/rubbery, lingers	unknown phenol (135, 150)
1245	fatty - sticky	nonanoic acid
1248	green/grassy/plastic	2-decenal
1312	plastic	2,6,7-trimethyldecane
1315	green	(E,E)-2,4-decadienal
1335	plastic	ND
1423	fecal	ND
1425	candies	ND
1475	fecal	a methylalkane
1488	rubbery	cyclopentadecane
1509	fecal	unknown
1516	fecal	unknown
1524	rotten plants	unknown
1587	rotten plants	unknown

Relative odour responses for each of the compounds are shown in Figure 4. ND, peak not detected by MS. MS identifications were by comparison with the NIST MS database and Kovats' retention indices. Numbers in parentheses for unknown compounds refer to the principal ions reliably observed, in decreasing order of intensity.

4.4 Conclusions

This study demonstrated that volatile compounds isolated by headspace analysis from cooked meat and fat change quantitatively with pH and that these changes are consistent with changes observed by sensory panellists. Many of these compounds are produced from the oxidation of lipids during cooking and are considered to be important contributors to meat odour and flavour. The findings indicate that even moderate increases in meat ultimate pH from 5.6 to 6.3 significantly alter the quality and quantity of odours and flavours of cooked sheepmeat. This conclusion can almost certainly be extended to meat of other species.

Chapter 5.

Effects of Titrating High Ultimate pH Meat to a Lower pH on Cooked Meat Odour and Flavour

5.1 Introduction

In the previous chapter is was clearly shown by sensory analysis and gas chromatography/mass spectrometry, that cooked meat of high ultimate pH was less flavourful and produced lower levels of volatile compounds in the cooked fat.

Possible reasons for enhanced flavour development at lower meat ultimate pH include more active proteolytic and lipolytic activity at lower pH to produce flavour precursors before cooking (Buscailhon, *et al.*, 1994), the known lower optimum pH for production of Maillard reaction products during cooking (Madruga and Mottram, 1995), and greater water-holding capacity at higher pH, which would in turn influence the release of volatile compounds and affect flavour perception (Lawrie, 1985).

To help identify the cause of enhanced flavour development of cooked sheepmeat at lower ultimate pH, this chapter investigates the effect on odour and flavour development of lowering the pH of high ultimate pH meat with hydrochloric acid (HCl) to values similar to the low- pH group, as described in the previous chapter.

If flavour develops on cooking then by titrating high ultimate pH meat to pH 5.6 before cooking should result in meat of equal odour and flavour intensity as untitrated low pH (5.6) meat.

Samples of raw minced *semimembranosus* muscles were analysed before and after titration with HCl, for soluble protein, non-protein nitrogen, total fat and protein, free amino acids, and free fatty acids as indicators of proteolysis and lipolysis. Free fatty acids and free amino acids also contribute to odour and flavour in their own right as well as

participating as precursors in meat flavour development. In addition, cooked *semimembranosus* muscles were assessed by sensory panel and gas chromatography/mass spectrometry in the same manner as the previous experiment (chapter 4).

Data are evaluated and compared with the results of the previous experiment and a comprehensive model is proposed to explain the ultimate pH related changes in cooked meat odour and flavour.

5.2 Materials and Methods

Sample selection and preparation

Most lamb legs contralateral to those used in the first part of this experiment (chapter 4) were used for the present pH titration study. However, three samples were replaced by legs from the pool of carcasses (Table 5-1) because of misplacement of samples in freezer storage. However, sample pH values still comprised of three non-overlapping pH groups (figure 5-1).

Table 5-1.

List of samples from Part 1 of this study that were replaced with alternate samples.

Samples replaced		Samples substituted	
Sample ID	pH at mincing	Sample ID	pH at mincing
42	5.64	18	5.68
15	6.88	2	6.33
27	6.94	24	6.44

Titration of high- and medium- pH meat with hydrochloric acid

Contralateral legs from the selected frozen carcasses were tempered in a +10°C-room and the *m. semimembranosus* excised. Each muscle was diced and passed twice through a 3 mm plate mincer with enough diced backfat from the same animal to produce a mince with a 20% (w/w) fat content. This was calculated on the basis of an estimated lean fat content of 5% (w/w) and the added weight of backfat.

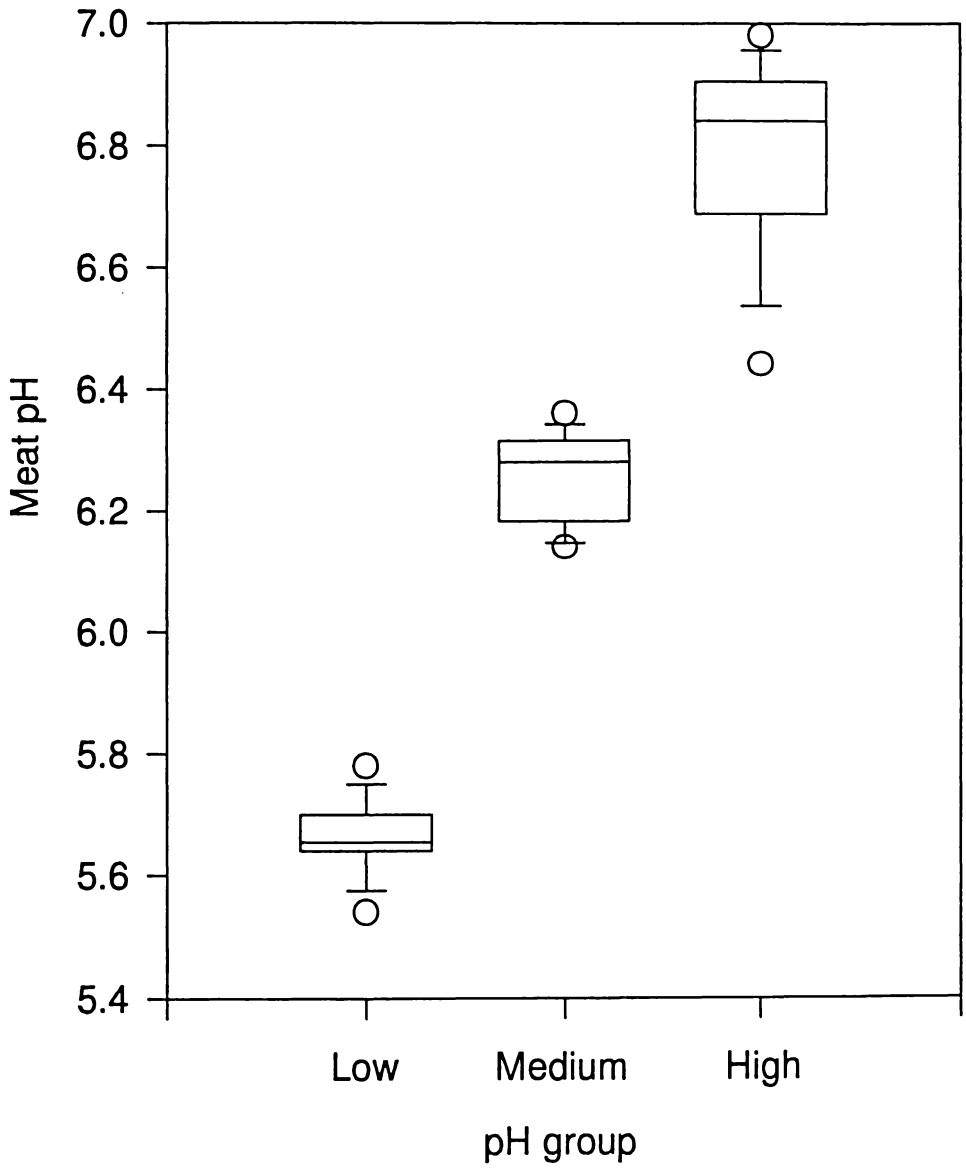


Figure 5-1.

Box plot of pH values for *semimembranosus* muscles in the three selected pH groups. The horizontal lines mark the 10th, 25th, 50th, 75th and 90th percentiles. The circles represent sample outliers.

Initial tests showed that titration with lactic acid, imparted a citrus-type flavour on cooked minced sheepmeat. HCl was therefore chosen. Muscles with the twenty highest pH values were individually titrated with 2M HCl. The remaining 10 samples, representing the Low pH group of Part 1 of this study (chapter 4), were not titrated. Before titration a 25 g subsample of each mince was taken for metabolite analysis (see later).

Titrations were done by placing each minced muscle into a domestic food processor, fitted with a sharp blade, and slowly adding between 1 and 6 mL of 2M HCl. Minces from the low- pH group were mixed in the food processor for a similar time. Change in pH was monitored during titration of each sample by measuring the pH of 1g of mince homogenised in 10mL of distilled water.

After titration, individual muscle minces were vacuum packed in oxygen impermeable foil-laminated plastic bags and stored overnight at +4°C. One hour before sensory analysis each mince was returned to the food processor, briefly mixed, retested for pH to ensure stability, and another subsample was taken for metabolite testing. All such subsamples were vacuum packed in O_2-impermeable foil-laminated plastic bags and stored at -80°C until need for analysis.

Sensory analysis

Sensory analysis generally followed the same procedures used in chapter 4 of this study with the following modification. In the period between the experiment in chapter 4 and this study, the Sensory Evaluation unit at the Meat Industry Research Institute of New Zealand Inc. (MIRINZ), where the sensory studies were carried out, changed the scoring scale for intensity sensory work. The scale was changed from 0 to 100, where 0 signified no odour or flavour and 100, extreme, to a new scale 0 to 9, with parallel interpretation. A near linear relationship would relate both scoring scales.

Gas chromatography/mass spectrometry

Procedures for purge-and-trap of rendered fat samples from each cooked mince sample were as described in Part 1 of this study. All peaks were integrated with MASSLAB

integration software, expressed as total-ion peaks areas and normalized to the internal standard, 2-octanone, to remove analytical variation of the purge-and-trap and GC/MS steps. Peak areas from each chromatogram, one for each sample, were then imported into a spreadsheet (Microsoft Excel). Using a combination of retention times, chromatogram profiles and mass spectral verification, peaks were matched across all samples. Any peaks attributable to column bleed were removed to leave a data set of 363 individual peaks. Peak areas were then converted to concentrations (ng g^{-1} of fat) by using the known concentration of the added 2-octanone internal standard.

Metabolite analyses

Sample preparation

Frozen subsamples at - 80°C were thawed to room temperature and mixed in their plastic bags to redistribute any drip that might have accumulated during the thawing process.

Free amino acids

Two grams of remixed subsamples was homogenised with 3 mL of high purity water using an Ultra-turrax (IKA-Labortechnik) blending probe. Residual homogenate was carefully washed from the probe head with an additional 1mL of water and washings combined with the homogenate to which had been added 45 nmole of the internal standard, norleucine. The combined homogenate was mixed by vortex and allowed to stand on ice for 15 minutes before centrifugation at 15,000 rpm for 30 minutes at +4°C. The supernatant was decanted and recentrifuged under the same conditions.

A 1 mL aliquot was retained for determination of soluble protein content. To a further 1.5 mL of supernatant was added 0.75 mL of 30 % (w/v) trichloroacetic acid, mixed by vortex, frozen to -20°C, thawed, re-vortexed and then centrifuged at 10,000 rpm for 10 minutes. The supernatant was then syringed through a 0.2 μm filter. A 1.0 mL and 0.5 mL aliquot was stored at -80°C for free amino acid and glucose assays, respectively.

For free amino acid analyses, samples were dispatched to an independent contracting laboratory and analysed using a Picotag (Waters, Inc.) precolumn derivitization HPLC

method to quantify 28 individual amino acids and the peptide carnosine. Results were expressed as μmole L^{-1} of extract and converted to μmoles per 100 g sample, mmoles per 100 g soluble (Biuret) protein and μmoles per 100 g Kjeldahl protein by the following formulae:

(i) μmole per 100 g sample (mince)

= (x/1000)*2.25*((4 + sample wt)/1.5)*(100/sample wt.)

Where: x/1000 converts values to μmole per mL of acid extract

2.25 = volume of homogenate (1.5 mL) + 0.75 mL TCA

(4 + sample wt) = weight of sample (~2.0 g) + 4 g water

100/sample wt = conversion to per 100g sample.

For example results, see Appendix C.

(ii) mmole per100 g soluble (biuret) protein

= ((μmole per100 g sample *100)/soluble protein (g%))/1000

Where: *100/soluble protein = conversion to per 100 g soluble protein

/1000 = converts to mmole

(iii) mmole per 100 g Kjeldhal protein

= ((μmole per100 g sample'*100)/Kjeldhal (g%))/1000

Where: *100/Kjeldahl protein = conversion to per 100 g Kjeldahl protein

/1000 = converts to mmole

Soluble protein

Soluble protein was determined, using the Biuret protein test (Bergmeyer, 1983) on an aliquot of supernatant used for the free amino acid test. Results were calculated as follows:

Soluble protein (g 100 g^{-1})

= Biuret protein (mg mL^{-1}) * (4 g + sample wt) * (100/sample wt)* (1/1000)

Where: (4 g + sample wt) = total weight of homogenate

(ie, 2 g sample + 4 g water)

Glucose analyses

Glucose analyses were done on an Hitachi clinical analyser and results expressed as mmoles L^{-1} of extract and converted to mmole 100 g^{-1} sample as follows:

Glucose (mmole 100 g^{-1})

= (x/1000)*2.25*((4 + sample wt)/1.5)*(100/sample wt.)

Where: x/1000 converts values to mmole per mL of acid extract

2.25 = volume of homogenate (1.5 mL) + 0.75 mL TCA

(4 + sample wt) = weight of sample (~2.0 g) + 4g water

100/sample wt = conversion to per 100 g sample.

Soluble and non-protein nitrogen (NPN)

Four grams of mince was homogenized for 20 seconds in 30 mL of 0.03M citrate buffer, pH 5.9, and incubated for 2 hours at 4°C. The homogenate was then centrifuged at 15,000 rpm for 40 minutes. One 10 mL fraction of clear supernatant was stored at -80°C for analysis of soluble nitrogen content.

For determination of NPN, 5 mL of 30%(w/v) trichloroacetic acid was added to10 mL of the supernatant and vortexed. The mixture was frozen to -20°C, thawed and centrifuged at 10,000 rpm for 10 minutes to obtain a clear supernatant. A 10 mL aliquot was stored at -80°C until required for analysis.

NPN, soluble nitrogen and total Kjeldahl protein were measured using standard methods (AOAC, 1994). Results were calculated as follows:

(i) NPN (g 100 g^{-1} sample)

= NPN (g 10 mL^{-1})*15 mL/10 mL *3.4 * 100/sample wt

Where: 10 mL = volume of supernatant used

15 mL = 10 mL homogenate + 5 mL trichloroacetic acid

3.4 = conversion to total volume of original extract

(4 g sample + 30 mL buffer = 34 mL)

(ii) Soluble nitrogen (g 100 g^{-1} sample)

= Nitrogen (g 10 mL^{-1}) * 3.4 * 100/sample wt

Where: 3.4 = converts value to total weight of original extract

(4 g sample + 30 mL buffer = 34 mL)

Free fatty acids and fat content

In outline, lipids were extracted from 4 g of mince by the Folch fat extraction method (Folch *et al.*, 1957) and an aliquot evaporated to dryness along with an internal standard. Solvent and an Amberlyst A26 resin were then added to bind the free fatty acids. The resin was washed, and the free fatty acids methylated, *in situ,* with boron trifluoride. The resultant fatty acid methyl esters were extracted with *iso*-octane(s) and quantified by gas chromatography with a BPX70 column (SGE, Australia). This method was a adaptation of Gandemer *et al* (1991). An aliquot of the Folch extract was also used for gravimetric measurement of fat content of the mince. A more detailed description follows:

Preparation of Amberlyst A26 anion exchange resin

The resin was shaken for 15 minutes with 200 mL 1M NaOH, washed 3 times with 300 mL of CO_2 -free distilled water with 10 minutes of shaking between each wash. Finally it was washed 3 times with 150 mL aliquots of methanol and stored under methanol until use.

Preparation of internal standard

A 2 mg tridecanoic acid (F13:0) mL^{-1} heptane stock was prepared by dissolving 0.10 g of the fatty acid in 50 mL heptane.

Recovery standards

Separate 2-mg mL^{-1} solutions of palmitic (F18:0) and stearic acid (F16:0) were prepared by weighing 0.04 g each into separate 20 mL volumetric flasks and making up to volume with heptane. A 1.0-mg mL^{-1} mixture of palmitic and stearic acids was prepared by mixing equal volumes of each solution.

Procedure

Internal standard and 0.125 mL of F13:0 standard were pipetted into a 30 mL Kimax screw cap test tube and a sample containing approximately 100 mg of lipid was added. The mixture was then evaporated to dryness under nitrogen at 35-40°C before addition of 15 mL of acetone:methanol (2:1). Washed Amberlyst A26 anion exchange resin, 200 mg, was added, the tube flushed with nitrogen, capped and shaken on a flat bed shaker for 30 minutes at 300 rpm.

The aqueous layer was carefully removed using a glass pasteur pipette attached to a water vacuum pump, and the resin washed five times with 5 mL portions of 2:1 (v/v) acetone:methanol. In the final rinse, some of the acetone/methanol was left with the resin to aid transfer to a 1.5 mL glass autosampler vial before drying under a gentle stream of nitrogen.

Boron trifluoride (300 μL of 14% in methanol) was added, the vial blanketed with nitrogen, capped with a Teflon-coated septa, and gently mixed before incubation overnight in the dark. *iso*-octane, 250 μL, was then added by injection through the septa and the vial shaken for 2 minutes. Saturated NaCl solution (1 mL) was added and the mixture blanketed with nitrogen, re-capped, shaken for a further 20 seconds, and allowed to settle. Approximately 80μL of the *iso*-octane layer was transferred to a gas chromatograph autosampler vial fitted with a 100 μL insert.

Fatty acid methyl esters were separated on a BPX 70, column (SGE, Australia) (30 m x 0.32 mm i.d, and 0.25 μm film) fitted to a HP 5890 II Gas Chromatograph. Chromatography conditions were as follows:

Injection volume:	1μL
Split flow:	100 mL min^{-1}
Carrier gas:	Helium
Column head pressure:	55 kPa
Column flow at 100°C:	1.26 mL min^{-1}
Split ratio:	79:1
Makeup gas:	Nitrogen, 30 mL min^{-1}
Air:	400 mL min^{-1}
Hydrogen:	30 mL min^{-1}
Injector temp:	240°C
Detector temp:	250°C

The temperature of the GC oven was maintained at 100°C for 4 minutes, increased to 130°C at 10°C min^{-1}, then to 150°C at 1°C min^{-1}, and finally to 200°C at 2.5°C min^{-1} with a final hold time of 13 minutes.

Calculations

A series of fatty acid methyl ester external standards spanning 0.05 to 2 mg mL^{-1} were used to determine individual fatty acid methyl esters (FAMES) concentrations in each sample. Calculations were done using Maxima Integration software (Water, Inc,). See Appendix B for detail of calculation.

Data analysis

Data from all metabolite assays, sensory evaluations and gas chromatography/mass spectrometry was analysed for variance by conventional methods using SigmaSTAT software.

5.3 Results and Discussion

Ultimate pH

Before titration the *semimembranosus* muscles had mean pH values (± standard deviation) of 5.66 (0.65) for the Low group, 6.26 (0.076) for the Medium and 6.79 (0.165) for the High.

Titration of high pH meat

Preliminary titrations were done on a high pH minced muscle to determine the concentration of hydrochloric acid to use that would give the least possible volume change to the mince but avoid high local concentrations of HCl during titration. Figure 5-2 shows a titration curve using increasing volumes of 2M HCl to lower the pH of 200 g of pH 7.0 mince to pH 4.2. The calculated buffering capacity of 30 μmole g^{-1} $pHunit^{-1}$ is similar to that found by others (Hamm, 1977). The pK_a of 5.5 was estimated from the slope of the curve.

Figure 5-3 shows the range of meat pH before and after titration with 2M HCl for all 30 experimental *semimembranosus* muscles. Apart from one outlying sample, pH 4.93, all meat samples were successfully titrated with HCl to within a range of 5.35 to 5.69. The mean (±standard deviation) for all samples was 5.56 (±0.15) after titration. The maximum change in pH for a sample was 1.86 pH units and the smallest 0.25 pH units. The author became adept at visually detecting a change in pH to around 5.6. There was a noticeable change in colour and texture from a dark red and sticky mince to light pink and dry mince (to touch). Minces maintained a stable pH after titration and storage overnight at 4°C. No further titration was required before sensory analysis.

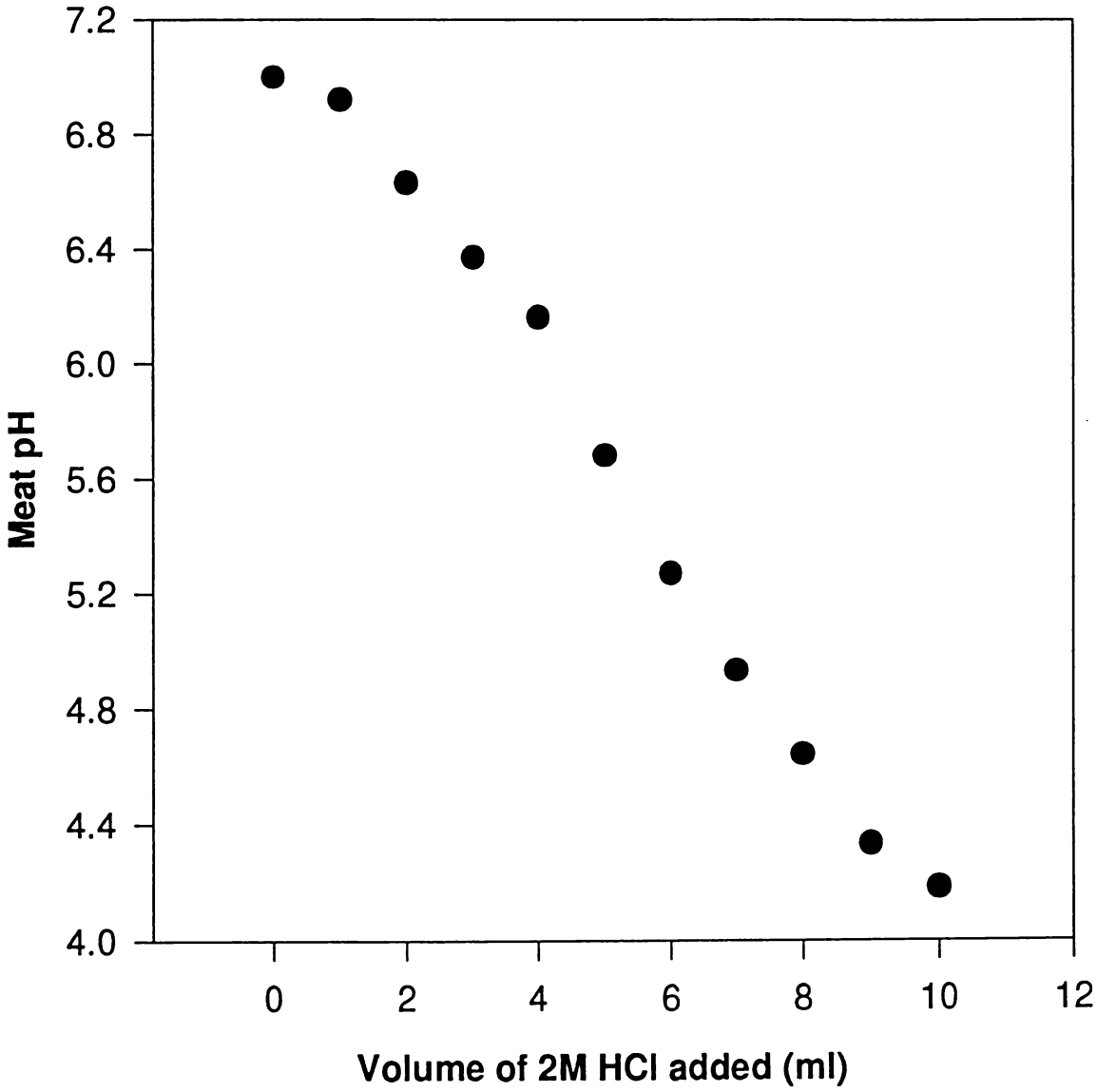


Figure 5-2.
Titration of a minced sheepmeat sample from an initial pH of 7.0 to 4.2 with increasing volumes of 2M HCl.

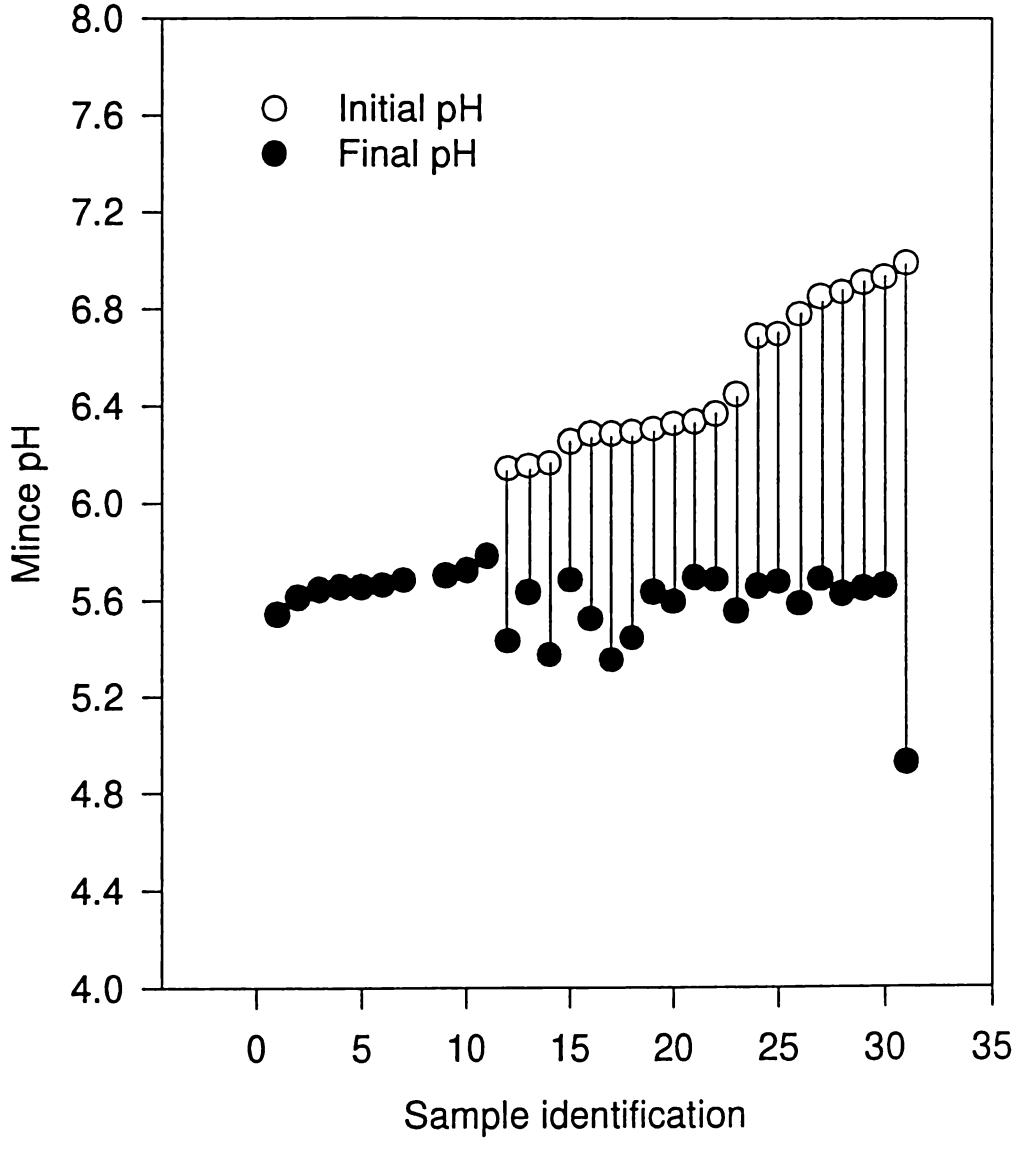


Figure 5-3.
Meat pH before and after titration with 2M Hcl for each minced *semimembranosus* muscle. The 10 samples with the lowest pH were not titrated.

Analytical repeatabilities for free fatty acids and free amino acids

A test for repeatability of analysis for each free fatty acid was done on a laboratory control sample of homogeneous beef. A control sample was included with each of the seven batches of test samples analysed.

Table 5-2.

Repeatability of free fatty acid determinations in a single sample of meat tested seven times.

Fatty Acid	Mean (μmole g^{-1})	SD (μmole g^{-1})	% CV
F8:0	1.3	0.7	55.4
F9:0	3.7	3.4	92.7
F10:0	3.1	0.8	25.0
F11:0	0.5	0.4	75.7
F12:0	20.4	1.8	8.7
F14:0	36.1	2.1	5.9
F14:1	12.3	0.6	4.8
F15:0	8.2	1.0	12.5
F16:0	336.5	16.7	5.0
F16:1	84.3	5.2	6.1
F17:0	12.9	0.9	6.9
F18:0	185.4	9.7	5.3
F18:1	835.3	46.9	5.6
F18:2	168.1	8.0	4.8
F19:0	0.7	0.3	46.0
F18:3	54.8	2.0	3.7
F18:4	10.7	1.4	12.9
F20:0	2.9	0.3	11.4
F20:1	6.0	0.5	8.6
F20:2	4.6	0.2	4.6
F21:0	1.3	0.2	12.5
F20:4	31.4	1.8	5.7
F20:3	2.2	0.3	15.6
F22:0	7.8	3.2	41.1
F20:5	22.8	0.9	3.9
F22:1	0.2	0.1	28.5
F24:0	1.3	0.2	17.1
F24:1	7.7	0.5	6.5
F22:6	5.7	0.3	5.5

SD = one standard deviation; % CV = percent coefficient of variation ((SD/mean)*100)

Except for fatty acids F8:0 to F11:0, F19:0, F22:0 and F22:1, all free fatty acid were within acceptable between-batch repeatability limits for a test of this nature. Seven free fatty acids that recorded high variability were all relatively low in concentration (Table 5-2).

Repeatability measurements for free amino acids was measured by testing four sets of duplicate samples with the batch of samples analysed. Table 5-3 lists the average variability recorded for each free amino acid according to the formula:

$$s = \sqrt{\sum D^2 / 2K}$$

where s = standard deviation

K = number of sets of duplicates

D = difference between duplicate determinations

Repeatability was good (≤ 14%) for all amino acids.

Table 5-3.

Repeatability of free amino acid determinations measured by testing four sets of duplicate samples.

Amino Acid/Peptide	Abbreviation	Mean	SD	% CV
		μmole litre^{-1}	μmole litre^{-1}	
aspartic acid	Asp	9.6	0.6	6.0
glutamic acid	Glu	763	25.6	3.3
hydroxyproline	OH-Pro	8.3	0.4	14.3
serine	Ser	78	4.0	5.1
asparagine	Asn	589	18.7	3.2
glycine	Gly	291	14.7	5.0
glutamine	Gln	7371	345	4.7
β-Alanine	BAla	36	2.4	6.5
taurine	Tau	461	20.4	4.4
histidine	His	38	1.1	3.0
threonine	Thr	91	3.8	4.1
alanine	Ala	531	21.2	4.0
carnosine (β-alanylhistidine)	Carnosine	1888	70.1	3.7
arginine	Arg	101	3.9	3.8
proline	Pro	84	3.0	3.5
1-methylhistidine	1MeH	7.1	0.2	2.4
3-methylhistidine	3MeH	1252	54.3	4.3
α-aminobutyric acid	AABA	40	1.5	3.8
tyrosine	Tyr	47	1.7	3.6
valine	Val	91	3.2	3.5
methionine	Met	44	1.3	2.9
cysteine	Cysta	4.5	0.3	5.7
isoleucine	Ile	53	1.6	3.0
leucine	Leu	111	3.6	3.3
phenylalanine	Phe	57	1.7	3.0
tryptophan	Try	14	0.5	3.7
ornithine	Orn	8	0.4	5.1
lysine	Lys	71	3.4	4.8

where SD = one standard deviation; % CV = coefficient of variation

Sensory analysis

The results of sensory analysis are summarised in Table 5-4. After the muscles were titrated with acid, the panellists found no significant difference between each of the pH groups for any of the attributes tested. This is in striking contrast to the results presented in chapter 4 where panellists detected significant decreases in odour and flavour with increasing ultimate pH before titration with HCl.

Table 5-4.

Mean sensory panel intensity scores for cooked *semimembranosus* mince from animals in three pH groups but titrated to constant pH. Samples were scored on a scale of 0 to 9 where 0 signified none and 9, extreme.

	Low (pH 5.66)	Medium (pH 6.26)	High (pH 6.79)	Low vs. Medium	Low vs. High	Medium vs. High
overall odour	5.1	4.8	5.0	NS	NS	NS
overall flavour	5.6	5.6	5.8	NS	NS	NS
sheepmeat odour	4.3	4.4	4.4	NS	NS	NS
sheepmeat flavour	5.2	5.3	4.9	NS	NS	NS
foreign odour	2.3	1.8	2.0	NS	NS	NS
foreign flavour	2.4	2.4	3.2	NS	NS	NS

NS, not significant ($P > 0.05$)

To compare sensory panel mean intensity scores before and after titration, each attribute was plotted graphically against initial meat pH (for an example see figure 5-4). (Approximate intensity scores can be compared between before and after titration with HCl by multiplying the 0 to 100 scale by a factor of 0.09. When this was done for the Low pH group, the intensity scores for each of the attributes were not considered significantly different between the two experiments).

The results of sensory analysis show that for each of the attributes tested, titration of high-pH meat to pH values similar to the low-pH group increased intensity scores to a similar level as observed when panellists tested the cooked minces before titration with HCl. This is summarized in Table 5-5 where the correlation coefficients (r^2) for relationship between each sensory attribute and initial pH approach zero after the minces were titrated.

Table 5-5.

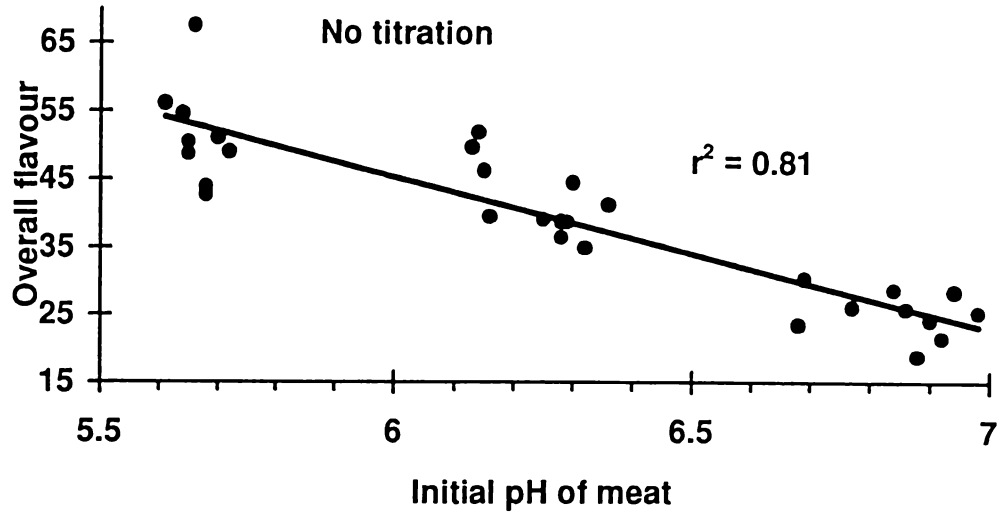
Comparison of correlation coefficient (r^2) values derived from plots of sensors attribute vs initial meat pH of samples before and after titration with HCl.

Attribute	r^2	
	Before titration	After titration
overall odour	-0.42***	-0.03 ^{NS}
overall flavour	-0.81***	+0.01^{NS}
sheepmeat odour	-0.06 ^{NS}	+0.01^{NS}
sheepmeat flavour	-0.60**	-0.08 ^{NS}
foreign odour	-0.06*	-0.06 ^{NS}
foreign flavour	+0.01^{NS}	+0.09 ^{NS}

Significance between low- and high- pH groups, titrated or not

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant ($P > 0.05$)

(a) Data derived from chapter 4



(b) Data from this chapter

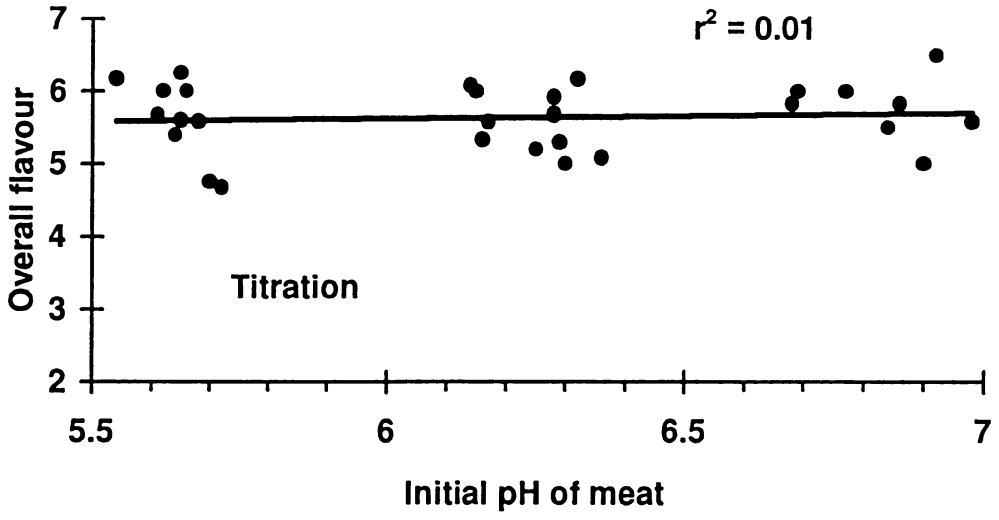


Figure 5-4.

Overall flavour sensory scores of cooked sheepmeat (a) before - scale 0 to 100 - and (b) after - scale 0 to 9 - titration with HCl to a (standard) low pH.

The most frequent descriptors from panellists' comments on odour and flavours before and after titration are shown graphically, by frequency of occurrence, in figure 5-5. Although rather subjective, this comparison shows interesting trends. In particular, the frequency of bland/fat descriptors, recorded often in high pH meat before titration with HCl, were recorded far less often when high- and medium-pH meat was titrated with HCl. Other notable trends were that the frequency of descriptors, "meaty" and "sweet", considered desirable attributes, decreased as meat pH increased (figure 5-5, upper), but were in equal proportions for the low, medium and high pH groups after titration (figure 5-5, lower). The descriptors, "livery", "rancid" and "buttery", not mentioned by panellists in the pre-titration experiment, were observed in each of the pH groups in the post-titration experiment. The rancid flavour may be similar to the stale/musty descriptor observed before titration.

Gas chromatography/mass spectrometry

Thermal desorption of the Tenax traps eluted more than 360 compounds (see Appendix A) that were recorded as total ion chromatogram peaks by GC/MS for each sample of rendered fat. Generally, total volatile compounds levels were similar for each of the pH groups after the meat was titrated with HCl (figure 5-6b). Similarly, of the 56 compounds found to decrease in concentration with increasing pH before titration (chapter 4), only four (1-pentanol, 3-penten-2-ol, 1-octen-3-ol and 1-phenylethanone) changed significantly between each pH group after titration with HCl. This indicates that their production is independent of pH. For comparison, examples of mean relative concentrations for some compounds before (part 1, chapter 4) and after titration are plotted in figure 5-6a. Only for the four compounds listed above do the after-titration curves significantly resemble the decrease in relative amounts observed before titration (figure 5-6b). The lack of change in the majority of volatile compounds after the meat was titrated with HCl reflect the sensory panellists' perception of meat tested after titration and supports the finding of others (Lineweaver and Pippen, 1961; Gasser and Grosch, 1990) that aldehydes, such as (*E,Z*)-2,4-decadienal, (*E,E*)-2,4-decadienal, nonanal, and 2-undecenal are significant contributors to cooked meat odour and flavour.

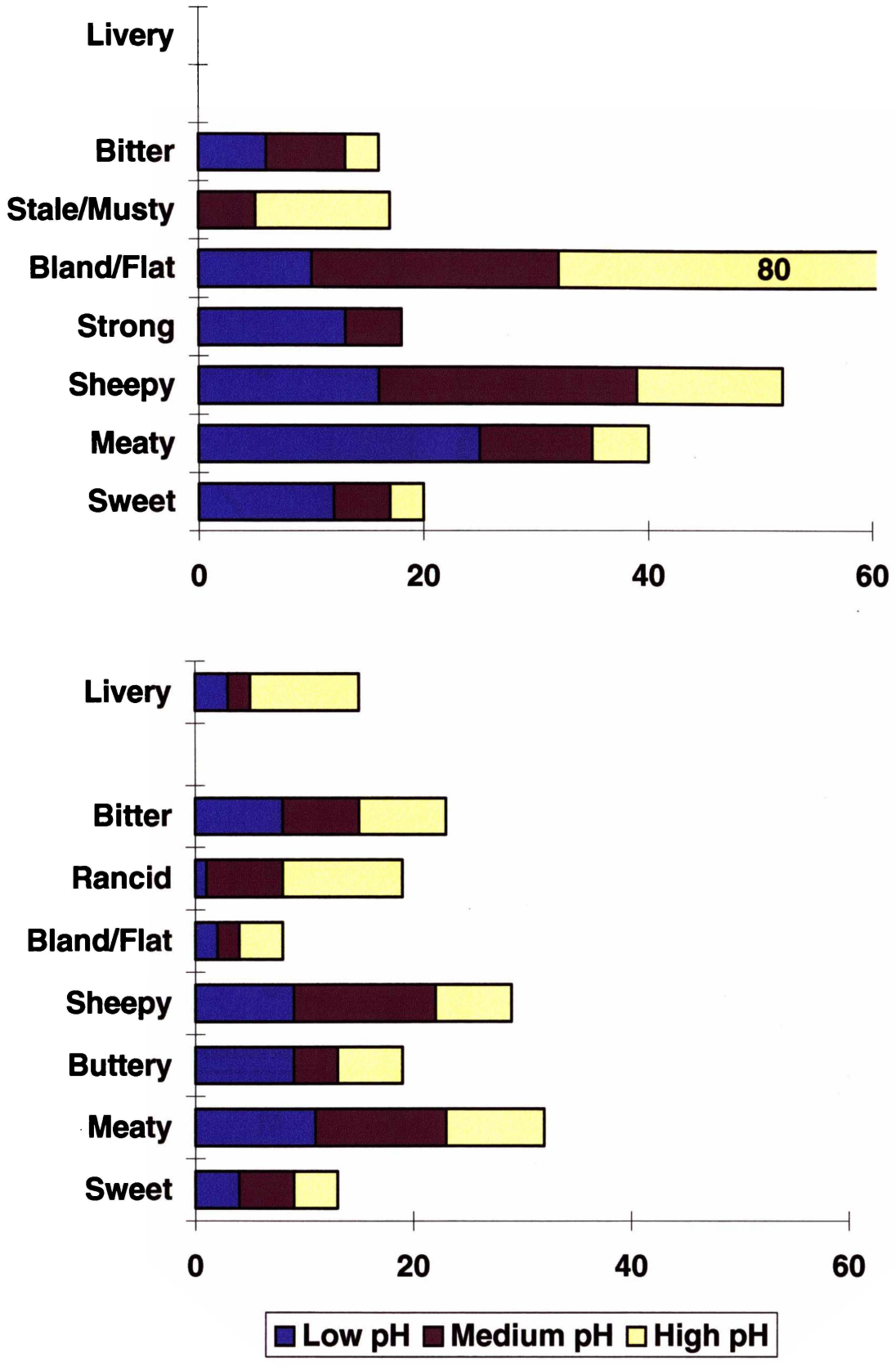


Figure 5-5.
Frequency of flavour descriptors before (from chapter 4, top) and after (bottom) titration with HCl for Low, Medium and High ultimate pH sheepmeat.

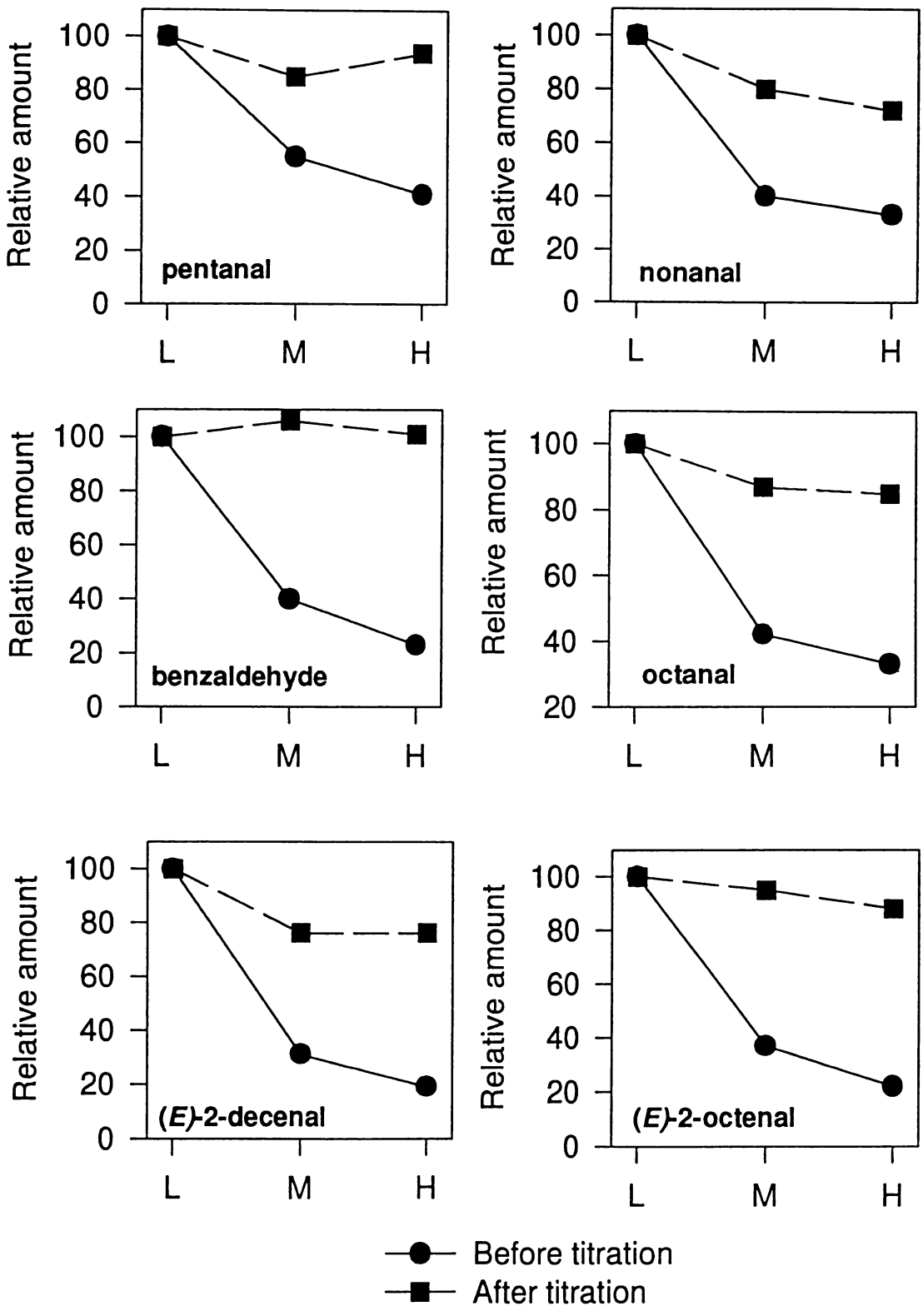


Figure 5-6a.
Example plots of relative amounts of volatile compounds that changed in concentration between Low (L), Medium (M), and High (H) pH groups before (part 1, chapter 4) titration with HCl compared with the same compounds after titration with HCl.

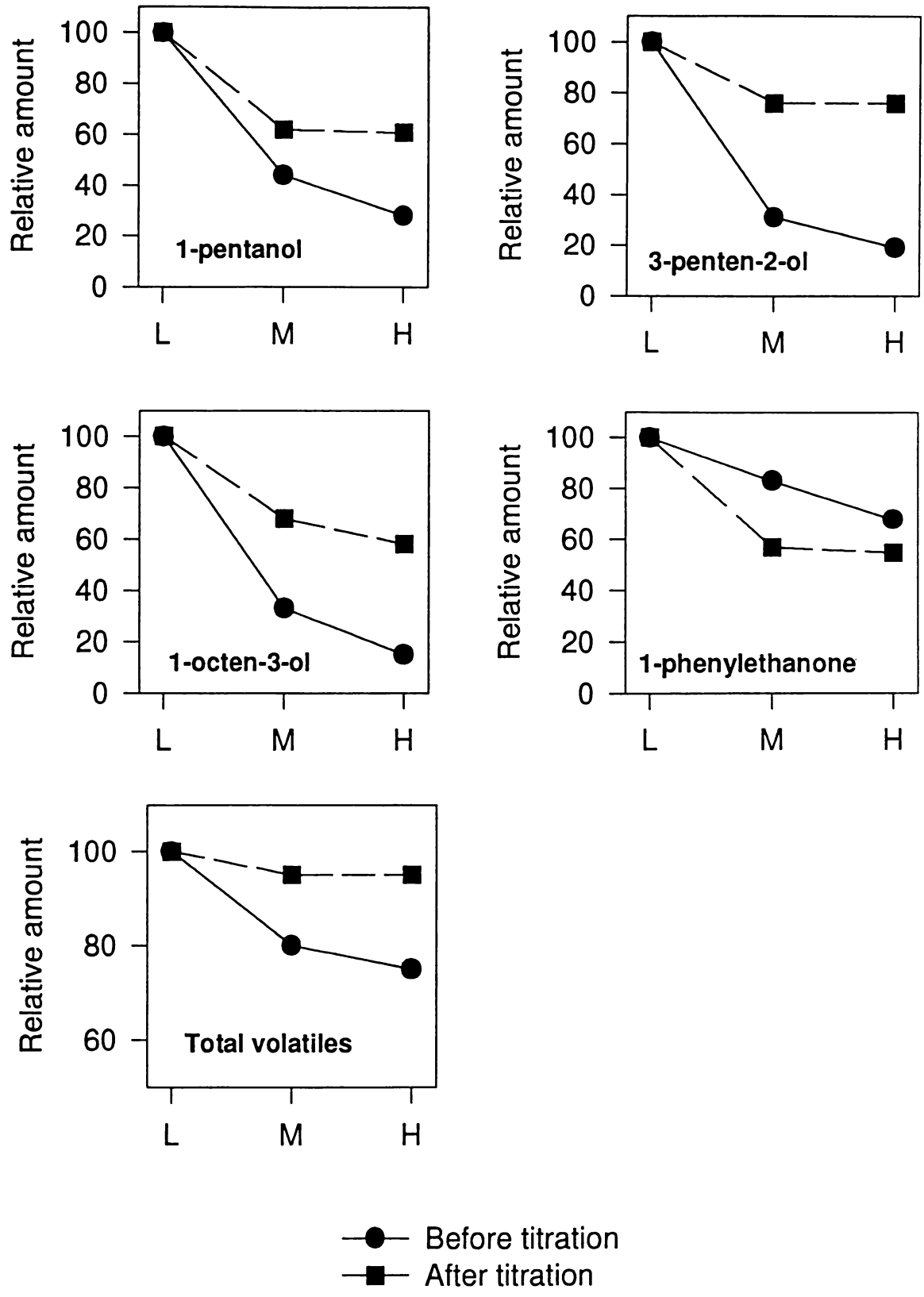


Figure 5-6b.
Plots of relative amounts of volatile compounds that changed in concentration between Low (L), Medium (M), and High (H) pH groups before (part 1, chapter 4) and after titration with HCl.

Twenty-nine additional compounds were found to change significantly in concentration between one or more of the pH groups after titration with acid (Table 5-6) but none of these compounds changed significantly in all three possible comparisons. Twelve compounds decreased significantly in concentration from the low- to medium-pH group, 15 compounds decreased significantly in concentration from the low- to high-pH group and 2 alcohols decreases significantly in concentration from the medium- to high-pH group. Conversely, 3 compounds increased significantly in concentration from the low- to medium-pH group, 14 compounds increased significantly in concentration from the low- to high-pH group, and 5 compounds increased significantly in concentration from the medium- to high-pH group. No relationship between post titration pH values and sensory data or GC/MS peaks could be found when data was analysed in a similar manner used for the pre-titration values (data not shown).

Of the 29 identified volatile compounds that significantly changed in concentration when samples were grouped according to their pre-titration pH values, 15 increased as pH increased -contrasting the results of the experiment (part 1, no titration) where only four compounds showed a significant increase in concentration with increasing pH among any of the treatment groups. Four of the five acids identified were all branch chain fatty acids and increased in concentration with increasing pH. The reason for this effect is unclear. Increased hydrolysis because of intense mixing in the food processor was probably not the cause as all minces from the three pH groups were mixed for similar periods. One possible explanation might be increased hydrolysis of triglycerides exposed to greater amounts of hydrochloric acid that had to be added to the higher pH samples compared to the low pH group samples that were not titrated with acid.

The branch chain fatty acids that increased in concentration with increasing pH all impart cheesy/sweet/fatty aroma (Brennand *et al.*, 1989) and would contribute to any foreign odour, although no significant change in foreign odour was detected by panellists. As mentioned in chapter 4, branched chain fatty acid are common in sheep adipose tissue and those branch chain fatty acids identified in Table 5-4 have been found previously in raw and cooking sheepmeat (Brennand and Lindsay, 1992a).

Table 5-6.

Mean approximate concentrations (nanograms per gram of rendered fat) of the twenty-nine compounds that changed significantly from low (L) to medium (M), low to high (H) and medium to high pH groups

Peak	Kovats' index	Compound	ng g^{-1}			significance		
			L	M	H	L vs M	L vs H	M vs H
		aldehydes						
127	902	3-(methylthio)-propanal	21	13	13	**	*	NS
268	1206	decanal	9	6	5	*	*	NS
		alcohols						
49	738	3-methylbutanol	2.5	2.4	1.7	NS	**	*
64	769	1-pentanol	107	67	66	**	**	NS
67	775	3-penten-2-ol	3.1	2.5	1.6	NS	**	*
68	781	2,3-butanediol	18	30	43	NS	(*)	NS
167	975	1-octen-3-ol	144	98	83	*	*	NS
		acids						
93	830	3-methyl butanoic acid	1.5	1.9	2.6	NS	(**)	NS
97	838	2-methyl butanoic acid	2.4	3.0	3.6	NS	(**)	NS
146	934	4-methyl pentanoic acid	0.9	2.1	2.4	(*)	(***)	NS
193	1035	4-methyl hexanoic acid	5	6	10	NS	(***)	(**)
291	1252	nonanoic acid	43	30	24	*	**	NS
		alkanes						
170	986	a methyl alkane	216	255	309	NS	(*)	NS
188	1024	a methyl alkane	25	29	35	NS	(**)	NS
197	1044	a methyl alkane	14	17	20	(**)	(*)	NS
199	1048	a methyl alkane	2.8	3.6	4.6	(*)	(***)	(*)
247	1146	a methyl alkane	11	14	15	*	**	NS
265	1200	decane	23	26	34	NS	(**)	(*)
327	1358	a methyl alkane	3	4	4	NS	(*)	NS
		alkenes						
261	1183	an alkene (55,43,41,69)	6.4	7.6	8.7	NS	(**)	NS
		ketones						
119	883	2-heptanone	12	6	7	**	*	NS
207	1062	1-phenylethanone	23	13	12	*	*	NS
		lactones						
128	902	4-hydroxybutanoic acid lactone	35	23	14	**	***	NS
		miscellaneous						
135	913	unknown	0.7	0.4	0.4	*	**	NS
144	930	2-Thujene ?	8.6	7.3	12.5	NS	*	(**)
219	1087	unknown (43,55,45,99)	55	27	22	**	**	NS
251	1162	unknown (57,71,85,112)	14	18	20	NS	(*)	NS
259	1179	unknown (57,69,83,130)	0.5	0.8	1.1	NS	(**)	NS
288	1242	unknown (70,83,41,110)	1.4	0.9	0.8	*	*	NS

[a] MS identifications were by comparison with the NIST MS data base and Kovats' retention indices. Numbers in parentheses refer to the principal ions observed, in decreasing order of intensity, for unidentified compounds. Asterisks represent significant decreases (increases) in concentration between pH groups. NS, not significant $P > 0.05$; *, $P < 0.05$.; **, $P < 0.01$.; ***, $P < 0.001$.

Other compounds behaved in a similar manner. Methional [3-(methylthio)-propanal], a Strecker degradation product of methionine (Ballance, 1961), also decreased significantly between the low- and medium-pH groups and the low- and high-pH groups after titration with acid. Although this change was not observed between pH groups before titration, this compound was identified by GC/OP as a contributor to the odour of cooked sheepmeat before titration with acid (see Table 4-6, chapter 4) and has been previously identified in the adipose tissue of lambs (Sutherland and Ames, 1995). Methional has also been implicated as a major contributor to the unpleasant odour when meat is irradiated in attempts to produce a stable room temperature product (Wick *et al.*, 1965).

The six alkanes that increased in concentration with increasing pH have high odour threshold values and therefore unlikely to contribute to the total aroma of meat. Two alcohols, 1-pentanol and 1-octen-3-ol, that impart a fruity/sweet and herbaceous or mushroom odour note, respectively, were found in significantly higher concentrations in the initially low pH group even after titration of the high pH group. These two alcohols are produced from thermal oxidative decomposition of linoleic acid (F18:2) (Forss, 1972) of phospholipid rather than triglyceride origin (Mottram and Edwards, 1983). Similarly, 3-penten-2-ol decreased from the low- to high- pH group both before and after titration with acid. However, this alcohol was found at far lower concentration compared to the former two alcohols and because of its high odour threshold would not significantly contribute to the overall odour of cooked sheepmeat.

Butyrolactone (4-hydroxybutanoic acid lactone) also decreased from the low- to medium- and low- to high-pH groups after titration with HCl. Butyrolactone has been identified in ovine adipose tissue previously (Caporaso *et al.*, 1977) and is produced during the thermal oxidation of saturated and unsaturated fatty acids (Watanabe and Sato, 1971). Low molecular lactones impart a herbaceous, fatty aromas.

Changes in metabolite concentration before and after titration with HCl

The average glucose concentration for the low pH group was about six and eight times greater than that found in the medium and high pH groups, respectively (Table 5-7). This reflects biochemical changes that occur in live muscle from an animal injected with

adrenaline or subject to preslaughter stress. The animal's glycogen reserve is depleted thus reducing the available source of glucose supply. Titration of the medium and high pH groups with acid had no influence on the glucose content of the raw mince since the glycogen reserves were already depleted.

The low glucose levels found in the medium- and high-pH groups compared with the low-pH group might conflict with panellists observation. After titration of the high-pH samples with acid, panellists could not detect the flavour differences between each of the pH groups as observed before titration. As mentioned in chapter two, glucose is an important ingredient in the Maillard reaction so one could expect that a reduction in glucose concentration would reduce the meat flavour. The reason for these apparent conflicting observations may be that only low levels of reducing sugars are required to form flavourful Maillard reaction products, or that in the case of mildly cooked meat, as in this study, the major contributors to flavour development are pH dependent precursors of Strecker aldehydes – α-amino acids – or a combination of both possibilities.

Absolute concentrations of metabolites, expressed on a sample weight basis, found in cooked mince cannot be directly compared with concentrations found in raw minces since cooking will change the proportions of fat, protein and moisture. Yet, glucose content of cooked mince was still proportionally higher in the low- pH compared with the higher pH groups. The cooking process did not greatly deplete glucose levels. The higher concentration of glucose in cooked mince from the low pH group would contribute to the sweet flavour of the meat and participate in the production of Maillard reaction products.

The amounts of total protein and total fat were unaffected by meat ultimate pH and titration with acid (neither was tested in cooked mince samples but would not be expected to change significantly). NPN levels in the soluble fraction were low and unaffected by acid titration and cooking. Soluble nitrogen levels (by Kjeldahl) were the same for each of the pH groups before titration, with a slight decrease in concentration for the high pH group after titration with acid. This difference was not reflected in the post-cooking concentrations between each of the groups.

Soluble protein levels, as measured by the Biuret method, increased significantly ($P < 0.05$) from the low- to medium- and low- to high-pH groups before titration with acid. The soluble protein values of the medium- and high-pH groups returned to the pre-titration low-pH group level after titration with acid. Similar findings have been reported for beef (Saffle and Galgreath, 1964) and turkey meat (Prusa and Bowers, 1984) and attributed to greater solubility of proteins at pH values distant from their isoelectric point (pH 5.5 for meat). However, such effects are also dependent on the vigor of the extraction process. Daum-Thunberg *et al.* (1992) found that under much milder extraction conditions than used in this study, the amount of soluble protein from chicken breast decreased with increasing pH. This suggests two processes are involved in the solubilization of meat protein. The first process, under mild extraction conditions, could be dependent on the swelling of myofibrils at higher pH values (Offer and Trinick, 1983). It is possible that the swelled myofibrils are more difficult to disperse, thus, less protein would be extracted at higher pH. The second process, under more rigorous extraction procedures, would overcome the resistance to dispersion of myofibrils and the charged proteins would solubilize more readily than at their isoelectric point, as predicted from theory.

The pH dependent solubility of meat proteins probably does not play a major role in flavour and odour development of cooked meat since rigorous homogenization of minces was not employed before cooking to release more soluble protein at higher meat ultimate pH. However, this does not preclude possible influence of pH related heat denaturation on cooked meat odour and flavour development.

There was no significant difference in concentration of soluble protein between each of the pH groups after titrated minces were cooked, although the absolute amounts were about eight times lower than pre-cooked levels because of heat induced denaturation of the soluble protein.

Table 5-7.

Mean concentrations of meat constituents that changed significantly for low (L), medium (M), and high (H) pH groups before and after titration with hydrochloric acid.

pH group	Before titration	After titration	After cooking
Glucose (μmole g $^{-1}$)			
L	1.85^{a}	1.85^{a}	2.05^{a}
M	0.32^{b}	0.36^{b}	0.64^{b}
H	0.22^{b}	0.22^{b}	0.33^{b}
NPN (g 100 g $^{-1}$)			
L	0.38^{a}	0.38^{a}	0.41^{a}
M	0.41^{a}	0.41^{a}	0.4 ^{a}
H	0.41^{a}	0.41^{a}	0.41^{a}
Soluble N (g 100 g $^{-1}$)			
L	1.00^{a}	1.00^{a}	0.45^{c}
M	1.04^{a}	1.05^{a}	0.42^{c}
H	1.04^{a}	0.96^{b}	0.41^{c}
Soluble protein (g 100 g $^{-1}$)			
L	4.47^{a}	4.47^{a}	0.65^{d}
M	5.64^{b}	4.85^{ac}	0.73^{d}
H	6.05^{b}	4.49^{ac}	0.66^{d}
Total protein (g 100 g $^{-1}$)			
L	17.8^{a}	17.8^{a}	
M	17.9^{a}	18.1^{a}	-
H	17.8^{a}	17.6^{a}	-
Total fat (g 100 g $^{-1}$)			
L	17.3^{a}	17.3^{a}	
M	17.3^{a}	16.7^{a}	-
H	16.9^{a}	16.9^{a}	-

Values in the same line or column for each analyte with different superscripts differ significantly ($P < 0.05$)

Changes in total free amino acid and individual free amino acid

The summation of all free amino acids tested (see Table 5-3), expressed per sample weight for each pH group, is listed in Table 5-8. The concentration of total free amino acids increased with increasing pH before titration with acid. After titration with HCl these levels were the same as pre-titration concentrations of the low-pH group, suggesting that total free amino acid content is related with the soluble protein content. This was confirmed when data were expressed per weight of soluble protein (Table 5-9); pH and titration had no effect.

The most abundant free amino acids (and peptide), of those tested, were: glutamine (45%), carnosine (14%), 3-methylhistidine (10%), glutamic acid (9%), taurine (4.2%), alanine (3.5%), asparagine (3.6%) and glycine (3%). The remainder were each less than 1% of the total extracted.

Free amino acids, expressed per sample weight, that changed significantly ($P < 0.05$) between at least one of the treatments are also listed in Table 5-8. The amino acids listed increased significantly ($P < 0.05$, at least between the low- and high-pH groups) in concentration with increasing pH, reflecting a similar change in the total free amino acids content when expressed per sample weight.

With rare exceptions, titration of the medium- and high-pH groups with HCl lowered the level of all of the amino acids listed in Table 5-8, to the low-pH group pre-titration levels.

The exceptional free amino acids that did not decrease in concentration after titration were hydroxyproline, serine, and arginine. For these three amino acids the concentrations remained significantly ($P < 0.05$) higher for the medium-pH group compared with the pre-titration low-pH group but this effect was not maintained after the titrated meat and the low-pH group samples were cooked. Nonetheless, all free amino acids listed in Table 5-8 showed slight increases in concentrations for the higher pH groups (after titration), only glutamic acid, hydroxyproline, glycine and cysteine were significant ($P < 0.05$).

When total free amino acids were expressed on a soluble protein basis, concentrations were unaffected by initial meat pH or titration with HCl, contrasting the changes observed when expressed on a sample weight basis. Also fewer individual free amino acids (9 out of the 14 that changed when expressed on a sample weight basis) increased with increasing pre-titration pH (Table 5-9).

The concentration of the majority of those free amino acids that increased with increasing pH, was reduced to the low- pH group (pre-titration) levels when titrated with HCl.

The absolute concentrations of total and individual free amino acids were higher after cooking, when expressed on a soluble protein basis, because heat denaturation had reduced the amount of extracted soluble protein (Table 5-7). Only glutamic acid and hydroxyproline concentrations increased significantly ($P < 0.05$) with increasing pH after cooking.

The free amino acid results show that solubilization of total- and a number of free amino acids (those listed in Tables 5-8 and 5-9) are regulated by meat pH probably in a similar manner as the regulation of extractable protein.

Table 5-8.

Mean concentrations of total free amino acids and individual free amino acids (both μmole 100 g^{-1} sample) that changed significantly for low (L), medium (M), and high (H) pH groups before and after titration with hydrochloric acid.

pH group		Before titration	After titration	After cooking
Total FAA (μmoles 100 g^{-1} sample)				
	L	3634^{acd}	3634^{acd}	2656^{d}
	M	4940^{abc}	4755^{ab}	3763^{d}
	H	5715^{b}	3242^{cd}	3567^{d}
Asp				
	L	3.04^{a}	3.04^{a}	2.00^{a}
	M	5.16^{a}	3.65^{a}	2.59^{a}
	H	11.93^{b}	3.76^{a}	3.86^{a}
Glu				
	L	266^{a}	266^{a}	292^{ad}
	M	342^{b}	322^{d}	367^{b}
	H	395^{bc}	369^{bd}	451^{c}
OHpro				
	L	2.2^{a}	2.2^{a}	2.3^{a}
	M	3.7^{b}	3.6^{b}	3.4^{b}
	H	3.4^{bc}	2.6^{ac}	3.7^{b}
Ser				
	L	21^{a}	21^{a}	17^{a}
	M	41^{bc}	32^{c}	26^{ac}
	H	53^{b}	23^{ab}	29^{ac}
Asn				
	L	134^{acd}	134^{acd}	72^{d}
	M	220^{ab}	164^{ac}	107^{d}
	H	294^{b}	115^{cd}	107^{d}
Gly				
	L	87^{ac}	87^{ac}	85^{ac}
	M	136^{bd}	124^{cd}	125^{be}
	H	157^{b}	94^{ce}	126^{b}

Table 5-8. cont.

	pH group	Before titration	After titration	After cooking
His				
	L	8.5^{a}	8.5^{a}	5.3^{a}
	M	11.1^{a}	9.7^{a}	7.7^{a}
	H	18.2^{b}	7.0^{a}	8.7^{a}
Thr				
	L	22^{ac}	22^{ac}	16^{c}
	M	35^{a}	24^{a}	20^{ac}
	H	49^{b}	19^{ac}	23^{ac}
Arg				
	L	23^{ac}	23^{ac}	12^{e}
	M	41^{bd}	35^{cd}	22^{ae}
	H	50^{b}	20^{ae}	19^{ae}
Pro				
	L	23^{a}	23^{a}	23^{a}
	M	35^{b}	26^{a}	27^{a}
	H	48^{c}	25^{a}	31^{ab}
Tyr				
	L	13^{ac}	13^{ac}	8^{c}
	M	18^{ab}	16^{ad}	11^{cd}
	H	20^{b}	10^{ac}	11^{c}
Val				
	L	23^{ac}	23^{ac}	15^{cd}
	M	35^{ab}	25^{a}	19^{c}
	H	44^{b}	17^{ac}	20^{c}
Cysta				
	L	0.8^{a}	0.8^{a}	0.3^{c}
	M	1.1^{a}	1.0^{a}	0.3^{c}
	H	2.7^{b}	1.2^{a}	0.7^{a}
Lys				
	L	14^{a}	14^{a}	9^{a}
	M	22^{ab}	18^{a}	12^{a}
	H	38^{b}	12^{a}	13^{a}

Values in the same line or column for each analyte with different superscripts differ significantly ($p < 0.05$)

Table 5-9.

Mean concentrations of total free amino acids and free amino acids (μmole 100 g^{-1} soluble protein) that changed significantly for low (L), Medium (M), and high (H) pH groups before and after titration with hydrochloric acid.

pH group	Before titration	After titration	After cooking
Total FAA (mmole 100g $^{-1}$ soluble protein)			
L	81^{a}	81^{a}	421^{b}
M	88^{a}	98^{a}	523^{b}
H	99^{a}	70^{a}	535^{b}
Asp			
L	0.06^{a}	0.06^{a}	0.28^{b}
M	0.08^{a}	0.08^{a}	0.35^{b}
H	0.20^{b}	0.06^{a}	0.43^{b}
Glu			
L	6.0^{a}	6.0^{a}	46^{c}
M	6.1^{a}	6.7^{a}	53^{c}
H	6.6^{a}	8.2^{b}	69^{d}
OHpro			
L	0.05^{a}	0.05^{a}	0.35^{c}
M	0.06^{ab}	0.07^{b}	0.52^{d}
H	0.06^{ab}	0.06^{ab}	0.56^{d}
Ser			
L	0.5^{ac}	0.5^{ac}	2.7^{d}
M	0.7^{b}	0.7^{b}	3.7^{d}
H	0.9^{b}	0.5^{c}	4.3^{d}
Asn			
L	3.0^{a}	3.0^{a}	11.5^{c}
M	3.9^{ab}	3.4^{a}	14.7^{c}
H	5.1^{b}	2.5^{a}	16.0^{c}
Gly			
L	1.9^{a}	1.9^{a}	13.6^{c}
M	2.4^{ab}	2.6^{b}	17.4^{c}
H	2.7^{b}	2.1^{ab}	18.9^{c}

Table 5-9. cont.

	pH group	Before titration	After titration	After cooking
His				
	L	0.19^{a}	0.19^{a}	0.87^{b}
	M	0.20^{a}	0.20^{a}	1.06^{b}
	H	0.32^{a}	0.19^{a}	1.30^{b}
Thr				
	L	0.50^{a}	0.50^{a}	2.50^{c}
	M	0.62^{ab}	0.50^{a}	2.82^{c}
	H	0.84^{b}	0.41^{a}	3.35^{c}
Arg				
	L	0.51^{a}	0.51^{a}	1.93^{c}
	M	0.73^{ab}	0.73^{ab}	3.00^{c}
	H	0.86^{b}	0.44^{a}	2.80^{c}
Pro				
	L	0.51^{a}	0.51^{a}	3.57^{c}
	M	0.62^{ab}	0.54^{a}	3.82^{c}
	H	0.80^{b}	0.80^{b}	4.68^{c}
Tyr				
	L	0.29^{ab}	0.29^{ab}	1.31^{c}
	M	0.32^{ab}	0.32^{ab}	1.50^{c}
	H	0.35^{a}	0.23^{b}	1.64^{c}
Val				
	L	0.52^{ab}	0.52^{ab}	2.39^{c}
	M	0.61^{ab}	0.51^{a}	2.62^{c}
	H	0.76^{a}	0.37^{b}	2.94^{c}
Cysta				
	L	0.02^{a}	0.02^{a}	0.06^{bc}
	M	0.02^{a}	0.02^{a}	0.04^{b}
	H	0.05^{b}	0.03^{a}	0.11^{bc}
Lys				
	L	0.31^{a}	0.31^{a}	1.39^{c}
	M	0.41^{ab}	0.37^{a}	1.67^{c}
	H	0.65^{b}	0.29^{a}	1.90^{c}

Values in the same line or column for each analyte with different superscripts differ significantly ($p < 0.05$)

Changes in total free fatty acid and individual free fatty acids levels

Total free fatty acids levels, calculated by the summation of all 29 free fatty acids measured, showed no significant change with increasing pH when expressed as mg g^{-1} of sample (Table 5-10). However, total free fatty acid content increased slightly after titration with acid ($P < 0.05$ for the high group only) when similarly expressed.

Table 5-10.

Mean concentrations of total free fatty acids (mg g^{-1} sample) that changed significantly for low (L), medium (M), and high (H) pH groups before and after titration with hydrochloric acid.

pH group	Total FFA		
	Before titration	After titration	After cooking
Low	1.13[a]	1.13[a]	-
Medium	1.10[a]	1.2[a]	-
High	1.20[a]	1.39[b]	-

Of the 29 individual free fatty acids tested, 9 significantly changed in concentration, either with increasing pH or after titration with hydrochloric acid (Table 5-11).

The free fatty acids listed in Table 5-11 increased in concentration with increasing pH ($P < 0.5$, at least between the low- and high-pH groups) before titration, except for F20:5 and F24:1. Seven of the nine free fatty acids were unsaturated (50 % of all unsaturated fatty acids measured).

For the majority of the 29 free fatty acids measured, titration with HCl had no significant effect on concentrations for each of the pH groups. The four exceptions were three unsaturated (F18:1, F20:5 and F24:1) and one saturated acid (F14:0) which increased significantly ($P < 0.05$). Resources were not available for measurement of free fatty acids in the cooked minces.

Table 5-11.

Mean concentrations of free fatty acids (both per gram of sample) that changed significantly for low (L), medium (M), and high (H) pH groups before and after titration with hydrochloric acid.

pH group	Free fatty acid ($\mu g\ g^{-1}$)		
	Before titration	After titration	After cooking
F14:0			
L	26.9^{a}	26.9^{a}	-
M	28.5^{ab}	31.8^{ab}	-
H	31.5^{bc}	37.8^{c}	-
F16:1			
L	15.8^{a}	15.8^{a}	-
M	16.4^{a}	18.3^{ac}	-
H	19.3^{bc}	21.7^{c}	-
F18:1			
L	375^{a}	375^{a}	-
M	374^{ab}	406^{ab}	-
H	427^{b}	491^{c}	-
F18:4			
L	14.9^{a}	14.9^{a}	-
M	16.4^{ab}	18.1^{ab}	-
H	19.7^{bc}	23.4^{c}	-
F19:0			
L	1.23^{a}	1.23^{a}	-
M	1.81^{bc}	1.73^{bc}	-
H	1.77^{c}	1.66^{bc}	
F20:1			
L	1.13^{a}	1.13^{a}	-
M	1.45^{b}	1.42^{b}	-
H	2.39^{c}	2.14^{c}	-
F20:2			
L	3.01^{a}	3.01^{a}	-
M	3.30^{ab}	3.40^{a}	-
H	4.28^{c}	4.26^{c}	-
F20:5			
L	20.7^{a}	20.7^{a}	-
M	16.4^{b}	21.3^{ac}	-
H	20.3^{a}	25.4^{d}	-
F24:1			
L	6.37^{ab}	6.37^{ab}	-
M	5.81^{ab}	6.85^{b}	-
H	5.78^{a}	7.90^{c}	

Values in the same line or column for each analyte with different superscripts differ significantly ($p < 0.05$)

When expressed per weight of extracted fat, total free fatty acid concentration was unaffected by meat ultimate pH (Table 5-12). After titration with HCl there was a significant ($P < 0.05$) increase in total free fatty acids content of the high-pH group only. Again this effect might be due to hydrolysis of some triglyceride caused by the added HCl.

Table 5-12.

Mean concentrations of total free fatty acids (mg g^{-1} fat) that changed significantly for low (L), medium (M), and high (H) pH groups before and after titration with hydrochloric acid.

pH group	Total FFA		
	Before titration	After titration	After cooking
Low	6.57^a	6.57^a	-
Medium	6.39^a	7.26^a	-
High	7.21^a	8.50^b	-

Similarly, when expressed per weight of extracted fat, 6 free fatty acids (5 were unsaturated) increased in concentration ($P < 0.05$ between at least one of the groups) with increasing ultimate pH (Table 5-13).

Titration with HCl increased the amounts of 1 saturated and 4 unsaturated free fatty acids.

The conflicting results of lower levels of some extracted free fatty acids from meat of lower ultimate pH and either increased or unchanged levels for meat adjusted to the same pH with acid, show that once generated in high pH meat, the levels of free fatty acids are unaffected by changes in pH, water holding capacity or protein solubility. Titration with HCl may cause increased hydrolysis of meat lipids, particularly freely available triglycerides, to produce free fatty acids. This might have been a consequence of rapidly mixing the minces in the food processor during titration with acid.

Table 5-13.

Mean concentrations of free fatty acids (μg g $^{-1}$ fat) that changed Significantly for low (L), medium (M), and high (H) pH groups before and after titration with hydrochloric acid.

pH group	Free fatty acid		
	Before titration	After titration	After cooking
C14:0			
L	157^{a}	157^{a}	-
M	166^{a}	191^{ab}	-
H	189^{a}	232^{b}	-
C16:1			
L	91^{a}	91^{a}	-
M	95^{a}	110^{abc}	-
H	115^{b}	133^{c}	-
C18:1			
L	2177^{a}	2177^{a}	-
M	2180^{a}	2440^{ab}	-
H	2560^{b}	3015^{c}	-
C18:4			
L	87^{a}	87^{a}	-
M	96^{a}	110^{ab}	-
H	119^{bc}	144^{c}	-
C19:0			
L	7.3^{a}	7.3^{a}	-
M	10.1^{b}	10.3^{b}	-
H	10.7^{b}	10.2^{b}	
C20:1			
L	6.5^{a}	6.5^{a}	-
M	8.3^{b}	8.4^{ab}	-
H	14^{c}	13^{c}	-
C20:2			
L	17.6^{a}	17.6^{a}	-
M	19.7^{a}	20.2^{a}	-
H	25.8^{b}	26.2^{b}	-
C20:5			
L	121^{ab}	121^{ab}	-
M	96^{a}	130^{b}	-
H	124^{ab}	156^{b}	-
C24:1			
L	37.4^{a}	37.4^{a}	-
M	34.0^{a}	41.5^{ab}	-
H	35.1^{a}	48.0^{b}	
			-

Values in the same line or column for each analyte with different superscripts differ significantly ($p < 0.05$)

The reason for increased levels of some free fatty acids with increasing meat ultimate pH (ie before titration) is more challenging to explain. Free fatty acids in meat are derived from triglycerides and phospholipids by enzyme hydrolysis or by thermal induced hydrolysis or oxidation during cooking. Fatty acid residues that are esterified to the glyceryl backbone of the phosphatide molecule can be liberated by the hydrolytic enzymes phospholipase A_1 and phospholipase A_2. Both enzymes can be generally classified into soluble and membrane bound forms. Soluble phospholipases have been isolated from lysosomes and usually have an acidic pH optima, whereas, the membrane bound forms require the presence of calcium ions for optimal activity at a neutral pH. Activity of these enzymes may help explain increases in some of the unsaturated free fatty acids found in meat of higher ultimate pH. It is possible that at high pH, the neutral proteases more readily degrade polyunsaturated rich phospholipids associated close to the membranes. At lower pH the soluble lysosomal phospholipases may be relatively less active because they are contained within the lysosomes and it is not until heat induced rupture of lysomal membranes during the cooking process do they become active enough to produce the abundance of fatty acid precursors for flavour development.

5.4 A Model to Explain the Ultimate pH Related Changes in Cooked Meat Odour and Flavour

The results of studies presented in chapters 4 and 5 show that the ultimate pH of meat has a marked effect on odour and flavour of cooked meat, as assessed by sensory panels and instrumental analysis. Lowering the pH of high ultimate pH meat with added hydrochloric acid to levels comparable to that of low, more desirable, ultimate pH meat generated meat of comparable odour and flavour, as assessed by sensory panellists. GC/MS analysis of the volatiles from the titrated meat showed that the majority of compounds found to decrease in concentration with increasing ultimate pH (before titration) did not decrease in concentration after the high pH minces were titrated with acid and subsequently cooked.

Analysis of potential flavour precursors such as soluble protein, free fatty acids and free amino acids, showed changes in concentration related to meat ultimate pH that could be

explained by changes in water holding capacity or protein solubility associated with the extraction process. Some increase in unsaturated free fatty acids at higher ultimate pH may be explained by minor alkaline phospholipase action.

From these results I propose that the continuous evolution of odours and flavours during cooking is more dependent on the dynamic conversion of precursors and reactants from the large supply available in the gross structure of meat than on the relatively low levels of free amino acids and free fatty acids available before cooking. Macy *et al,* (1964) showed that a solution of a diffusate powder from a lean beef water extract developed a meaty flavour upon heating but nearly all amino acids, and the histidine dipeptides, anserine and carnosine which made up 60% of the diffusate powder, showed considerable loss. Yet, the same authors (Macy *et al.,* 1970) found a 38% increase in total free amino acids produced from cooking whole meat. The results of this study support this finding - free amino acid concentrations either increased or remained the same after cooking. This indicates participation of meat fibre proteins in flavour formation, providing a far greater supply of precursors and reactants than water-soluble free amino acids and peptides.

Proteolytic enzymes such as calpains, cathepsins and other lysomal enzymes are implicated in the tenderization of meat during ageing (Koohmaraie *et al.,* 1988; Ouali, 1990). Spanier *et al.* (1990) showed that proteolytic enzymes are still active during the cooking process. Cathepsin B (an exopeptidase, pH optima 5.5) and L (an endopeptidase, pH optima 6.2) still maintained 21 % of their raw meat activity when meat was cooked to an end-point temperature of 70°C. These thiol proteinases, and others such as arylamidase and cathepsin C, have low pH optima and would assist in the production of amino acid and peptide precursors at lower pH values compared with high ultimate pH meat both before, but perhaps more importantly, during the cooking process (Asghar and Bhatti, 1987).

The calpain neutral proteinases exhibit a pH optima of 7 *in vitro*, but may still play a significant role in flavour precursor development *in vivo*. An inhibitor, calpastatin, binds

to calpains forming a stable inactive complex at pH 7 but dissociates and becomes ineffective at pH 5.8 (Dransfield, 1993). However, calpains' *in vivo* activity at temperatures above 45 to 50°C is not known so their involvement in heat induced production of flavour precursors needs investigation.

Spanier *et al.* (1990) showed that enzymes are also redistributed to new intracellular locations during aging, so the possibility exists that similar changes in enzyme location may be bought about by direct influence of pH on membrane integrity and alteration of myofibrilar structure to make proteins more accessible to proteinases during the heating process.

Alteration of myofibrilar structure by low pH to make proteins more accessible to proteinases was proposed by Buscailhon *et al.* (1994) to explain interaction between pH and time on the content of non protein nitrogen in dry-cures hams. Further, Young *et al.* (1992) showed that onset of gelation of myofibrillar fractions was induced at lower temperatures as pH declined. Myofibrillar gelation at pH 5.5 was induced at 45°C whereas at pH values >6.0 gelation required temperatures close to 60°C. A decrease in negatively charged groups (COO^{-}) of muscle proteins occurs most when meat is cooked close to its isoelectric point (pH 5.5) but is negligible in meat at high (6.8) pH (Hamm and Deatherage, 1960). This decrease in carboxyl groups is thought to involve ester linkages with hydroxy groups of serine, threonine or tyrosine rather than decarboxylation.

Phospholipids play a major role in the supply of lipid oxidation products for the Maillard reaction and flavour development by virtue of their high level of unsaturated fatty acids and close association with structural proteins as an integral part of cell membranes (Owen *et al.*, 1975). Mottram and Edwards (1983) showed that removal of intramuscular triglycerides and phospholipid by chloroform/methanol extraction significantly reduced the meaty aroma and volatile compounds of the cooked meat residue compared to meat selectively removed of triglycerides only or to unextracted meat. Further work with model mixtures containing cysteine and ribose, with and

without phospholipids and triglyceride added, confirmed the involvement of phospholipids with Maillard reactants in the development of flavour volatiles. An interesting outcome was that many Maillard reaction products showed marked reductions on the addition of lipid. The type of compounds reduced were dependent on the type of lipid used. (Farmer and Mottram, 1990).

Oxidative rancidity can also be pH dependent. Phospholipids, because of their high proportion of polyenoic acids and close association with strong potential pro-oxidant catalysts (the metal-complexed globulins), which reside in and near cellular membrane structures, are the major contributors to oxidative rancidity. Owen *et al.* (1975) observed a higher degree of oxidative rancidity in porcine muscle at low- compared with high-pH meat. This effect was attributed to the potentially pro-oxidant muscle pigments, myoglobin and oxymyoglobin, being stabilized at high pH by removal of oxygen by enzyme reducing systems such as succinic dehydrogenase and cytochrome oxidase. In addition, oxymyoglobin is more stable at its isoelectric point of pH 6.8 than at lower pH. As pH decreases, the heme catalysis of lipid oxidation will also make available important precursors for flavour development. Thus, in meat of high ultimate pH heat enhanced lipid oxidation will play a less prominant role in generation of flavour precursors than meat of low ultimate pH.

Internal cooking temperatures of high ultimate pH meat is 3 to 4°C higher than meat of a lower pH (Lewis *et al.,* 1967) and the rate of heating of high pH meat can be up to twice that of low pH meat (Sayre *et al*, 1964). These temperature effects are thought to be due to differences in meat water holding capacity. Lower pH meat has greater cooking loss than high pH meat and therefore would tend to decrease the surface and internal temperature by evaporative cooling. This offers the possibility that proteinases and phospholipases are heat-denatured sooner at the higher pH when cooked. This would result in lower levels of flavour and odour precursors being produced in higher ultimate pH meat.

Also, there is an increase in osmotic pressure from pre- to post-rigor state. Ouali, (1990) found that as muscle pH fell, osmotic pressure increased exponentially and reached its maximum value at completion of rigor. This change was highly correlated (r = 0.97) with pH. The gradual decrease in osmotic pressure observed thereafter was accounted for by the gradual increase in meat pH seen during ageing. Change in osmotic pressure equates to changes in ionic strength that dissociates contractile proteins and consequently alters the structure of myofibrils (Ouali, 1984) making them more susceptible to proteolytic attack (Wu, 1987). Thus, myofibrils could be more stable at higher ultimate pH because of the lower osmotic pressure.

Another possible cause of the differences in odour and flavour intensities at higher meat pH is the evolution of H_2S during cooking. Johnson and Vickery (1964) showed increased amounts of H_2S was produced from meat of higher pH. This was directly related to the pH of meat, irrespective of whether the pH increase was varied artificially with acid or alkali or biologically by starvation (glycogen depletion). Such high levels of liberated H_2S might influence flavour and odour generation during cooking. Some evidence of this was provided by van den Ouweland *et al.* (1989) who showed formation of a number of heterocyclic compounds, present in meat volatiles, when reacting H_2S with unsaturated aldehydes in aqueous solutions. Polyunsaturated fatty acids and their thermal degradation products also compete with other components for Strecker degradation-derived H_2S (Farmer and Mottram, 1990).

5.5 Summary

In summary, it is proposed that the ultimate pH related changes to sheepmeat odour and flavour development occur mainly at time of cooking due to pH and heat initiated disruption of myofibrillar protein structure and lysomal membranes, activation of acidic proteinase and phospholipase enzymes, and heat induced autoxidation of lipid, principally polyenoic rich- phospholipids. At this stage it is not known which of the above mentioned mechanisms plays the dominant role, but might collectively work in the same direction to produce more flavourful meat at lower ultimate pH during cooking. Biochemical changes that occur in raw high ultimate pH meat that cause flavour and odour changes in cooked meat could be overcome by titration to lower, more desirable pH, with acid.

Chapter 6.

Effects of CO_2 Packaging on Sheepmeat Odour and Flavour

6.1 Introduction

Although a very important issue, meat ultimate pH is one of a number of meat properties of interest to the New Zealand meat industry. New Zealand's geographic position remote from Northern Hemisphere markets is an extra challenge that the meat industry has had to address to remain internationally competitive. Traditionally, New Zealand has exported most of its sheepmeat as frozen product. However, in a drive to market table-ready cuts of beef and lamb, a carbon dioxide controlled atmosphere packaging system (CAP) was developed so raw product can be shipped chilled (-1.5°C) and still maintain a hygienic storage life of at least 16 weeks.

In a brief report, Gill (1988a) noted that the use of a carbon dioxide controlled atmosphere for chilled meat storage "stripped" ovine flavour from sheepmeat and "gamey" flavour from venison. He also commented that with prolonged storage, red meat develops "livery" flavours due to peptides formed during the hydrolysis of proteins. Jeremiah *et al.* (1992) found an unidentifiable "off" aromatic in pork stored for between 6 and 24 weeks in CO_2 at -1.5°C. A livery aromatic was also perceived at 12 and 15 weeks, but was not detected at later times.

These observations support anecdotal comments from export markets that New Zealand chilled meat packaged in a CO_2 atmosphere has less desirable flavour and odour attributes than fresh product.

As an adjunct to the meat ultimate pH work (chapters 4 and 5), carbon dioxide controlled atmosphere packaging of lamb legs was studied to investigate the changes in cooked meat odour and flavour that may occur during long term chilled storage in a carbon dioxide atmosphere.

This chapter discusses the results of a preliminary study comparing the cooked odour and flavour of minces from lamb legs stored chilled (-1.5°C) in a CO_2 atmosphere or stored frozen (-35°C) in vacuum packs. Sensory panels evaluated the cooked minces for various odour and flavour attributes after 4, 8 and 14 weeks storage.

In addition to the conventional panel assessment, samples of both raw and cooked minces (at 14 weeks) were also evaluated using a new technology, called a volatile chemical analyser or olfactometer - more commonly called an electronic nose - that in many ways functions in a way closely analogous to the sense of smell. This technology might be useful for providing a better understanding flavour and odour changes in foods, giving better consistency than sensory panels and as a reliable instrument for product quality control.

Brief review of electronic nose technology

Research over the past 10 years has produced working models of a volatile chemical analyser or olfactometer (Dodd *et al.*, 1991). These combine gas sensor arrays, and various pattern-recognition techniques to map odours.

The human nose can be trained to differentiate between about 10,000 aromas covering a wide range molecular sizes, structures and concentrations. Volatile compounds, not all of which are odorous, interact with about 10 million olfactory receptors imbedded in the nasal membranes that are connected to the olfactory bulb in the brain via a nerve pathway. The molecular characteristics of a specific volatile compound will determine the type of response induced. Thus, it can be extremely sensitive to very low concentration of some chemicals but insensitive to others. Interpretation of the information supplied to the brain relies on training and past experience.

The electronic nose functions in a way that emulates the human nose. The sensing system consists of an array of chemical polymer or metal oxide sensors that interact with volatile chemicals that are passed over their surfaces to produce a change in electrical resistance that is measured and recorded by a computer. The differential responses that the array

produces form a "fingerprint" of the volatile chemicals. These "fingerprint" patterns are processed by pattern-recognition computer algorithms, such as multivariate statistical analysis and neural networks. Processed data are usually output as two dimension maps that allow interpretation of differences in aroma response the electronic nose may have measured between samples.

Like the human sense of smell, the electronic nose cannot identify individual odour components within a mixture or accurately measure the concentration. All odorous compounds are volatile by nature yet not all volatile compounds are odorous. Variations exist in the sensitivity of the human nose to detect certain odours, and there are similar variations in sensitivity and selectivity between the human nose and the electronic nose. Not all compounds detected by the human nose as odorous are similarly detected by the electronic nose. Conversely, some compounds detected by the electronic nose are insensitive to the human sense of smell.

There are a number of emerging and competing sensor technologies but I will briefly review only the two technologies that are currently commercially available, chemical polymer sensors and metal oxide sensors.

Chemical polymers, such as polypyrole, are electrolytically deposited across the gap between two thin gold electrodes on a standard electronic substrate (figure 6-1). The conductivity of the sensor is responsive to the presence or absence of substituents on the pyrrole rings and to their electron-donating or withdrawing character. Sensing depends on a change in conductance of the conductor on interaction with the analyte. These reversible interactions occur at room temperature.

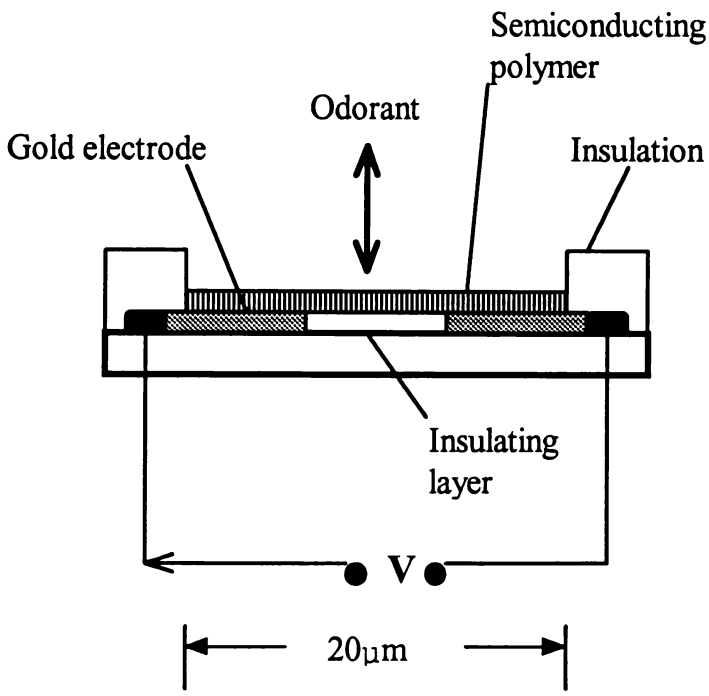
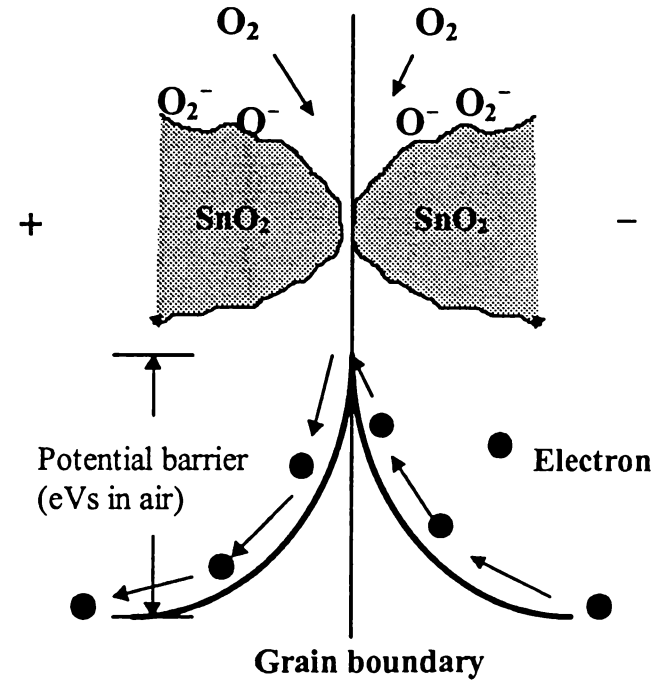


Figure 6-1.
Typical design of an odourant-sensing device based on an electrodeposited conducting polymer film (Dodd *et al.*, 1991).

Metal oxides, such as tin oxide (SnO_2), are likewise semiconducting materials and can be odorant-sensitive. When the sensor is heated to a high temperature (400+°C) in the absence of oxygen, free electrons flow easily through the grain boundaries of the SnO_2 particles. In air, oxygen is absorbed onto the tin dioxide particle surface and traps free electrons due to oxygen's intrinsic electron affinity. Charged oxygen species (O_2^- or O^-) form a resistance at grain boundaries of the metal oxide by binding electrons. This potential barrier, measured in electron volts (eV), restricts the flow of electrons, causing the electric resistance to increase (figure 6-2a).

(a) In presence of clean air



(b) In presence of an odorant.

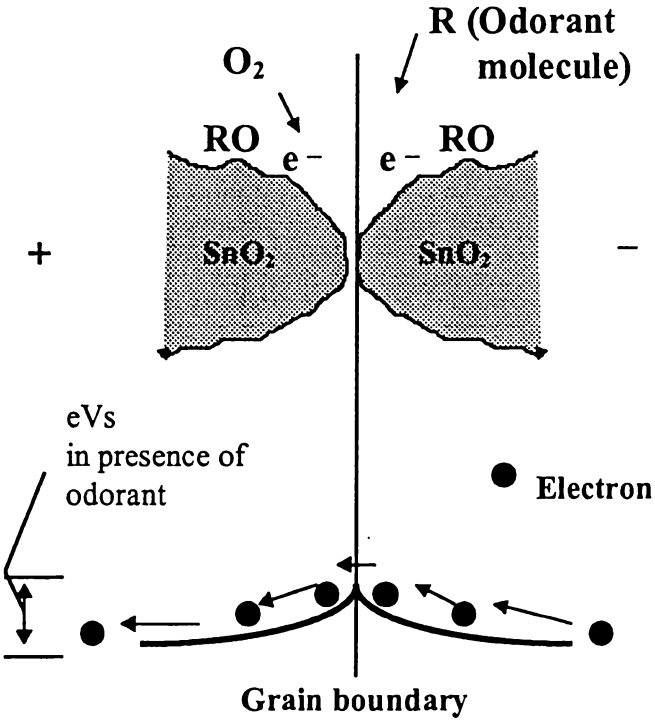


Figure 6-2.

Schematic of the changes in the potential barrier at the grain boundary of a tin oxide sensor (a) in the presence of clean air and (b) in the presence of an odorant.

Gas-phase odorant molecules (R) react irreversibly with the chemisorbed oxygen (O^-), to produce combined molecules (RO) and liberated electrons (e^-). These electrons lower the potential barrier between oxide grains and allow current to flow more easily. (figure 6-2b). The result is a reduction in the electrical resistance. The reaction between gases and surface oxygen species at the surface differs depending on the sensor element's temperature and activity of the sensor material. Metal oxides can also be 'doped' with noble metals such as Pd, Pt, or Rh that induce differences in the electronic responses of the oxides (Gopel *et al.*, 1995). By judicious choice of an array of sensors, differential responses to volatile compounds can be obtained.

Whatever the nature of the sensor, the changes in resistance are recorded for each sensor response and a "fingerprint" pattern is generated to give a visual display of differences between samples. However, this raw pattern is often of only limited use, as many differences in sensor responses between samples cannot be easily discerned. Multivariate statistical analysis or artificial neural network analysis of sensor array data is required for reliable interpretation. The basic design of an electronic nose is drawn below (figure 6-3).

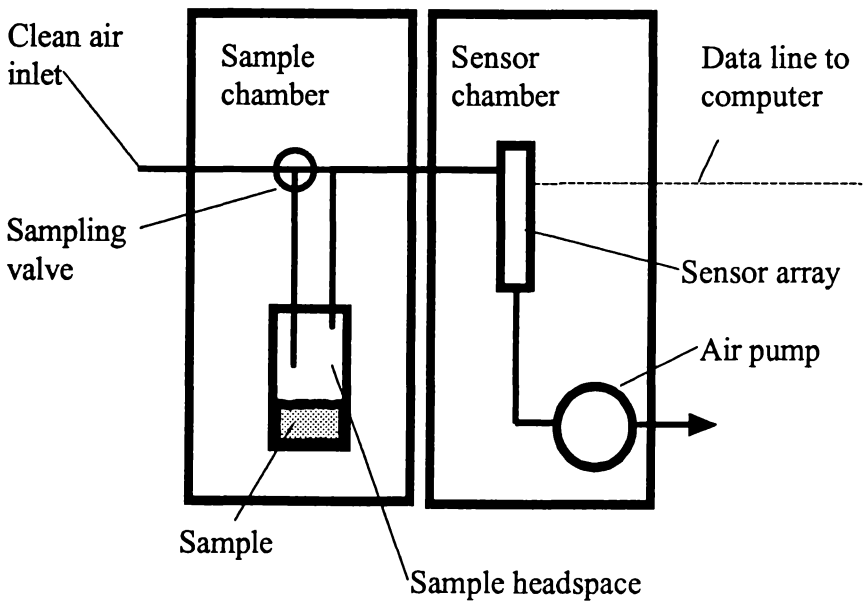


Figure 6-3.

Basic scheme of an electronic nose. Initially a steady state equilibrium of sensor responses is attained with clean air. Then by redirecting the clean air inlet flow, the equilibrated headspace above the sample is swept across the sensor surfaces. The change in sensor responses is recorded and further processed by the computer.

6.2 Materials and Methods

Animals

Carcasses from 40 Coopworth lambs from one flock were used. The animals, weighing on average 35 kg (range 29 to 44 kg), were head-only stunned, then conventionally slaughtered by throat cut. Carcasses, which were not electrically stimulated, were held at between approximately 4 and 6°C for 6 hours followed by 22 hours at 3°C.

Sample preparation

Both legs of each carcass were removed and the ultimate pH of each *semimembranosus* muscle was measured using a pH probe. Twenty-seven pairs of legs with ultimate pH values ranging from 5.66 to 5.92 (mean = 5.77 , SD = 0.08) were randomly sorted into three replicate groups of nine. Left and right legs in each of the groups were then divided into two subgroups for the two packaging treatments. Each subgroup had similar numbers of left and right legs (9 all told) with the contralateral leg in the other subgroup.

Legs of one subgroup (from each of the three replicate groups) were packed in aluminum foil-lined gas impermeable polypropylene bags containing 1.5 L of CO_2 (containing less than 500 ppb oxygen) per kg of meat, and stored at -1.5°C. The contralateral legs were then placed in the same type of bag, vacuum-packed, and stored at -35°C.

Microbial assessment

At chosen times packaged legs from each storage treatment were removed from storage. Before analysis, the frozen legs were tempered in a +10°C room. Each bag was then opened and a 5 cm^2 area of the meat surface was swabbed for determination of aerobic plate count. The meat was also visually assessed for any deterioration.

Sensory analysis

For sensory analysis, each leg was deboned at +10°C and the entire muscle and fat content diced, then minced by two passes through a 3-mm plate mincer. A 50 g subsample of each mince was wrapped in aluminium foil, vacuum-packed in foil-laminated oxygen impermeable plastic bags, and immediately stored at -80°C for later metabolite tests.

To minimize lipid oxidation, samples were prepared less than 1 hour before cooking and kept at 4°C in sealed (not vacuum-packed) plastic bags with a stated oxygen transmission rate of 30 mL m^{-2} 24 hr^{-1} at 1 atm, 23°C, and 75% relative humidity.

Procedures for sensory evaluation were as described previously (chapter 4), except that evaluation at each storage time was done as consecutive daily sessions over 3 days. This procedure tested three left and right pairs of legs on each of the three days.

Twelve panellists were asked to score for overall odour and sheepmeat odour as well as for other defined odour descriptors listed in the score sheet presented to the panellists (figure 6-4). They were then asked to score for overall flavour and sheepmeat flavour as well as for those flavour descriptors listed in the score sheet in Figure 1. All attributes were scored on a scale of 0 to 9 where 0 = no odour or flavour and 9 = intense.

pH measurement

As previously described, pH before storage was measured on the *semimembranosus*.

After storage, the pH of each sample was measured immediately before sensory analysis by homogenizing duplicate 1-g samples of leg mince in 10 mL of distilled water. For these pH measurements, the measurement was done about 2 hours after the deboned leg muscle had been minced, to allow time for dissolved CO_2 to evolve from the minced meat and not affect the true meat pH.

Evaluation of Sheepmeat

Panellist Number: _____ Booth Number:_____

Date: _____ am/pm

Rate each sample for the attributes listed below using the scale of 0 (absent) to 9 (intense)

Sample Number	1		2		3		4		5		6	
	Odour	Flavour	Odour	Odour	Odour	Flavour	Odour	Flavour	Odour	Flavour	Odour	Flavour
Overall												
Sheepmeat												
Foreign/Other:	-	-	-	-	-	-	-	-	-	-	-	-
Sweet												
Sour												
Bitter												
Metallic												
Roasty												
Beefy/meaty												
Livery/offaly												
Stale/musty												
Rancid												
Other												

Comments

Figure 6-4.

Sensory panellist score sheet used to evaluate odour and flavour descriptors for cooked lamb leg mince.

Electronic nose analysis

The headspace gas of selected samples of raw and cooked minces from chilled-CO_2 packed and frozen-vacuum packed meat were tested using a FOX 4000 electronic nose (ALPHA M.O.S. SA, Toulouse, France). Initially, the array of 18 sensors was equilibrated with humidified instrument grade air. Ten gram samples of minced meat were spread evenly over the bottom of a 20 mm x 20 mm, 120 mL glass jar and tightly sealed with teflon-lined sampling cap (figure 6-3). The jar was then purged with humidified instrument grade air and incubated for 10 minutes at 30°C. The equilibrated headspace above the sample was dynamically purged with instrument grade humidified air across the surface of three chambers of six sensors at a flow rate of 150 mL min^{-1}. Sampling of each headspace was for three minutes with an inter-sample cleaning of the sensors with instrument grade air for 10 minutes.

Changes in resistence of each sensor were recorded and processed as the change in resistence (ΔR) divided by the resistence of each sensor measured at time zero (R_0), using the proprietory software supplied with the instrument.

Statistical analysis

Sensory panellists' data contained a large number of zero scores, and preliminary analysis showed animal variability was far less than panellist variability for attributes other than overall and sheepmeat odour and flavour. Therefore, in these instances the data were summarized over animals for panellist x storage treatment, and subsequently analysed in three ways, as follows:

- ANOVA of slopes over time for each storage treatment using panellists as replicates to test for differences between storage treatments in terms of any linear change with time (i.e. interaction between storage method and storage time).
- Average of slopes, over storage treatments, for each panellist. Then a *t* -test was done of the mean slope over panellist vs zero to test for the main effect of time.
- Average of the panellist score over all times for each storage method. Then ANOVA using panellists as replicates to test for an interaction between storage treatments.

Overall and sheepmeat odour and flavour sensory data, for which there were no zero data, were analysed by REML (Genstat), which allows for fixed and random effects. Aerobic plate count and pH data were analysed by standard analysis of variance (ANOVA) (Genstat).

Electronic nose data was evaluated by Principle Component and Canonical Discriminant Analysis using Unistat Statistical software (version 3.0a, Unistat, London, England).

6.3 Results and Discussion

Meat pH

A surprising result was the significant difference between initial pH (before storage) and the pH measured after storage (figure 6-5). With frozen storage the increase (0.13 pH units) may have been due to the freeze/thaw cycle, in which case such a change has not been reported before. A more likely explanation is that the initial pH was measured on the *semimembranosus* muscle whereas the post-storage pH measurements were done on whole-leg minces containing many muscles. Thus, the difference could be explained by inter-muscle variability in pH.

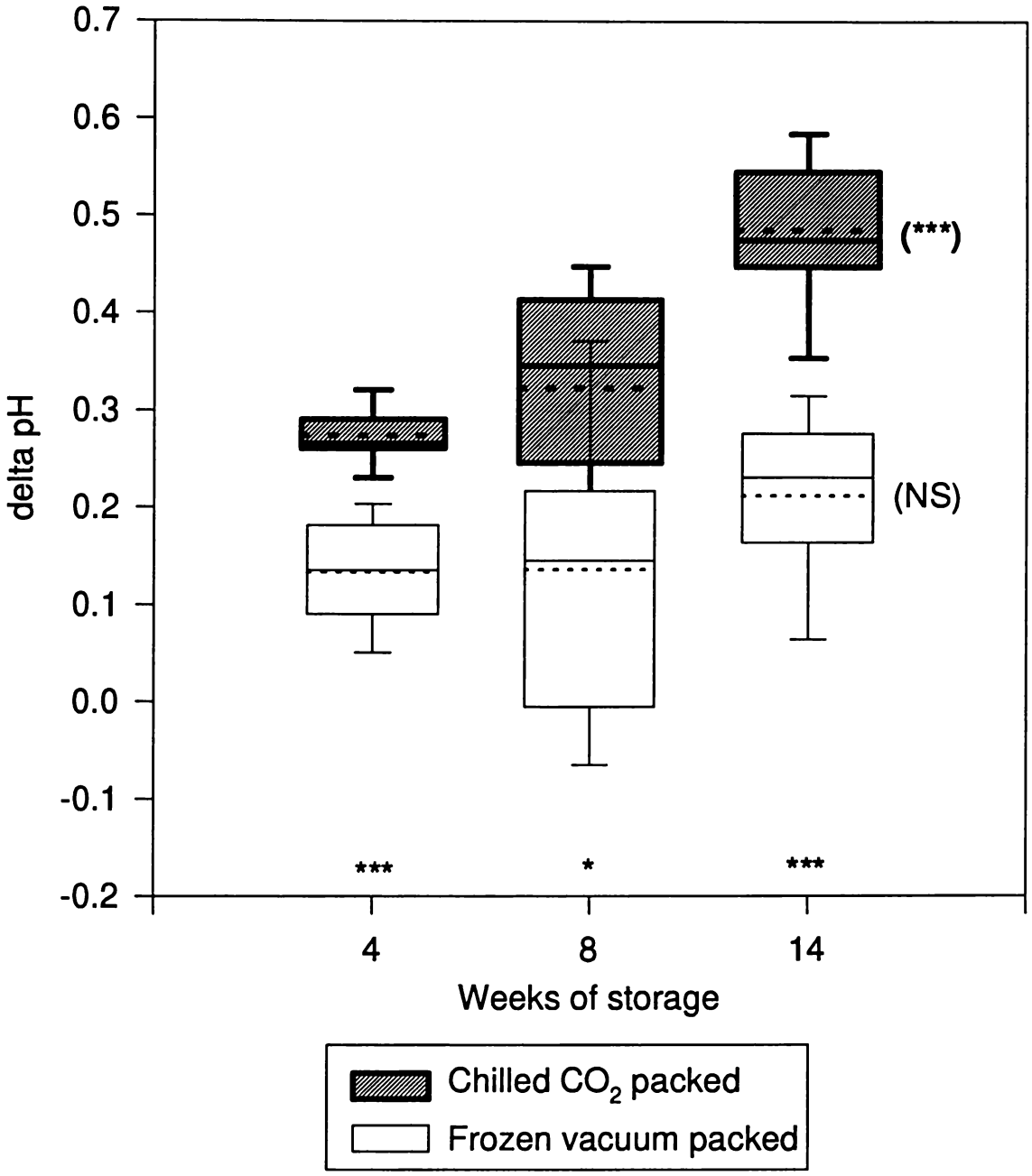
The change in meat pH (ΔpH: pH at storage time - initial pH before storage) increased with storage time for meat stored in a CO_2 atmosphere at -1.5°C ($P<0.001$) but not for frozen-stored meat. The greatest change (mean Δ pH = 0.48) occurred after 14 weeks of chilled storage (figure 6-5). There were also significant differences in Δ pH between the storage treatments at 4 ($P < 0.001$), 8 ($P < 0.05$) and 14 ($P < 0.001$) weeks storage.

This pH effect was not caused by microbial spoilage as there was no significant difference ($P > 0.05$) in aerobic plate counts between storage treatments at each of the storage times. However, there was an increase ($P < 0.01$) in aerobic plate counts with time for the CO_2 chill pack meat (figure 6-6). An aerobic plate count of 4.0 CFU cm^2 (log_{10}) is not considered high enough to have any effect on meat pH or cooked meat flavour and odour (Gill *et al.*, 1979).

Parrish *et al.* (1969) observed an increase in meat pH during aging of chilled (2°C) vacuum-packed beef after 28 days storage. Boakye and Mittal (1993) also observed a pH increase in unpacked and vacuum-packed beef after 16 days at 2°C, with vacuum-packed samples having a greater pH change than unpackaged. A comparison of lamb shoulders vacuum-packed or packed in modified atmospheres (80% O_2/20% CO_2; 50% CO_2/50% N_2; and 100% CO_2) showed a significant increase in pH over 28 days of storage only in the latter two storage atmospheres (Doherty *et al.*, 1996). Moore and Gill (1987) compared vacuum and CO_2-packed lamb stored for 16 weeks at -1.5°C and observed a similar significant increase in meat pH over time for both storage treatments. They also noted a relationship between increasing pH and declining display colour stability. They believed that the pH increase was due to an increase in basicity as a result of tissue breakdown (presumably decarboxylation) rather than a consumption of lactic acid or other acid metabolites.

It is believed that meat pH should fall when stored in a CO_2 atmosphere because of the formation of carbonic acid (Gill, 1988b). Some evidence of this was provided by Ledward (1970), who measured changes in the surface pH of samples upon removal of meat from the packaging after 14 days storage at 0°C under various CO_2 pressures. After the meat was removed from the packaging the surface pH increased, and this increase was greatest (0.12 pH units) with the greatest CO_2 storage pressure. Internal pH levels were not measured in Ledward's study, so the depth of CO_2 penetration is not known.

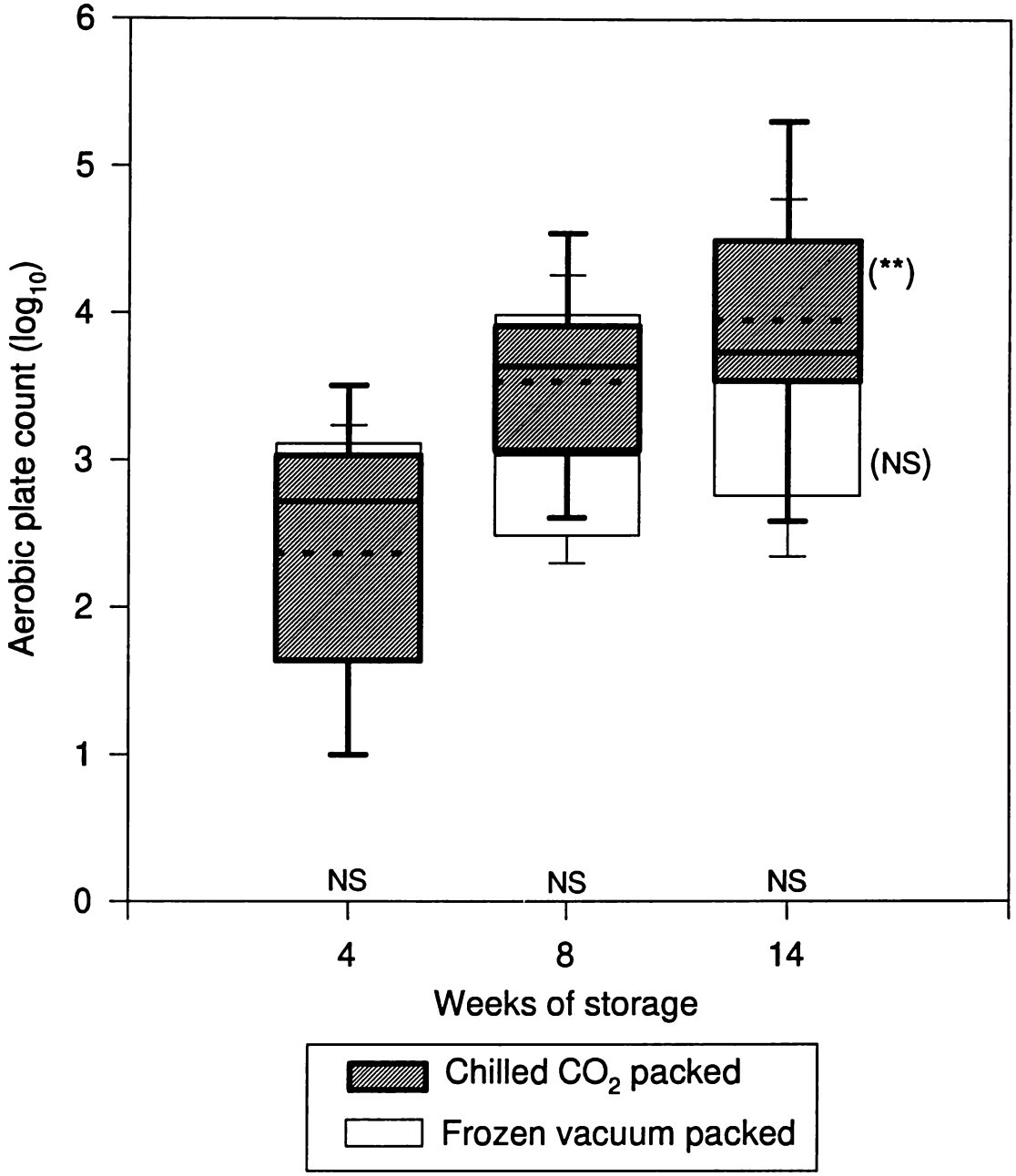
In the present study no attempt was made to determine the variability of pH with muscle depth. Such a determination would be appropriate in future experiments. In any event, once the carbon dioxide has evolved from meat after exposure to a normal atmosphere (because of an increase in meat temperature and a decrease in pCO_2), it will no longer affect the final pH of the meat before cooking. The relationships between the observed increase in meat pH of CO_2-packed meat over time, the storage temperature, the storage atmosphere, the decrease in pH due to dissolved CO_2, and the buffering capacity of meat need further investigation.



The horozontal lines mark the 10th, 25th, 50th, 75th and 90th percentiles.
Dashed lines represent data means.
Asterisks at the bottom of the graph at each storage time represent the significance of the difference between each treatment at each storage time.
Asterisks in parenthesis to the right of the graph represent the time effect for eact storage treatment.
In addition, the overall treatment effect (summed over time) was $P < 0.001$.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS $P > 0.05$.

Figure 6-5.

Box plot of the change in meat pH ($pH_{initial}$ - pH_{final}) for CO_2-packed meat stored at minus 1.5°C and vacuum-packed meat stored at minus 35°C, for 4, 8 and 14 weeks.



The horozontal lines mark the 10th, 25th, 50th, 75th and 90th percentiles. Dashed lines represent data means.

Asterisks at the bottom of the graph at each storage time represent the significance of the difference between each treatment at each storage time.

Asterisks in parenthesis to the right of the graph represent the time effect for eact storage treatment.

In addition, the overall treatment effect (summed over time) was $P < 0.01$.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, $P > 0.05$.

Figure 6-6.

Box plot of the change in aerobic plate count (CFU cm^2) for CO_2-packed meat stored at minus 1.5°C and vacuum-packed meat stored at minus 35°C, for 4, 8 and 14 weeks.

Changes in sensory attributes during storage

Because of limited resources, a fresh meat control could not be included in this experiment, so changes in odour and flavour over the first 4 weeks of storage in CO_2 atmosphere at -1.5°C could not be studied. Rather, vacuum-packed contralateral lamb legs stored at -35°C acted as the control group.

Sensory analysis of cooked odour attributes showed that there was a significant decrease ($P < 0.05$) in overall, sheepmeat and sweet odour attributes over time (Table 6-1). A livery/offaly odour was also significantly higher in the CO_2-packed legs stored at -1.5°C compared with the vacuum-packed lamb legs stored at -35°C for the same period. The development of a livery note was also found in CO_2-packed, chilled pork (Jeremiah *et al.*, 1992).

Sheepmeat flavour tended to decrease over storage time but only at the 10% level of significance (Table 6-2), a finding that warrants further investigation with more replicates over time. The type of storage had a significant ($P < 0.05$) effect on "sweet" and "roasty" flavours, with chilled CO_2-packed meat being less "sweet" and "roasty" than the frozen control.

The most significant change occurred with a livery/offaly flavour. There was a significant storage effect ($P < 0.01$) with the average score for this attribute for the chilled CO_2-packed meat being more than double that of the frozen control. There was also a significant storage x time interaction ($P < 0.05$) for this attribute, indicating different trends with time between the two storage treatments.

As mentioned in Section 6-2, many of the panellists could not detect some of the attributes in many of the samples, as indicated by 0 scores, yet these data points were included in the standard statistical analysis (Tables 6-1 and 6-2). The inclusion of these data points has helped mask the significance of some of the changes that may have occurred. For example, variation in sensitivity toward a particular attribute can occur between panellists. Therefore, the livery/offaly attribute data were analysed in greater depth.

Table 6-1.

Mean panel scores for odour attributes of chilled-CO_2 packed and frozen (-35°C)-vacuum packed lamb legs stored for 4, 8 and 14 weeks.

Attribute		Weeks of storage				Significance		
		4	8	14	Storage type mean	Linear time effect	Storage effect	Storage x linear time
Overall odour	Vac	5.90	5.27	5.16	5.44	*		
	CO_2	5.88	5.10	5.12	5.37			
Sheepmeat odour	Vac	4.48	4.20	4.00	4.23	*		
	CO_2	4.58	4.15	3.54	4.09			
Sweet odour	Vac	1.89	1.76	1.48	1.71	*		
	CO_2	1.57	1.67	1.15	1.46			
Sour odour	Vac	0.22	0.16	0.37	0.25			
	CO_2	0.34	0.37	0.74	0.48			
Bitter odour	Vac	0.27	0.31	0.34	0.31			
	CO_2	0.17	0.43	0.42	0.34			
Metallic odour	Vac	0.66	0.60	0.36	0.54			
	CO_2	0.62	0.61	0.48	0.57			
Roasty odour	Vac	0.55	0.74	0.59	0.62			
	CO_2	0.41	0.58	0.35	0.45			
Beefy/meaty odour	Vac	0.22	0.21	0.17	0.20			
	CO_2	0.16	0.13	0.21	0.17			
Livery/offally odour	Vac	0.58	0.46	0.61	0.55		*	
	CO_2	0.91	0.44	1.00	0.78			
Stale/musty odour	Vac	0.58	0.23	0.63	0.48			
	CO_2	0.53	0.26	0.87	0.55			
Rancid odour	Vac	0.17	0.17	0.32	0.22			
	CO_2	0.18	0.16	0.51	0.28			
Other odour	Vac	0.66	0.63	0.52	0.60			
	CO_2	0.58	0.59	0.66	0.61			

*, significant $P < 0.0$); **, $P < 0.01$

Table 6-2.

Mean panel scores for flavour attributes of chilled-CO_2 packed and frozen (-35°C)-vacuum packed lamb legs stored for 4, 8 and 14 weeks.

Attribute		Weeks of storage				Significance		
		4	8	14	Storage type mean	Linear time effect	Storage effect	Storage x linear time
Overall flavour	Vac	5.56	5.40	5.44	5.46			
	CO_2	5.81	5.27	5.78	5.62			
Sheepmeat flavour	Vac	4.60	4.52	4.27	4.46	†		
	CO_2	4.47	4.19	3.73	4.13			
Sweet flavour	Vac	2.31	2.29	1.97	2.19		*	
	CO_2	1.85	1.89	1.37	1.70			
Sour flavour	Vac	0.66	0.75	0.93	0.78			
	CO_2	0.80	0.84	1.00	0.88			
Bitter flavour	Vac	0.58	0.55	0.76	0.63			
	CO_2	0.72	1.49	1.38	1.20			
Metallic flavour	Vac	0.09	0.10	0.12	0.10			
	CO_2	0.22	0.11	0.51	0.28			
Roasty flavour	Vac	0.64	1.00	0.70	0.78		*	
	CO_2	0.51	0.78	0.45	0.58			
Beefy/meaty flavour	Vac	0.59	0.60	0.61	0.60			
	CO_2	0.28	0.60	0.53	0.47			
Livery/offally flavour	Vac	0.93	0.69	0.76	0.79		**	*
	CO_2	2.0	1.31	2.53	1.94			
Stale/musty flavour	Vac	0.41	0.29	0.75	0.48			
	CO_2	0.65	0.35	0.88	0.62			
Rancid flavour	Vac	0.24	0.21	0.48	0.31			
	CO_2	0.55	0.72	0.99	0.74			
Other flavour	Vac	0.63	0.53	0.56	0.57			
	CO_2	0.61	0.56	0.70	0.62			

†, significant $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$

The frequency of panellists who scored the livery/offaly attribute was averaged for each sample and plotted against weeks of storage for both treatments. Figure 6-7 shows that significantly more panellists ($P < 0.01$ at 4 and 8 weeks; $P < 0.001$ at 14 weeks) detected

the livery/offaly flavour in the CO_2-packed meat compared with the vacuum-packed control. At 14 weeks this represented 66% of panellists detecting this attribute in CO_2-packed meat compared with 33% of panellists for the vacuum-packed meat. There was also an increase ($P < 0.001$) in the frequency of panellists who scored the livery/offaly attribute with time in the CO_2-packed meat only. The average livery/offaly flavour intensity scores from only those panellists who could detect this attribute were significantly ($P < 0.001$) greater for the CO_2-packaged meat after 14 weeks of storage (figure 6-8). In contrast, the average livery/offaly flavour intensity score remained unchanged over the 14 week storage period for the vacuum-packed meat stored at -35°C.

These results suggest that panellists have different flavour thresholds for this particular attribute (and probably other attributes) and help explain the high variability of the overall sensory data. This finding can be extrapolated to the general consumer. Each will be different in his or her ability to detect these flavour changes.

The reason(s) for the flavour changes that develop in chill-stored CO_2-packaged meat have not been fully elucidated. Aging of meat, independent of storage method, has been shown to enhance cooked meat flavours, changes that have been attributed to increases in the concentration of flavour precursors, particularly peptides and free amino acids derived from proteolysis (Coppock and MacLeod, 1977; Nishimura *et al.*,1988; Jeremiah *et al.*, 1991; Farmer, 1992).

In the present study no change in overall flavour or increase in favourable odour and flavour attributes was detected in either packaging treatment from 4 to 14 weeks of storage. Flavour changes could have possibly occurred over the first 4 weeks of storage, but because there was no pre-storage group included in the sensory evaluation part of this study, unfortunately no comparison can be made. Alternatively, increases in desirable odour and flavour notes may have been masked by increases in undesirable notes resulting in no net change in the overall odour and flavour score.

Greater increases in the pH of CO_2-packed meat over vacuum-packed meat stored at similar temperatures have been observed by others (e.g. Doherty *et al.*, 1996). These results and the results of the present study suggest that storage in CO_2 increases the meat pH on aging and might be related to the increase in undesirable livery/offaly odour and flavour and the decrease in sheepmeat odour observed by panellists.

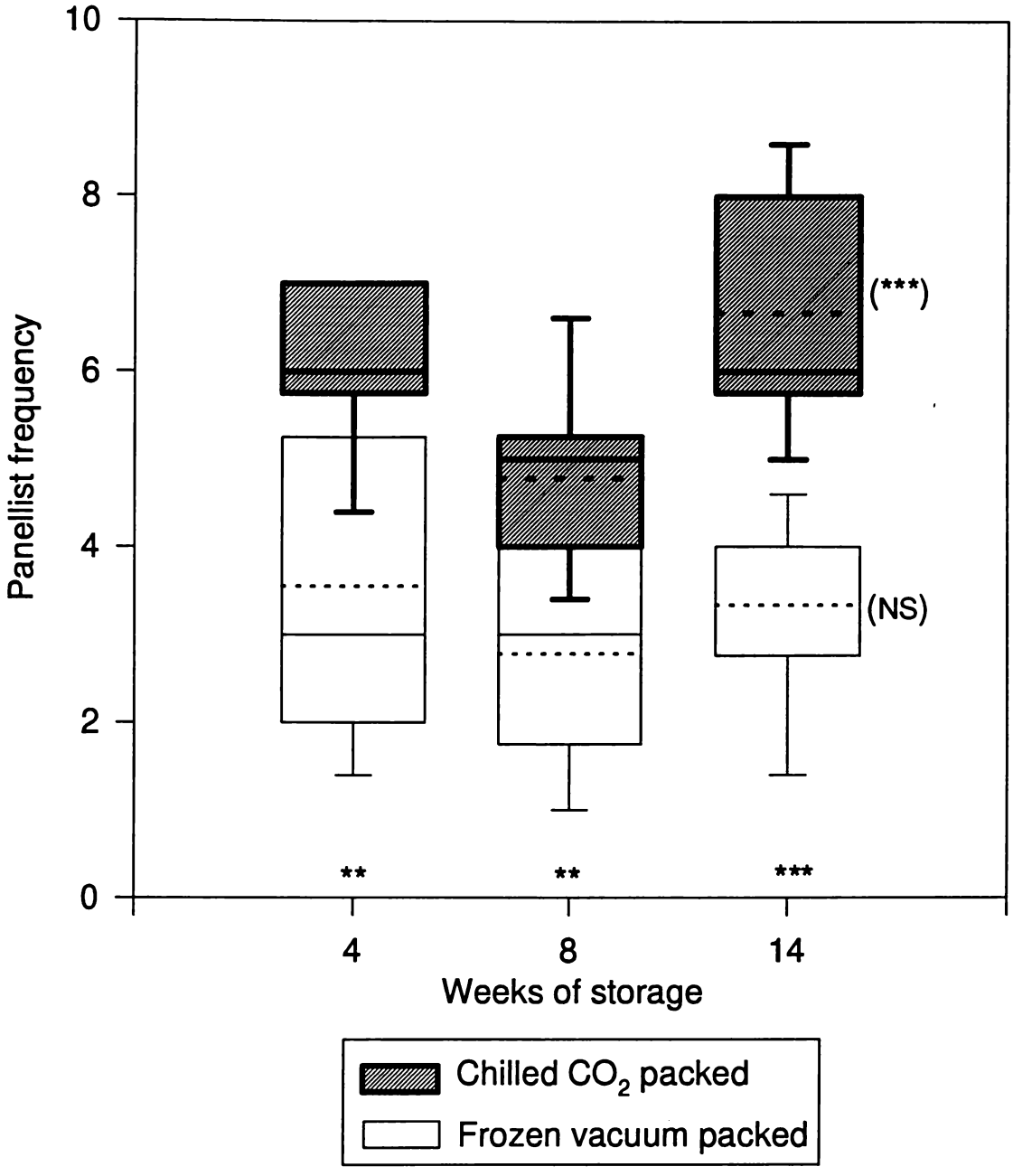
Meat of high (> 6.0) ultimate pH has a decreased overall and sheepmeat odour and flavour compared with meat of low (5.6) ultimate pH (chapter 4). However, the mechanisms involved in the generation of high meat ultimate pH are most certainly different to those responsible for the increases in aged meat pH observed in this study. In addition, the effects that storage-related pH changes have on cooked meat odour and flavour may not be the same as those observed in high ultimate pH meat (decreased odour and flavour at high ultimate pH).

Whatever the cause(s) of flavour changes in high ultimate pH meat and meat stored chilled in CO_2 atmosphere, pH appears to be a common factor.

Of particular interest, and possible concern to the New Zealand meat industry, is the change in meat pH that occurs when meat of initially high ultimate pH is stored over similar periods in a CO_2 atmosphere. Is the increase in meat pH observed during long-term CO_2 storage added to the initially high ultimate pH? What effect will this have on livery/offaly flavour development?

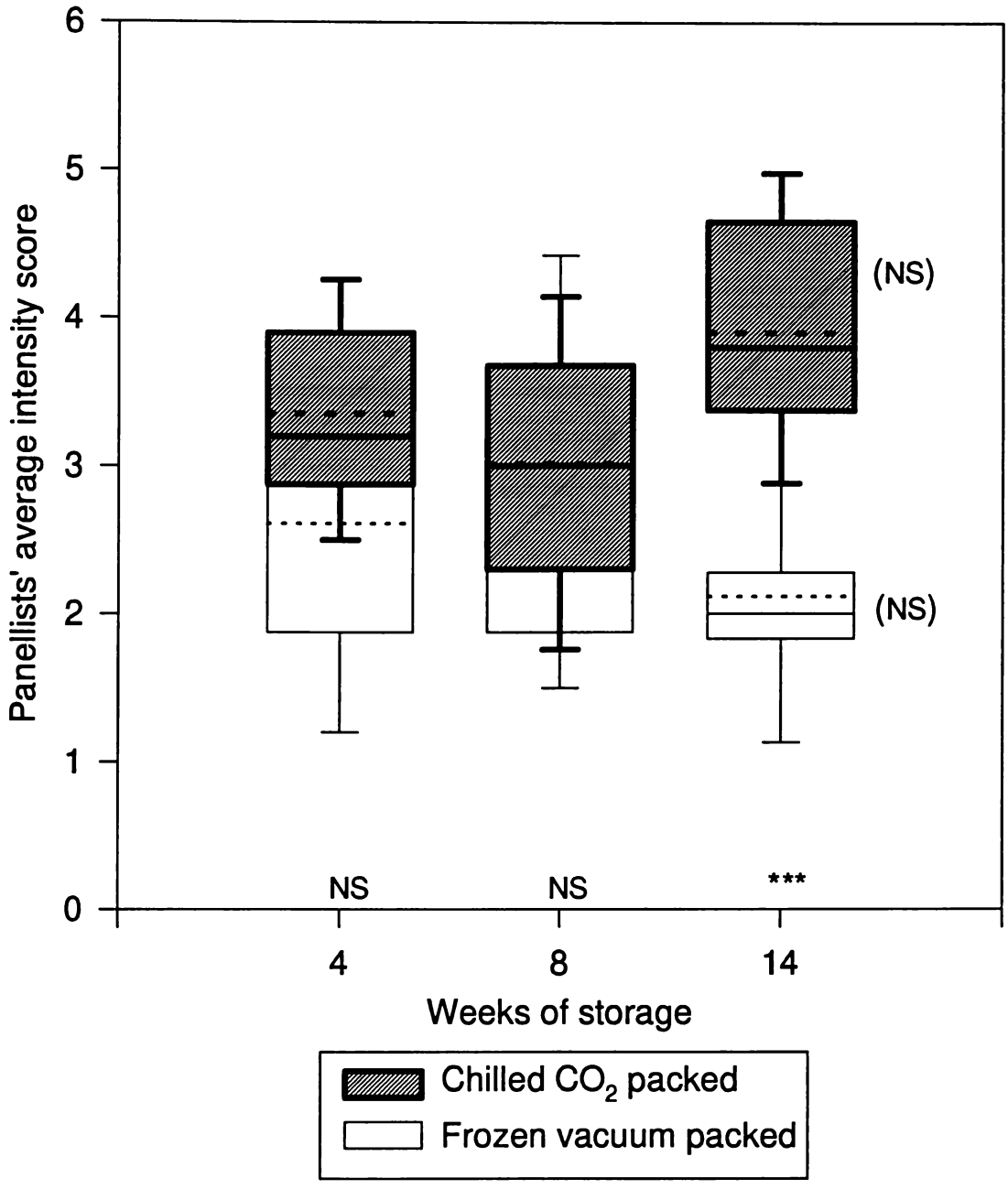
There is some justification for this concern. The solubility of CO_2 increases by about 360 mL kg^{-1} with each ultimate pH unit rise (Gill, 1988b). If storage of meat in a CO_2 atmosphere induces development of livery flavour notes, then it is not unrealistic to suggest that this process may be enhanced in meat of initially high ultimate pH. In addition, results from this study support the suggestion by Gill (1988a) that CO_2 reduces sheepmeat odour. Thus it is also possible that high ultimate pH meat packed in a CO_2 atmosphere will have a much reduced sheepmeat flavour in addition to the reductions in odour and flavour caused by the high ultimate pH itself.

Surveys show that 30% of sheepmeat produced in New Zealand has ultimate pH values greater than pH 5.8, so it is possible that up to one-third of all chilled lamb exported from New Zealand will have an unpredictable and significant level of undesirable 'livery flavour' and a much reduced sheepmeat odour and flavour. In this study a limited range of meat ultimate pH was used, so this hypothesis could not be tested.



The horozontal lines mark the 10th, 25th, 50th, 75th and 90th percentiles.
Dashed lines represent data means.
Asterisks at the bottom of the graph at each storage time represent the significance of the difference between each treatment at each storage time.
Asterisks in parenthesis to the right of the graph represent the time effect for eact storage treatment.
In addition, the overall treatment effect (summed over time) was $P < 0.001$.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, $P > 0.05$.

Figure 6-7.
Box plot of frequency of panellists who scored for "livery flavour" in CO_2-packed meat stored at minus 1.5°C and vacuum-packed meat stored at minus 35°C, for 4, 8 and 14 weeks.



The horozontal lines mark the 10th, 25th, 50th, 75th and 90th percentiles. Dashed lines represent data means.
Asterisks at the bottom of the graph at each storage time represent the significance of the difference between each treatment at each storage time.
Asterisks in parenthesis to the right of the graph represent the time effect for eact storage treatment.
In addition, the overall treatment effect (summed over time) was $P < 0.01$.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, $P > 0.05$.

Figure 6-8.
Box plot of average intensity scores for "livery flavour" for those panellists who detected this attribute in CO_2-packed meat stored at minus 1.5°C and vacuum-packed meat stored at minus 35°C, for 4, 8 and 14 weeks.

Detection of raw and cooked meat volatiles using an Electronic Nose

Responses for each of the eighteen metal oxide sensors of the Electronic Nose are shown graphically in Figure 6-9. After an initial 30 second equilibration period, headspace gas above the equilibated sample was swept through each of the three sensor chambers linked in series. Sampling of the headspace was maintained for 180 seconds until each sensor had reached equilibrium. After 210 seconds, the sampling valve was switched to again allow clean air to purge the sensors, seen as a drop and gradual decline in sensor response. Sensor responses of two dissimilar samples were used to select the most appropriate point along the response curve where the overall sensor responses for each sample were the most dissimilar. In this case 200 seconds was chosen.

The FOX 4000 software was then used to extract the sensor resistance ($\Delta R/R_0$) values of each sensor for every sample in the data set. The data matrix (18 sensors x number of samples) was imported into the Unistat statistical software package for principle components analysis and canonical discriminat analysis.

Principle components analysis of electronic sensory data of raw minces from lamb legs stored for 14 weeks showed clear descrimination between both storage methods and from blank sampling jars (figure 6-10a). There was tighter grouping of frozen-vacuum-packed samples, and sample replicates, compared with samples and their replicates (triplicates in the majority of cases) from chilled CO_2 -packed meat. Treatments are coded F (frozen) and C (CO_2 -packed). Close inspection of the CO_2 packed samples also showed good clustering of replicate samples (denote by subscripts; a,b,c). Eighty-seven percent of the data variability was explained by the first component which increased to 99% by the fifth component.

Principle components analysis is a more robust method for detecting relationship between data sets because it is an unsupervised procedure. An alternative statistical method is canonical discriminat analysis (CDA), is a supervised learning procedure. The operator assigns each sample in the data set to a treatment group. The validity of the statistical grouping is then checked by removing a number of the samples from the

'calibration' data set and presenting them to the analysis as 'unknowns'. This process is known as cross-validation. The reliability of the fit is determined by the closeness the 'unknowns' associate to their parent grouping (denoted as © in figure 6-10b). For the raw mince samples, canonical discriminant analysis also clearly discriminated between each of the storage treatments and the blank jars. Cross-validation of randomly selected replicate samples showed 'unknowns' associate with their respective parent group. This confirms that the Alpha M.O.S electronic nose could reliably measure differences in volatile compounds between chilled CO_2 -packed and frozen vacuum-packed raw meat.

As with the human nose, the electronic nose does not have the ability to identify the compounds responsible for this difference. It is assumed that CO_2 alone is not responsible for the observed difference, as the samples were equilibrated long enough in atmospheric air after mincing to allow dissolved CO_2 to dissipate from the meat.

Three samples of cooked mince from each of the two storage methods, used for one of the sensory analysis sessions, were also evaluated by the electronic nose under similar conditions used for the raw minces. Principle components and canonical discriminant analysis of the cooked meat also shows good discrimination between storage treatments and blank jar for such a small exploratory data set (figure 6-11). One of the three CO_2 samples clustered away from the other two samples but these effects are not unexpected wit such a small data set.

These preliminary results show that the ALPHA M.O.S electronic nose is sensitive enough to detect differences in headspace volatiles in both raw and cooked minces from meat subjected to different storage treatments. These results suggest that, under these experimental conditions, the electronic nose may be more discerning and possible more reproducible than sensory panellists. What relationship the electronic nose's measurements have to human perception of odour has yet to be ascertained. However, what is certain is that this new and exciting technology will become a prominent tool in food and other industries where odour is a significant component.

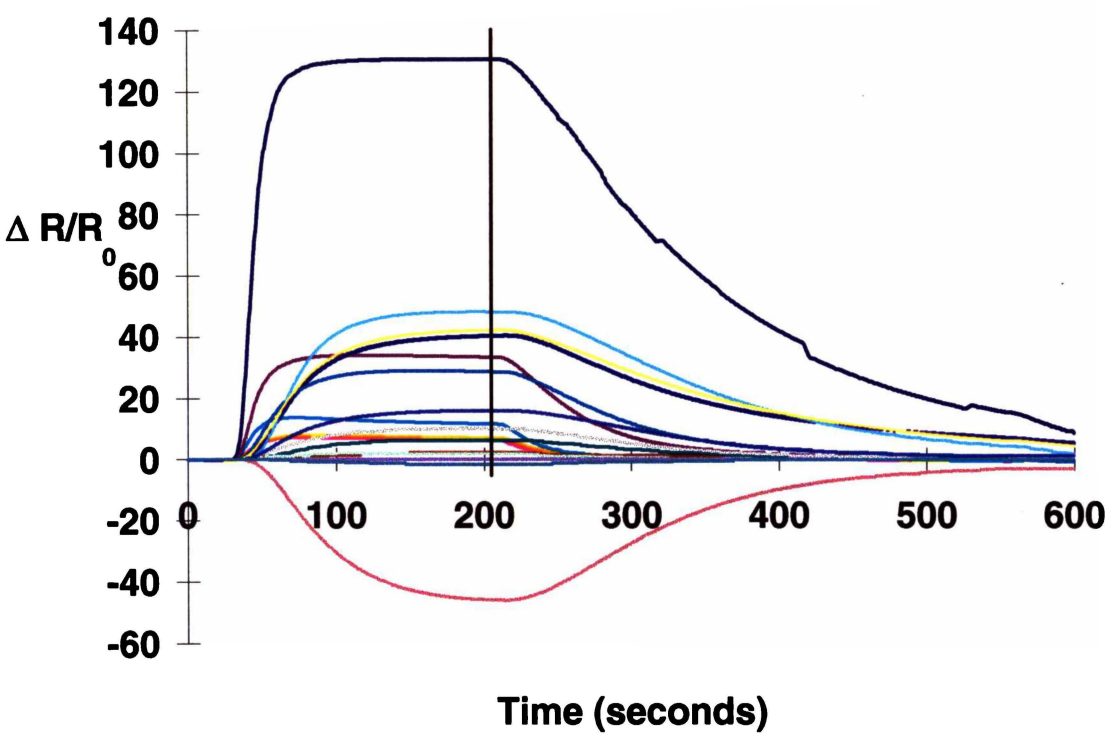
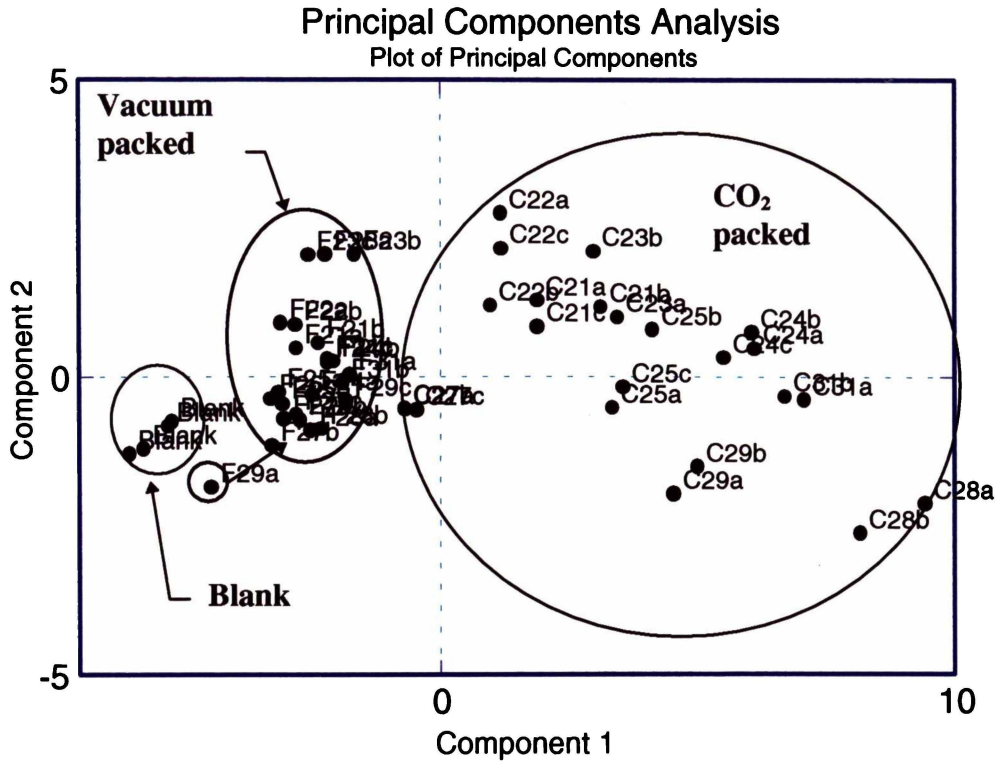


Figure 6-9.

Sensor responses expressed as the change in resistence (Δ R) divided by the resistence at zero time (R_0) for each of 18 metal oxide sensors during the analysis of headspace volatiles from raw minced sheepmeat after storage in CO_2 atmosphere for 14 weeks at -1.5 ^{0} C. The vertical line at 200 seconds represents the time at which the response for each sensor was used for Principle Component and Canonical Discriminant Analysis.

(a)



(b)

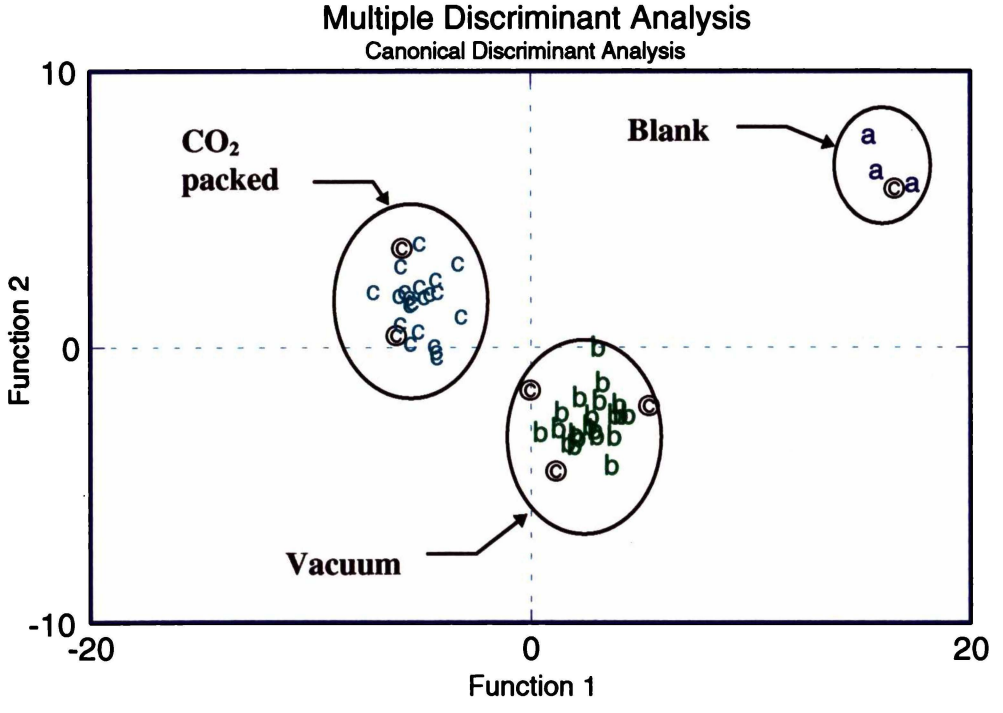
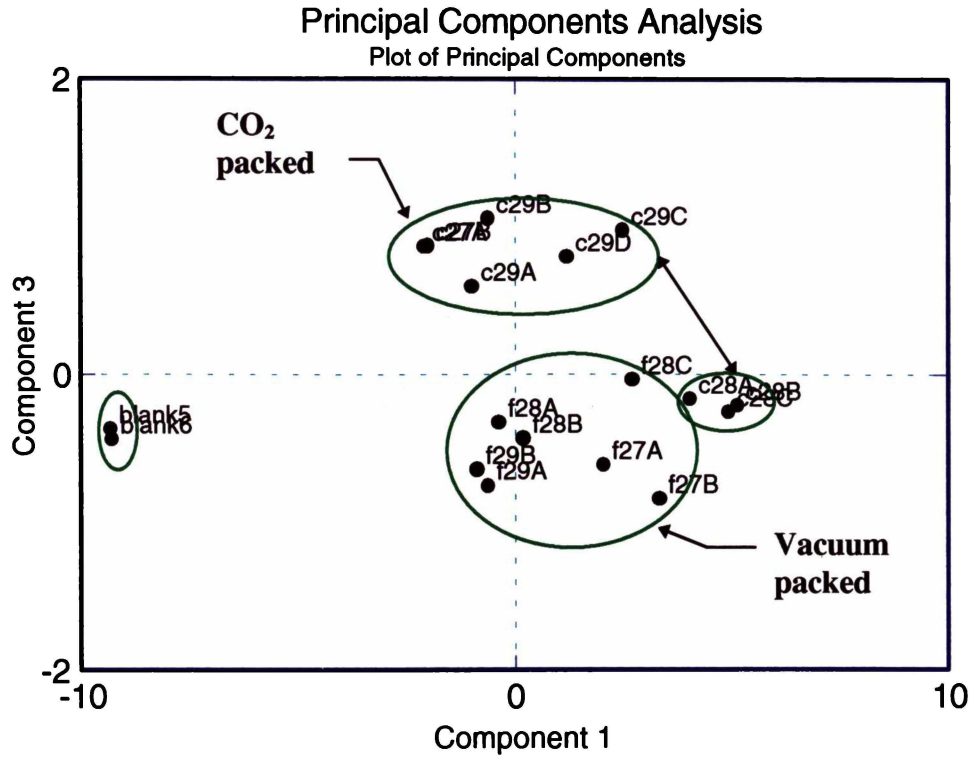


Figure 6-10.

(a) Principal Component Analysis plot and (b) Canonical Discriminant Analysis plot of raw minces from lamb legs stored at -1.5 °C in CO_2 atmosphere and lamb legs stored at -35 °C vacuum-packed, and stored for 14 weeks. In (b) cross validation with unknowns (©) showed the analysis was valid.

(a)



(b)

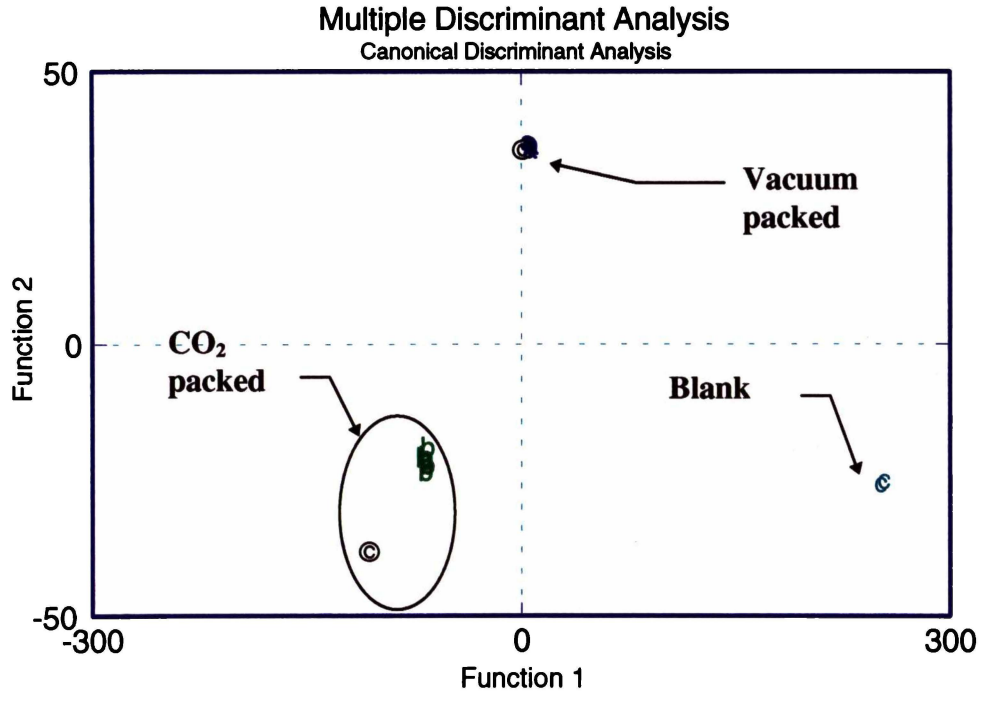


Figure 6-11.

(a) Principal Component Analysis plot and (b) Canonical Discriminant Analysis plot of cooked minces from lamb legs stored at -1.5 °C in CO_2 atmosphere and lamb legs stored at -35 °C vacuum-packed, and stored for 14 weeks. In (b) cross validation with unknowns (©) showed the analysis was valid.

Chapter 7.

Review and Conclusions

This thesis combined sensory and GC/MS and GC/olfactometry techniques to study the effects of meat ultimate pH on the odour and flavour of cooked sheepmeat. Sensory analysis and an emerging technology, an electronic nose, were also used to investigate the changes in cooked meat odour and flavour during long term storage of chilled lamb legs in a carbon dioxide atmosphere. This multidisciplinary approach has helped to explain some of the variations in meat flavour quality important to the New Zealand meat industry.

The topics in this thesis are linked by a common thread: meat odour and flavour and its relationship to meat pH. New Zealand farming and pre-slaughter abattoir practices contribute to changes in the eating quality of cooked meat by affecting meat ultimate pH. Animal contact with humans and farm dogs, and social regrouping and yarding all contribute to the psychological and physical stress of animals during pre-slaughter handling. Stress causes a depletion in muscle glycogen reserves, resulting in a meat ultimate pH far higher than is desirable for good meat quality. A major part of this thesis has addressed the effect meat ultimate pH has on cooked meat odour and flavour.

Most previous studies of pH-related changes in cooked meat odour and flavour have relied on sensory panels. This approach is somewhat subjective and variability between panellists and between panel sessions can be high enough to make interpretation of experimental results difficult. A more objective approach is to use instrumental analysis, such as GC/MS, of volatile compounds that evolve during cooking. Yet, in isolation, the latter technique may produce vast quantities of data that cannot be related to the flavour experience of the consumer. In this thesis an attempt has been made to link sensory perception and instrumental analysis, to aid understanding of the processes involved in changes in cooked meat odour and flavour arising from meat ultimate pH. Instruments and techniques used for this purpose included gas chromatography/mass spectrometry, gas chromatography/olfactometry, and a new technology - an electronic nose.

In electing to pursue the pH study with GC/MS, it was important to first develop methods suitable for the materials under analysis. The elution of volatile compounds from cooked meat fat trapped on Tenax-TA porous polymer using supercritical fluid carbon dioxide was compared with the more classical techniques of thermal desorption and diethyl ether elution. Volatile compounds released from foods and trapped on Tenax-TA porous polymer traps can be successfully eluted at relatively low temperatures with supercritical carbon dioxide. The advantage of this elution method is that any potentially thermally labile compounds present are not likely to be affected by the relatively low temperatures of 33°C compared with conventional thermal desorption temperatures, which can be up to 250°C.

The repeatability of supercritical carbon dioxide elution was comparable to that of thermal desorption or diethyl ether elution. However, the combined use of Tenax traps and subsequent supercritical (carbon dioxide) elution had limitations when applied as a pre-chromatographic step. High carbon dioxide flow rates through the chromatographic column during elution distorted the peak shape of the more volatile early-eluting compounds. Overcoming this problem required very low column cryofocusing temperatures (≤ -40°C) to ensure effective trapping. In addition, the high gas flow rate, particularly at high pressures, caused large quantities of eluted compounds to be lost from the sample injection split vent of the gas chromatograph. Thus, sensitivity (the amount of analyte loaded onto the column) was less than that attained with thermal desorption. Installing a flow restriction valve at the split vent outlet of the split/splitless injector of the Hewlett Packard GC went some way to increasing the quantity of volatile compounds entering the capillary column. Another problem stems from high flow rates: the flow through the capillary column can be too great for some mass spectrometer vacuum pumps, limiting the usefulness of supercritical carbon dioxide as an on-line elution procedure for Tenax traps.

It was concluded that the thermal desorption of volatile compounds from cooked meat was the most appropriate elution method among those tested. Thermal desorption gave the best resolution of early eluting peaks, was the most sensitive, did not produce

noticeable thermal artifacts, and did not require modifications to the gas chromatograph for effective operation.

This technique, was therefore used in a series of experiments directed at investigating the relationship between meat ultimate pH, chemical volatiles and flavour.

Stress-related changes in meat ultimate pH adversely affected cooked meat odour and flavour. Sheepmeat with a moderate or high ultimate pH (mean pH 6.3 and 6.8, respectively), induced by pre-slaughter adrenaline injection, had a lower overall cooking odour and flavour intensity than sheepmeat of a more acceptable pH (5.66). Also, desirable odour and flavour notes decreased and undesirable ones increased as ultimate pH increased. Purge-and-trap GC/MS of fat rendered during cooking of sheepmeat revealed 57 (out of a total of 325) volatile compounds that significantly decreased in concentration with increasing pH. Aldehydes dominated the 57. GC/olfactometry identified 54 odour-active compounds, 10 of which were also responsive to changes in meat ultimate pH. Most of these compounds were again aldehydes. Therefore, these compounds play a major role in cooked sheepmeat odour and flavour quality.

In an effort to understand how changes in pH cause these odour/flavour and related chemical changes, experiments were undertaken where high ultimate pH raw meat was titrated with hydrochloric acid to pH levels close to that of low ultimate pH (5.5). Sensory panellists found that when sheepmeat so adjusted was cooked it had comparable odour and flavour intensity of normally low ultimate pH meat. Most of the volatile compounds found by GC/MS analysis to decrease in concentration with increasing ultimate pH, no longer decreased in concentration after this downward pH adjustment. These results and parallel analyses of soluble protein, free amino acids and free fatty acids led to the conclusion that pH-related changes in the odour and flavour of cooked sheepmeat are probably mediated by direct pH effects during the cooking process rather than by the development of odour and flavour precursors in raw meat before cooking began.

These results, and evidence from the literature, were incorporated in a model to explain the pH-related changes in cooked meat odour and flavour: A low, and normal, pH value of 5.5 is close to the (average) isoelectric point of meat proteins. When meat is cooked myofibrillar gelation at pH 5.5 occurs at a lower temperature than at pH values greater than 6.0. Intracellular osmotic pressure is also higher at pH 5.5 compared with meat of higher pH. Changes in osmotic pressure equates to changes in intracellular ionic strength that dissociate contractile proteins and consequently alters the structure of myofibrils. This makes them more susceptible to proteolytic attack. Some proteolytic enzymes have low pH optima and still show a degree of activity at near cooking temperatures. Low pH and heat also enhance autoxidation and enzymatic lipolysis of membrane-bound phospholipid.

Proteolysis, and autoxidation and lipolysis of lipids generate odour and flavour precursors.

The flavour precursors so liberated, such as fatty acids and amino acids, are flavourful in their own right. They also generate a wide range of volatile compounds (via the Maillard reaction and Strecker degradation of amino acids) implicated in odour and flavour development in cooked meat. These reaction mechanisms are also influenced by pH.

Meat pH is also affected by long-term storage. Sheepmeat stored chilled in a carbon dioxide atmosphere with a very low oxygen concentration maintains hygienic acceptability for more than 16 weeks. However it was found that during such storage, meat pH increased and cooked meat flavour and odour quality changed. Panellists detected a significant decrease in overall and sheepmeat odour and "sweet" and "roasty" flavour after 14 weeks of carbon dioxide storage. Of particular concern is a significant increase in the intensity of a "livery/offaly" flavour for minces from carbon dioxide stored legs compared with the frozen controls, after 14 weeks storage.

In addition to the conventional panel assessment, samples of both raw and cooked minces were also evaluated using an electronic nose. Preliminary results showed that an electronic nose, based on an array of metal oxide sensors, is sensitive enough to detect differences in the headspace volatile compounds of both raw and cooked minces from meat subjected to the different storage treatments. What relationship the electronic nose's measurements have to human perception of these particular odours and to GC/MS analysis of volatile compounds from raw and cooked meat has yet to be defined.

The compounds and mechanisms responsible for these odour and flavour changes during storage in carbon dioxide and their relationship with the observed rise in meat pH on storage warrants further investigation using instrumental analysis. This relationship is important since current work has shown that high ultimate pH meat has a reduced cooked odour and flavour intensity compared with meat of normal pH (chapter 4 and 5). Meat with an initial higher ultimate pH stored chilled in a CO_2 atmosphere might have a very much greater deterioration of odour and flavour.

Conclusions arising from the comparison of thermal desorption, diethyl ether elution and supercritical carbon dioxide elution of Tenax-TA traps were:

- The three methods have comparable repeatability.
- Supercritical carbon dioxide (SCO_2) elution has limitiations. It requires very low column cryofocusing temperatures to ensure effective trapping and resolution of early eluting compounds. High CO_2 gas flow rate, particularly at high pressures, reduces the amount of analyte loaded onto the capillary column, and might be too high for some mass spectrometer vacuum pumps to handle. These limitations could be overcome by modifications to the gas chromatograph.
- Thermal desorption of cooked meat volatile compounds from Tenax-TA traps gave the best resolution of early eluting peaks, was the most sensitive, did not produce noticable thermal artifacts, and did not require excessive modifications to the gas chromatograph for effective operation.

Conclusions arising from the studies of ultimate pH related changes of cooked sheepmeat odour and flavour were:

- Stress-induced moderate or high ultimate pH sheepmeat has a significantly lower overall cooking odour and flavour intensity, as assessed by a trained sensory panel, than sheepmeat of a lower pH (5.66).
- Desirable odour and flavour notes decrease and undesirable ones increase as ultimate pH increases.
- Results of purge-and-trap GC/MS of rendered fat during cooking clearly indicate that meat of high ultimate pH has lower concentrations of a number of volatile compounds than meat of more acceptable ultimate pH. The most common of these compounds are aldehydes.
- GC/olfactometry identified a number of odour active compounds that are also responsive to changes in meat ultimate pH. Most of these compounds are again aldehydes.
- Titration of higher ultimate pH meat to values similar to low-pH meat produces meat of comparable odour and flavour intensity, as assessed by a trained sensory panel, to that of low-pH untitrated meat.
- GC/MS analysis of rendered fat during cooking of titrated high ultimate pH meat indicate that concentrations of volatile compounds are similar to those of low-pH untitrated meat.
- Titration of higher ultimate pH meat to values similar to low-pH meat lowered levels of soluble protein and some free amino acids to levels found in untitrated meat of low ultimate pH. This is because solubility is greater at pH values further from the protein's isoelectric point (pH 5.5 of meat proteins).
- No major changes in total free fatty acids or most individual free fatty acids occur with change in meat ultimate pH.

- Ultimate pH related changes to sheepmeat odour and flavour development probably occured mainly at the time of cooking due to pH and heat initiated disruption of myofibrillar protein structure and lysomal membranes, activation of acidic proteinase and phospholipase enzymes, and heat induced autoxidation of lipid, principally phospholipid.

Conclusions arising from the studies of the effect of extended chilled storage on the odour and flavour of cooked sheepmeat were:

- Meat pH values increase significantly between 4 and 14 weeks storage for chilled CO_2-packed meat but not for the vacuum-packed frozen control.
- Significant decreases in "overall", "sheepmeat", and "sweet odours" of cooked meat with storage time were detected by sensory panellists.
- Panellists can also detect a significant decrease in "sweet" and "roasty" flavours in the CO_2-packed lamb legs stored at-1.5°C compared with the frozen-stored controls.
- There is aslo a significant increase in intensity of a "livery/offaly" flavour for minces from the CO_2-packed stored legs compared with the frozen controls, after 14 weeks storage.
- Preliminary results show that the electronic nose is sensitive enough to detect difference in headspace volatiles in both raw and cooked minces from meat subjected to different storage treatments.

Appendix A.

Mass Spectral Analysis

This appendix contains:

- a typical GC/MS chromatogram of volatile compounds trapped from the headspace of cooked sheepmeat rendered fat (figure A-1)
- the formula used to calculate the approximate amount (ng g^{-1} fat) of each volatile compound (figure A-2).
- a complete list of TIC and final amounts of 363 compounds detected for two representative samples. (the full data set is available from the author on request) (figure A-3)
- mass spectra of unknown compounds found to change in concentration with increasing meat ultimate pH (Chapter 4) (figure A-4a and b).
- mass spectra of unknown compounds found to change in concentration between the low-, medium-, and high pH groups after titration with HCl (Chapter 5) (figure A-5).

An addendum to this thesis containing a database of Kovats' indices and authentic compounds used to help identify compounds separated by Gas Chromatography and listed in Chapters 4 and 5 is available on request. Please contact:

The Librarian, Meat industry Research Institute of New Zealand (Inc.).
P.O. Box 617, Hamilton, New Zealand.

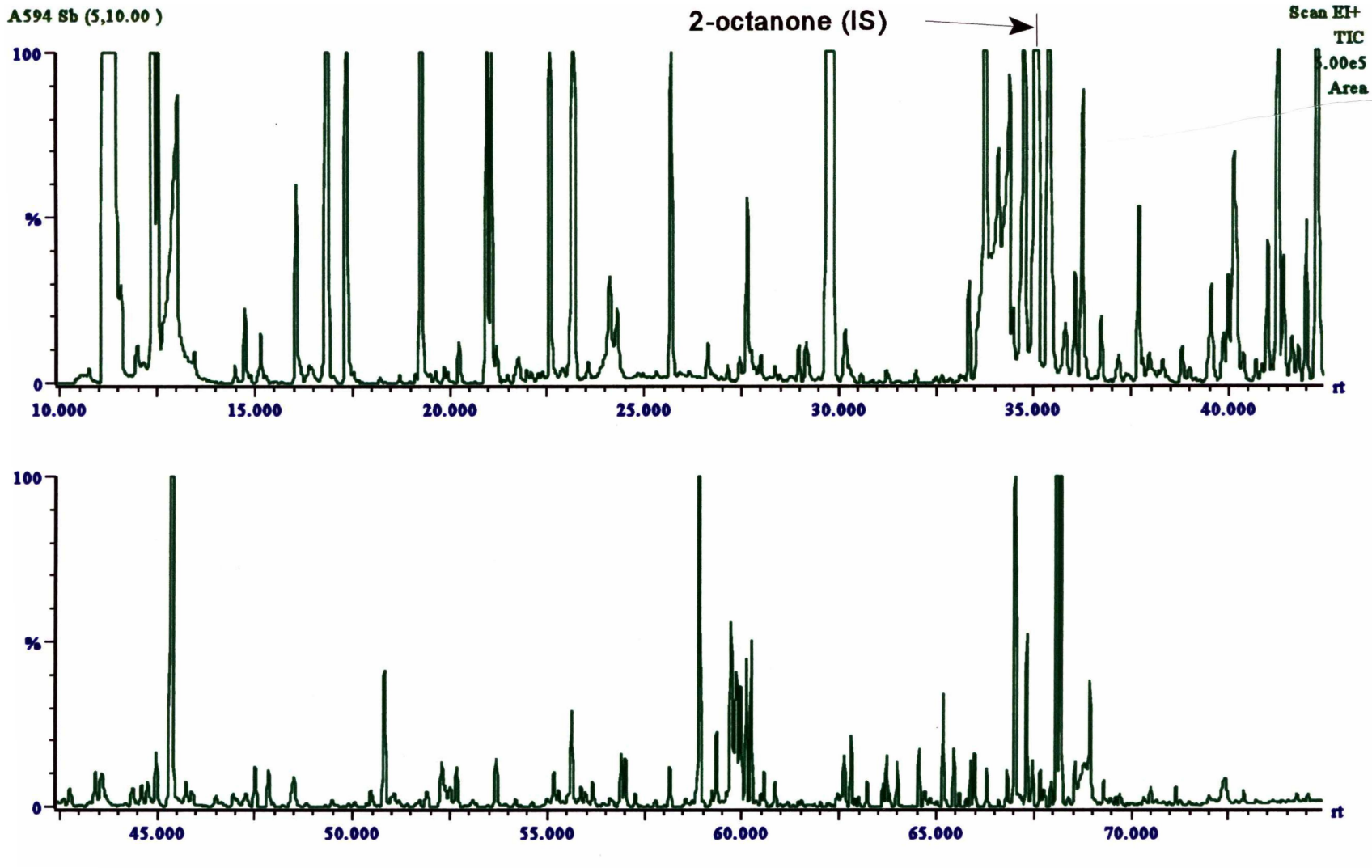


Figure A-1.
Typical GC/MS chromatogram (TIC) of volatile compounds from cooked sheepmeat.

Example data and calculations of GC/MS results.

Concentration (ng per g fat) = (((2.05*TIC)/$TIC_{internal\ standard}$)/2)*1000

where 2.05 = ug 2-octanone internal standard added to fat
TIC = Total ion count of unknown
$TIC_{internal\ standard}$ = Total ion count of internal standard (peak#169)
2 = sample weight

				Total ion counts			ng per g fat		
	Peak id	RT	Kovats'	11	1a	1b	11	1a	1b
1	4	10.801	518	642504	118375	127226	8.98	2.14	2.30
2	7	11.217	547	1090023	912161	760032	15.24	15.19	13.71
3	8	11.467	562	23946970	11962436	17946888	334.72	199.15	323.78
4	9	11.634	571	933102	495109	972096	13.04	8.24	17.54
5	11	12.034	591	8506938	151282	201760	118.91	2.52	3.64
6	13	12.301	603	51368	6837260	5462670	0.72	113.82	98.55
7	14	12.451	610	3106212	1614069	5080966	43.42	26.87	91.67
8	15	12.518	613	52574392	2680805	3193263	734.86	44.63	57.61
9	17	12.884	626	11815836	10868866	14532957	165.16	180.94	262.19
10	18	13.401	643	3965029	95233	123818	55.42	1.59	2.23
11	19	13.651	651	30503	23563	7432	0.43	0.39	0.13
12	24	14.385	670	147821	150196	156534	2.07	2.50	2.82
13	25	14.618	675	2924432	1715873	1668568	40.88	28.57	30.10
14	26	14.768	678	717480	414926	55497	10.03	6.91	1.00
15	28	15.018	683	1247577	778444	788021	17.44	12.96	14.22
16	29	15.085	685	111846	46150	70164	1.56	0.77	1.27
17	34	15.885	699	2069633	2532356	2450784	28.93	42.16	44.21
18	35	15.985	701	1118680	302922	299890	15.64	5.04	5.41
19	36	16.235	705	350998	109013	225981	4.91	1.81	4.08
20	37	16.335	707	285504	339477	239542	3.99	5.65	4.32
21	38	16.552	710	158789	169282	155655	2.22	2.82	2.81
22	39	16.618	711	9312163	6209744	5746464	130.16	103.38	103.67
23	40	16.668	712	1867839	2324281	2396884	26.11	38.69	43.24
24	41	16.818	714	193559	197335	151361	2.71	3.29	2.73
25	43	17.135	719	10198075	6423925	6179154	142.54	106.94	111.48
26	44	17.252	721	174023	81437	92028	2.43	1.36	1.66
27	47	18.135	733	34254	49537	26789	0.48	0.82	0.48
28	49	18.485	738	139653	126540	147374	1.95	2.11	2.66
29	52	18.885	743	39539	46877	52212	0.55	0.78	0.94
30	53	19.035	745	26175466	19413284	18635930	365.87	323.19	336.21
31	54	19.252	748	724434	438875	685330	10.13	7.31	12.36
32	55	19.402	750	51028	43437	62276	0.71	0.72	1.12
33	57	19.619	753	246147	218443	218781	3.44	3.64	3.95
34	58	19.736	755	159202	75627	67585	2.23	1.26	1.22
35	59	19.752	755	101922	92395	121594	1.42	1.54	2.19
36	60	19.986	758	328589	368971	353485	4.59	6.14	6.38
37	61	20.269	762	50543	42601	30400	0.71	0.71	0.55
38	62	20.419	764	114505	49727	49883	1.60	0.83	0.90
39	63	20.686	767	2063318	1450040	1640390	28.84	24.14	29.59

A-2 and A-3.
Calculation of the amount of each volatile compound and a list of TIC and final concentrations of two representative samples.

				Total ion counts			ng per g fat		
	Peak id	Sample # → RT	Kovats'	11	1a	1b	11	1a	1b
40	64	20.819	769	4639479	3480317	3392786	64.85	57.94	61.21
41	65	20.952	771	637943	647738	679802	8.92	10.78	12.26
42	66	21.119	773	411831	431726	401693	5.76	7.19	7.25
43	67	21.236	775	160143	111088	150465	2.24	1.85	2.71
44	68	21.719	781	943355	215935	245472	13.19	3.59	4.43
45	71	22.119	787	674913	397211	61016	9.43	6.61	1.10
46	72	22.369	790	20886190	10180489	9794130	291.94	169.48	176.70
47	73	22.636	794	162166	163956	171134	2.27	2.73	3.09
48	74	22.903	797	6857965	3821653	3777543	95.86	63.62	68.15
49	75	22.903	797	7536586	9433101	8908100	105.34	157.04	160.71
50	76	23.186	801	72015	21091	46890	1.01	0.35	0.85
51	77	23.286	803	453896	348977	403545	6.34	5.81	7.28
52	78	23.403	804	31522	38233	72192	0.44	0.64	1.30
53	80	23.62	808	163201	1590	25584	2.28	0.03	0.46
54	82	23.82	810	476075	91487	343101	6.65	1.52	6.19
55	83	23.87	811	1000000	3588524	1667787	13.98	59.74	30.09
56	85	24.136	815	750030	1453222	620821	10.48	24.19	11.20
57	88	24.586	821	62631	57034	72896	0.88	0.95	1.32
58	89	24.703	823	41449	25616	48742	0.58	0.43	0.88
59	90	24.803	825	113039	79218	74320	1.58	1.32	1.34
60	92	25.037	828	59244	29399	44469	0.83	0.49	0.80
61	93	25.153	830	121222	109476	94535	1.69	1.82	1.71
62	94	25.187	830	57624	23921	31250	0.81	0.40	0.56
63	95	25.42	834	1928568	661215	1288943	26.96	11.01	23.25
64	97	25.737	838	212419	127352	131606	2.97	2.12	2.37
65	98	25.903	841	164243	58217	65831	2.30	0.97	1.19
66	99	26.053	843	74371	11681	13146	1.04	0.19	0.24
67	100	26.153	845	46961	26458	18115	0.66	0.44	0.33
68	101	26.353	847	413522	402249	379128	5.78	6.70	6.84
69	102	26.47	849	271490	140457	93185	3.79	2.34	1.68
70	103	26.537	850	81424	40316	61121	1.14	0.67	1.10
71	105	26.87	855	713105	281638	275167	9.97	4.69	4.96
72	107	27.387	863	2167549	2132750	1992666	30.30	35.51	35.95
73	119	28.704	883	452577	435065	390362	6.33	7.24	7.04
74	120	28.887	886	1158574	563860	495725	16.19	9.39	8.94
75	121	28.921	887	525872	367983	344631	7.35	6.13	6.22
76	123	29.221	892	503303	207086	194372	7.03	3.45	3.51
77	124	29.404	894	1749769	2118914	2161171	24.46	35.27	38.99
78	125	29.437	895	3471088	713946	449796	48.52	11.89	8.11
79	126	29.554	897	16962206	15243513	14758479	237.09	253.77	266.26
80	127	29.871	902	1082272	432306	624625	15.13	7.20	11.27
81	128	29.887	902	1784625	1920549	1652673	24.94	31.97	29.82
82	129	29.987	904	1259299	645800	698119	17.60	10.75	12.59
83	130	30.021	904	391375	145880	204639	5.47	2.43	3.69
84	132	30.304	909	18989	116491	137082	0.27	1.94	2.47
85	135	30.571	913	32140	36440	43822	0.45	0.61	0.79
86	138	30.971	919	164724	68509	57161	2.30	1.14	1.03
87	140	31.271	924	135455	27460	21115	1.89	0.46	0.38
88	141	31.338	925	94964	67109	15489	1.33	1.12	0.28
89	142	31.421	926	62989	45631	23508	0.88	0.76	0.42

A-2 and A-3.

Calculation of the amount of each volatile compound and a list of TIC and final concentrations of two representative samples.

				Total ion counts			ng per g fat		
	Peak id	RT	Kovats'	11	1a	1b	11	1a	1b
90	143	31.554	928	124839	74304	75219	1.74	1.24	1.36
91	144	31.688	930	453921	351848	317247	6.34	5.86	5.72
92	146	31.904	934	92695	83210	86686	1.30	1.39	1.56
93	147	32.104	937	189288	48390	33335	2.65	0.81	0.60
94	149	32.138	938	238032	59450	105443	3.33	0.99	1.90
95	150	32.221	939	61618	60925	33344	0.86	1.01	0.60
96	151	32.371	941	46471	101569	105712	0.65	1.69	1.91
97	152	32.488	943	336328	61202	71175	4.70	1.02	1.28
98	153	32.538	944	110909	102902	115068	1.55	1.71	2.08
99	154	32.738	947	159727	57214	36661	2.23	0.95	0.66
100	155	32.888	950	679663	362468	344493	9.50	6.03	6.21
101	157	33.055	952	1129034	1224413	1148611	15.78	20.38	20.72
102	158	33.205	955	577555	334779	260689	8.07	5.57	4.70
103	159	33.388	958	563515	723974	484460	7.88	12.05	8.74
104	160	33.455	959	9953678	4821035	4584715	139.13	80.26	82.71
105	161	33.571	961	270940	216748	207600	3.79	3.61	3.75
106	162	33.688	962	108035	73684	30544	1.51	1.23	0.55
107	163	33.821	965	925351	631989	441297	12.93	10.52	7.96
108	164	33.971	967	1711216	818181	832813	23.92	13.62	15.02
109	165	34.105	969	370366	315986	409574	5.18	5.26	7.39
110	167	34.472	975	6625437	4974466	5185920	92.61	82.81	93.56
111	168	34.822	981	126237712	269170272	252664720	1764.49	4481.06	4558.30
112	IS 169	**35.122**	**986**	**73332040**	**61570188**	**56815296**	**1025.00**	**1025.00**	**1025.00**
113	170	35.155	986	27429044	6240033	7164518	383.39	103.88	129.25
114	172	35.455	991	1065872	678539	684886	14.90	11.30	12.36
115	173	35.522	992	231345	372573	251499	3.23	6.20	4.54
116	174	35.772	996	6168075	3619599	3604935	86.21	60.26	65.04
117	175	35.955	999	3774881	2739719	2861303	52.76	45.61	51.62
118	176	36.055	1001	130023	180638	131262	1.82	3.01	2.37
119	177	36.289	1004	59703	51823	57028	0.83	0.86	1.03
120	178	36.422	1007	1032302	846645	840299	14.43	14.09	15.16
121	179	36.539	1008	36016	43168	56194	0.50	0.72	1.01
122	181	36.805	1013	530651	697176	749070	7.42	11.61	13.51
123	182	36.839	1013	357229	275382	184303	4.99	4.58	3.32
124	184	37.072	1017	424065	190086	235734	5.93	3.16	4.25
125	185	37.122	1018	282511	189497	187501	3.95	3.15	3.38
126	187	37.389	1022	923075	483189	399860	12.90	8.04	7.21
127	188	37.489	1024	3238597	1100084	1075983	45.27	18.31	19.41
128	189	37.655	1027	1273540	1713613	1665368	17.80	28.53	30.04
129	190	37.805	1029	393127	370118	303370	5.49	6.16	5.47
130	191	37.906	1031	2674071	2140979	2184423	37.38	35.64	39.41
131	192	38.005	1032	500674	398933	334814	7.00	6.64	6.04
132	193	38.172	1035	404341	331667	332578	5.65	5.52	6.00
133	195	38.489	1040	1530309	421907	503349	21.39	7.02	9.08
134	197	38.706	1044	1824560	686572	674964	25.50	11.43	12.18
135	198	38.839	1046	614517	316320	295972	8.59	5.27	5.34
136	199	38.956	1048	361610	148856	117600	5.05	2.48	2.12
137	200	39.089	1050	199423	154126	104555	2.79	2.57	1.89
138	202	39.239	1053	1527937	1369700	1321564	21.36	22.80	23.84
139	203	39.289	1053	398291	200511	255724	5.57	3.34	4.61

A-2 and A-3.

Calculation of the amount of each volatile compound and a list of TIC and final concentrations of two representative samples.

				Total ion counts			ng per g fat		
	Peak id	Sample # → RT	Kovats'	11	1a	1b	11	1a	1b
140	204	39.506	1057	172067	69760	64850	2.41	1.16	1.17
141	205	39.656	1059	1491990	579731	642450	20.85	9.65	11.59
142	206	39.656	1059	2150170	2701056	2503112	30.05	44.97	45.16
143	207	39.839	1062	977653	1010974	975356	13.67	16.83	17.60
144	208	39.873	1063	2143027	1335116	1654514	29.95	22.23	29.85
145	209	39.906	1064	869257	1048105	552706	12.15	17.45	9.97
146	210	40.056	1066	872905	1006455	1471435	12.20	16.76	26.55
147	211	40.256	1069	102669	12260	6000	1.44	0.20	0.11
148	212	40.373	1071	218746	327270	468660	3.06	5.45	8.46
149	213	40.556	1074	701764	964831	1501098	9.81	16.06	27.08
150	214	40.673	1076	4454544	9654032	9589244	62.26	160.72	173.00
151	215	40.789	1078	190369	79323	104995	2.66	1.32	1.89
152	216	40.906	1080	65955	45847	67749	0.92	0.76	1.22
153	217	41.123	1084	773074	236219	350088	10.81	3.93	6.32
154	218	41.123	1084	786354	560223	423407	10.99	9.33	7.64
155	219	41.306	1087	1750314	2366263	2474260	24.47	39.39	44.64
156	220	41.473	1090	294747	84532	96190	4.12	1.41	1.74
157	221	41.673	1093	1791643	841991	853680	25.04	14.02	15.40
158	223	41.94	1098	12188294	7759700	8658024	170.36	129.18	156.20
159	224	42.023	1099	499646	586763	578550	6.98	9.77	10.44
160	225	42.173	1102	119472	89422	93715	1.67	1.49	1.69
161	226	42.306	1104	54017	69035	98592	0.76	1.15	1.78
162	227	42.456	1107	834800	234230	251865	11.67	3.90	4.54
163	228	42.606	1109	155090	17924	28708	2.17	0.30	0.52
164	229	42.74	1111	23290	68231	12565	0.33	1.14	0.23
165	230	42.823	1113	76031	10101	53341	1.06	0.17	0.96
166	231	42.94	1115	112638	8000	108010	1.57	0.13	1.95
167	232	43.09	1117	796444	174621	328799	11.13	2.91	5.93
168	233	43.273	1121	276220	360749	247661	3.86	6.01	4.47
169	234	43.373	1122	315000	352263	13890	4.40	5.86	0.25
170	235	43.573	1126	119231	43966	29975	1.67	0.73	0.54
171	237	43.707	1128	40268	44280	14151	0.56	0.74	0.26
172	239	43.89	1131	164755	35526	43551	2.30	0.59	0.79
173	240	44.023	1134	552012	185900	183112	7.72	3.09	3.30
174	242	44.273	1138	456050	205773	193994	6.37	3.43	3.50
175	243	44.323	1139	202251	65533	73589	2.83	1.09	1.33
176	244	44.457	1141	531296	241016	328779	7.43	4.01	5.93
177	245	44.507	1142	229371	328322	335507	3.21	5.47	6.05
178	246	44.674	1145	713667	1640361	1423176	9.98	27.31	25.68
179	247	44.724	1146	1476912	683126	665193	20.64	11.37	12.00
180	248	45.057	1152	4530607	4401794	3942718	63.33	73.28	71.13
181	249	45.257	1156	11513610	9061396	8275240	160.93	150.85	149.29
182	250	45.39	1158	1592254	957772	865827	22.26	15.94	15.62
183	251	45.59	1162	2084370	798951	749125	29.13	13.30	13.51
184	253	45.79	1166	43846	39646	30384	0.61	0.66	0.55
185	254	45.94	1168	27367	65626	15467	0.38	1.09	0.28
186	256	46.19	1173	359743	557698	559438	5.03	9.28	10.09
187	258	46.357	1176	433311	208402	131124	6.06	3.47	2.37
188	259	46.524	1179	116865	24450	31024	1.63	0.41	0.56
189	260	46.624	1181	52963	22594	30476	0.74	0.38	0.55

A-2 and A-3.

Calculation of the amount of each volatile compound and a list of TIC and final concentrations of two representative samples.

				Total ion counts			ng per g fat		
	Peak id	Sample # → RT	Kovats'	11	1a	1b	11	1a	1b
190	261	46.741	1183	859098	256483	269080	12.01	4.27	4.85
191	262	46.841	1185	255764	103547	83648	3.57	1.72	1.51
192	263	46.957	1187	368836	149151	132362	5.16	2.48	2.39
193	264	47.024	1189	27567	45940	16953	0.39	0.76	0.31
194	265	47.191	1192	2484228	653960	716695	34.72	10.89	12.93
195	266	47.224	1192	713836	209271	157146	9.98	3.48	2.84
196	267	47.391	1196	32010	10560	34632	0.45	0.18	0.62
197	268	47.524	1198	600796	276481	198312	8.40	4.60	3.58
198	269	47.657	1201	352743	58326	75502	4.93	0.97	1.36
199	270	47.724	1202	43848	16722	18653	0.61	0.28	0.34
200	271	47.907	1205	294669	245779	170285	4.12	4.09	3.07
201	272	47.957	1206	69732	87388	33496	0.97	1.45	0.60
202	273	48.124	1210	133233	278018	205842	1.86	4.63	3.71
203	274	48.191	1211	971130	660837	667675	13.57	11.00	12.05
204	277	48.558	1218	243538	70000	74122	3.40	1.17	1.34
205	279	48.758	1222	15793	60206	56692	0.22	1.00	1.02
206	280	48.824	1224	77775	87995	46782	1.09	1.46	0.84
207	281	48.974	1227	492628	121956	142165	6.89	2.03	2.56
208	282	49.158	1230	184240	47220	59528	2.58	0.79	1.07
209	283	49.274	1233	91037	46693	49879	1.27	0.78	0.90
210	284	49.324	1234	173273	15442	25358	2.42	0.26	0.46
211	285	49.441	1236	60195	49224	59872	0.84	0.82	1.08
212	286	49.608	1240	219229	14021	20615	3.06	0.23	0.37
213	287	49.641	1240	83271	23768	35112	1.16	0.40	0.63
214	288	49.741	1242	77260	53531	76005	1.08	0.89	1.37
215	290	50.058	1249	97481	23751	58534	1.36	0.40	1.06
216	291	50.224	1252	2290422	1669955	1551252	32.01	27.80	27.99
217	292	50.274	1253	193652	71656	47559	2.71	1.19	0.86
218	293	50.491	1258	1771272	1590532	1521050	24.76	26.48	27.44
219	294	50.675	1262	229481	125887	131876	3.21	2.10	2.38
220	295	50.758	1264	292817	261938	235583	4.09	4.36	4.25
221	296	50.875	1266	249983	169783	123715	3.49	2.83	2.23
222	297	51.108	1271	74062	66582	10200	1.04	1.11	0.18
223	298	51.175	1273	1597544	151401	19602	22.33	2.52	0.35
224	299	51.225	1274	394928	23456	142768	5.52	0.39	2.58
225	300	51.408	1278	174383	170708	140344	2.44	2.84	2.53
226	301	51.575	1282	375777	234702	193579	5.25	3.91	3.49
227	302	51.608	1282	233332	98565	138377	3.26	1.64	2.50
228	304	51.975	1290	1474493	1797960	1579622	20.61	29.93	28.50
229	305	52.025	1292	154128	402994	380655	2.15	6.71	6.87
230	306	52.175	1295	162373	245545	216671	2.27	4.09	3.91
231	307	52.358	1299	1501838	1028542	740825	20.99	17.12	13.37
232	309	52.775	1309	121683	44698	29218	1.70	0.74	0.53
233	310	52.958	1313	16911	9105	15026	0.24	0.15	0.27
234	311	53.142	1317	151921	58234	56730	2.12	0.97	1.02
235	312	53.408	1324	653562	460840	452246	9.14	7.67	8.16
236	313	53.408	1324	532378	222632	214296	7.44	3.71	3.87
237	314	53.658	1330	575384	133211	124963	8.04	2.22	2.25
238	315	53.742	1332	50186	8012	12003	0.70	0.13	0.22
239	317	53.975	1337	126675	21090	30793	1.77	0.35	0.56

A-2 and A-3.

Calculation of the amount of each volatile compound and a list of TIC and final concentrations of two representative samples.

				Total ion counts			ng per g fat		
	Peak id	RT	Kovats'	11	1a	1b	11	1a	1b
240	319	54.125	1341	119491	20448	26276	1.67	0.34	0.47
241	322	54.325	1346	152576	83374	77848	2.13	1.39	1.40
242	323	54.492	1350	71139	31186	37472	0.99	0.52	0.68
243	325	54.692	1355	191755	168322	119333	2.68	2.80	2.15
244	327	54.809	1358	427033	149156	189477	5.97	2.48	3.42
245	328	55.042	1364	22064176	9451261	3758103	308.40	157.34	67.80
246	331	55.309	1371	1177517	554348	449182	16.46	9.23	8.10
247	332	55.359	1372	1608025	1308962	1227440	22.48	21.79	22.14
248	333	55.408	1374	236882	301200	201421	3.31	5.01	3.63
249	334	55.542	1377	2039848	1087048	933632	28.51	18.10	16.84
250	335	55.742	1383	790271	631359	587070	11.05	10.51	10.59
251	336	55.909	1387	339881	135056	144570	4.75	2.25	2.61
252	337	56.109	1392	38760	13780	46894	0.54	0.23	0.85
253	338	56.209	1395	109516	47847	66987	1.53	0.80	1.21
254	339	56.376	1400	352847	102554	131386	4.93	1.71	2.37
255	340	56.409	1401	149040	164685	42774	2.08	2.74	0.77
256	341	56.659	1407	1974932	1923656	880699	27.60	32.02	15.89
257	342	56.759	1410	669894	445251	370531	9.36	7.41	6.68
258	343	56.993	1417	47079	33718	110118	0.66	0.56	1.99
259	344	57.043	1418	247636	303060	5223	3.46	5.05	0.09
260	345	57.193	1422	34591	10885	7820	0.48	0.18	0.14
261	346	57.326	1426	19369	8161	2575	0.27	0.14	0.05
262	349	57.543	1432	75207	231957	32720	1.05	3.86	0.59
263	351	57.809	1440	19400	76268	13966	0.27	1.27	0.25
264	352	57.893	1443	298700	90995	2358	4.18	1.51	0.04
265	353	57.943	1444	80087	39461	12830	1.12	0.66	0.23
266	355	58.193	1452	7891	10437	4974	0.11	0.17	0.09
267	356	58.326	1456	176401	266087	85818	2.47	4.43	1.55
268	357	58.409	1458	153908	89145	50841	2.15	1.48	0.92
269	358	58.51	1461	485184	196614	133300	6.78	3.27	2.40
270	360	58.693	1467	11378338	7446331	6867140	159.04	123.96	123.89
271	361	58.81	1470	242414	296016	92951	3.39	4.93	1.68
272	362	59.026	1477	567344	370194	246108	7.93	6.16	4.44
273	363	59.126	1480	1310075	150140	99296	18.31	2.50	1.79
274	364	59.276	1485	49507	13843	17847	0.69	0.23	0.32
275	365	59.376	1488	435747	238316	233718	6.09	3.97	4.22
276	366	59.51	1492	7985331	4911808	4508644	111.62	81.77	81.34
277	367	59.66	1497	6447614	3451818	3228265	90.12	57.46	58.24
278	368	59.76	1500	4128513	2477172	2326528	57.71	41.24	41.97
279	369	59.91	1505	5251636	3859581	2315353	73.40	64.25	41.77
280	370	60.026	1509	3904641	2034219	1880615	54.58	33.87	33.93
281	371	60.177	1514	1471582	3179945	289380	20.57	52.94	5.22
282	372	60.293	1518	145410	235589	52863	2.03	3.92	0.95
283	373	60.377	1520	896507	470591	463182	12.53	7.83	8.36
284	374	60.51	1525	5190	22025	16123	0.07	0.37	0.29
285	375	60.627	1529	695648	196147	190277	9.72	3.27	3.43
286	378	60.91	1538	61326	77556	56195	0.86	1.29	1.01
287	380	61.093	1545	26986	79609	55743	0.38	1.33	1.01
288	381	61.21	1549	37123	51184	25170	0.52	0.85	0.45
289	382	61.243	1550	37934	201250	146614	0.53	3.35	2.65

A-2 and A-3.

Calculation of the amount of each volatile compound and a list of TIC and final concentrations of two representative samples.

				Total ion counts			ng per g fat		
	Peak id	Sample # → RT	Kovats'	11	1a	1b	11	1a	1b
290	384	61.377	1555	1144818	298413	104813	16.00	4.97	1.89
291	385	61.51	1559	222581	191259	49312	3.11	3.18	0.89
292	386	61.677	1565	452227	436300	131623	6.32	7.26	2.37
293	387	61.877	1572	77107	73155	15119	1.08	1.22	0.27
294	388	61.994	1576	39245	48922	11452	0.55	0.81	0.21
295	390	62.144	1582	80583	56888	60352	1.13	0.95	1.09
296	391	62.26	1586	805930	498121	421214	11.26	8.29	7.60
297	392	62.344	1589	470147	198050	169754	6.57	3.30	3.06
298	393	62.427	1592	1279115	1148597	1004442	17.88	19.12	18.12
299	394	62.61	1599	3163199	1814686	1213680	44.21	30.21	21.90
300	395	62.71	1603	311714	139396	122219	4.36	2.32	2.20
301	396	62.827	1607	23632	42338	4589	0.33	0.70	0.08
302	398	63.027	1615	247882	362299	263096	3.46	6.03	4.75
303	399	63.177	1621	31386	36645	10182	0.44	0.61	0.18
304	400	63.227	1622	26485	16741	14597	0.37	0.28	0.26
305	401	63.261	1624	33125	28270	17570	0.46	0.47	0.32
306	402	63.444	1631	20711	12939	10281	0.29	0.22	0.19
307	404	63.611	1637	486760	223766	184262	6.80	3.73	3.32
308	405	63.744	1643	208051	163634	25842	2.91	2.72	0.47
309	406	63.844	1647	63524	23716	4153	0.89	0.39	0.07
310	407	63.927	1650	113486	100489	29413	1.59	1.67	0.53
311	408	63.994	1652	68887	46098	32806	0.96	0.77	0.59
312	409	64.161	1659	208162	105225	62208	2.91	1.75	1.12
313	410	64.261	1663	68472	80717	23003	0.96	1.34	0.41
314	411	64.361	1667	337491	201036	161728	4.72	3.35	2.92
315	412	64.527	1674	495257	225602	162332	6.92	3.76	2.93
316	413	64.644	1679	439521	263875	196982	6.14	4.39	3.55
317	414	64.761	1684	166186	56381	64475	2.32	0.94	1.16
318	415	64.844	1687	320657	190237	144384	4.48	3.17	2.60
319	416	64.994	1693	4949649	2139252	1788985	69.18	35.61	32.27
320	417	65.044	1696	393865	191267	107156	5.51	3.18	1.93
321	418	65.261	1705	58608	27909	6461	0.82	0.46	0.12
322	419	65.394	1710	313077	112250	66810	4.38	1.87	1.21
323	420	65.494	1715	50463	2146	1962	0.71	0.04	0.04
324	421	65.594	1719	423965	159846	145108	5.93	2.66	2.62
325	423	65.778	1727	227043	19277	7263	3.17	0.32	0.13
326	425	65.928	1734	48348	38107	10271	0.68	0.63	0.19
327	426	66.111	1742	37466	15839	7245	0.52	0.26	0.13
328	427	66.144	1743	360131	71244	57708	5.03	1.19	1.04
329	428	66.211	1746	74620	22074	7103	1.04	0.37	0.13
330	429	66.378	1754	45140	215719	152950	0.63	3.59	2.76
331	430	66.428	1756	106215	70228	49526	1.48	1.17	0.89
332	431	66.595	1764	157238	150991	99655	2.20	2.51	1.80
333	432	66.711	1769	321056	185776	112136	4.49	3.09	2.02
334	433	66.845	1775	32150858	13741708	13450905	449.39	228.77	242.67
335	434	66.928	1779	947230	368824	336871	13.24	6.14	6.08
336	435	67.011	1783	399115	142140	137502	5.58	2.37	2.48
337	436	67.145	1789	11093991	3474243	3446069	155.07	57.84	62.17
338	437	67.195	1791	249239	84446	77861	3.48	1.41	1.40
339	438	67.295	1796	1742909	608574	593012	24.36	10.13	10.70

A-2 and A-3.

Calculation of the amount of each volatile compound and a list of TIC and final concentrations of two representative samples.

				Total ion counts			ng per g fat		
	Peak Id	RT	Kovats'	11	1a	1b	11	1a	1b
340	439	67.561	1809	579639	107194	74989	8.10	1.78	1.35
341	440	67.611	1811	391863	620317	476884	5.48	10.33	8.60
342	441	67.761	1818	688443	265407	245503	9.62	4.42	4.43
343	442	67.895	1825	66789444	25831926	25039058	933.55	430.04	451.73
344	443	68.011	1830	25347690	7387162	7461528	354.30	122.98	134.61
345	445	68.228	1841	71166	34028	25488	0.99	0.57	0.46
346	446	68.378	1848	4249763	1447883	1385265	59.40	24.10	24.99
347	447	68.428	1851	1116373	315387	270553	15.60	5.25	4.88
348	448	68.528	1856	480516	122748	96834	6.72	2.04	1.75
349	449	68.762	1868	5810719	2627800	2760257	81.22	43.75	49.80
350	450	68.895	1875	61393	104327	109524	0.86	1.74	1.98
351	451	69.128	1886	669024	248783	122494	9.35	4.14	2.21
352	452	69.178	1889	325429	126256	83915	4.55	2.10	1.51
353	453	69.345	1898	144106	211263	155134	2.01	3.52	2.80
354	455	69.445	1903	96555	26717	20410	1.35	0.44	0.37
355	456	69.512	1907	322316	79999	64537	4.51	1.33	1.16
356	457	69.578	1910	131309	26409	22921	1.84	0.44	0.41
357	459	69.728	1918	44590	14863	18734	0.62	0.25	0.34
358	460	69.812	1922	214735	10335	16947	3.00	0.17	0.31
359	461	69.862	1925	80298	7518	2963	1.12	0.13	0.05
360	462	69.945	1930	136586	8833	10706	1.91	0.15	0.19
361	463	70.062	1936	79193	9918	1951	1.11	0.17	0.04
362	464	70.145	1940	1168639	200889	227201	16.33	3.34	4.10
363	465	70.329	1950	624755	185330	106444	8.73	3.09	1.92

A-2 and A-3.

Calculation of the amount of each volatile compound and a list of TIC and final concentrations of two representative samples.

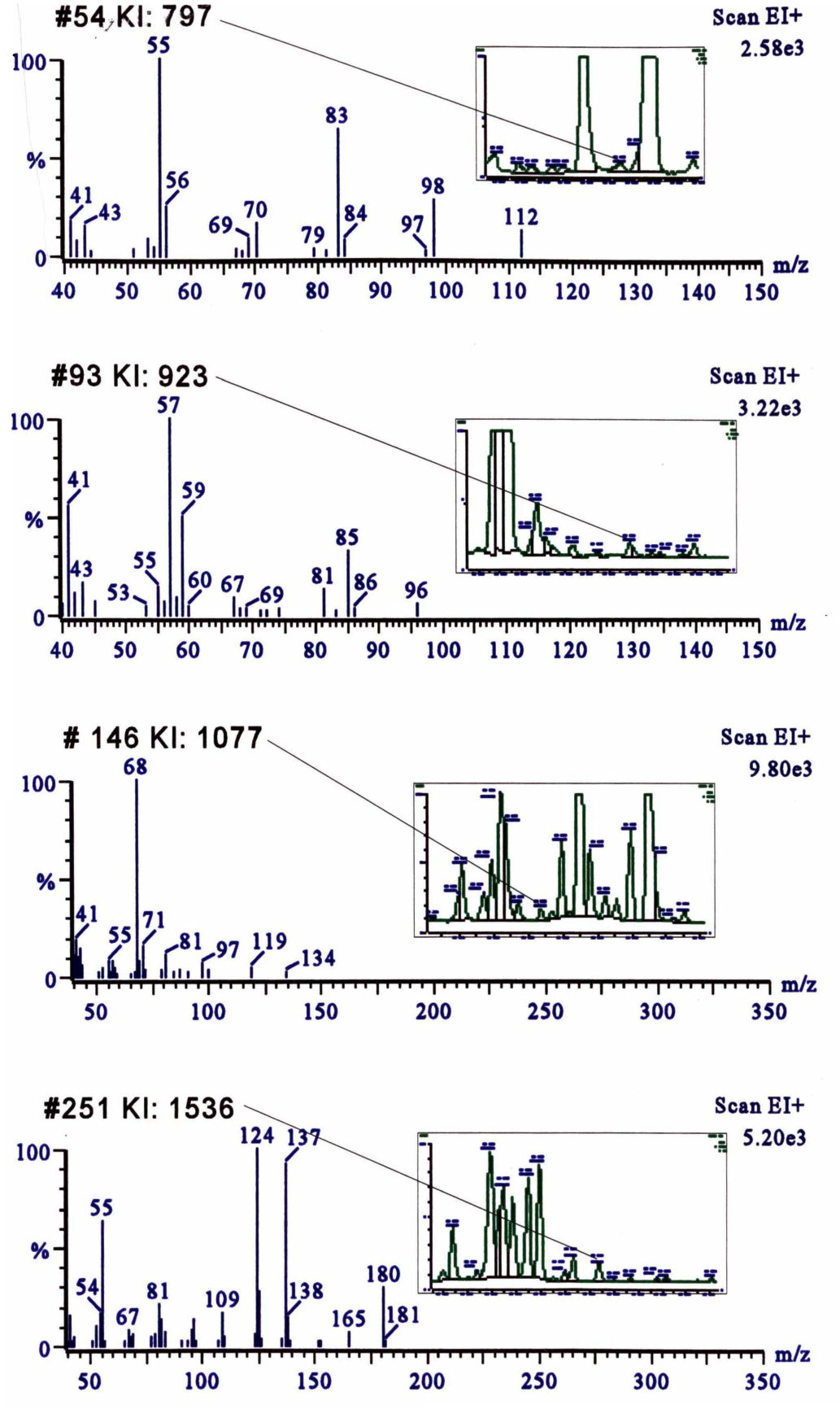


Figure A-4a

Mass spectra of unknown compounds found in the headspace of cooked sheepmeat that changed significantly with increasing meat ultimate pH (chapter 4).

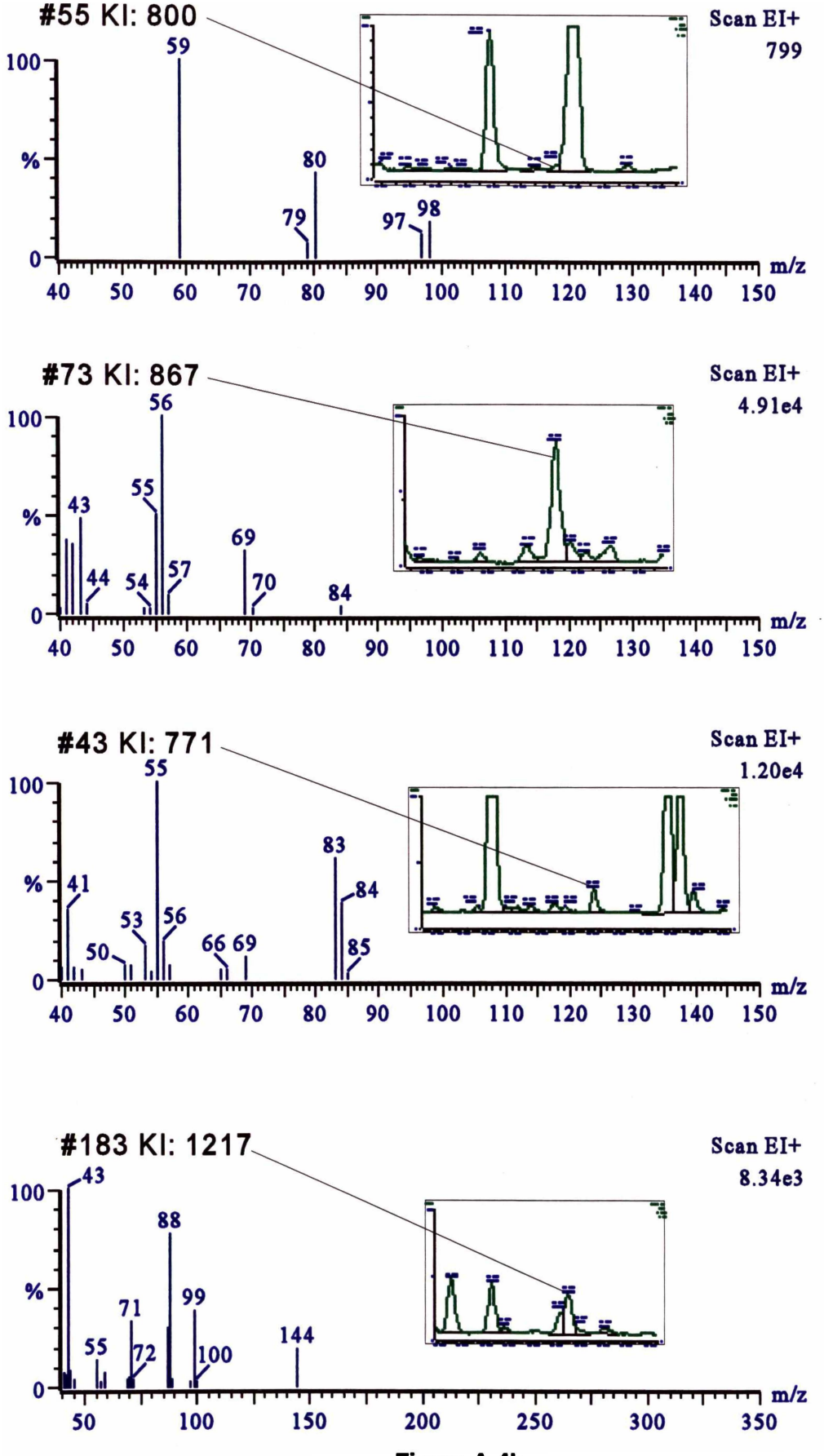


Figure A-4b.

Mass spectra of unknown compounds found inthe headspace of cooked sheepmeat that changed significantly with increasing meat ultimate pH (chapter 4).

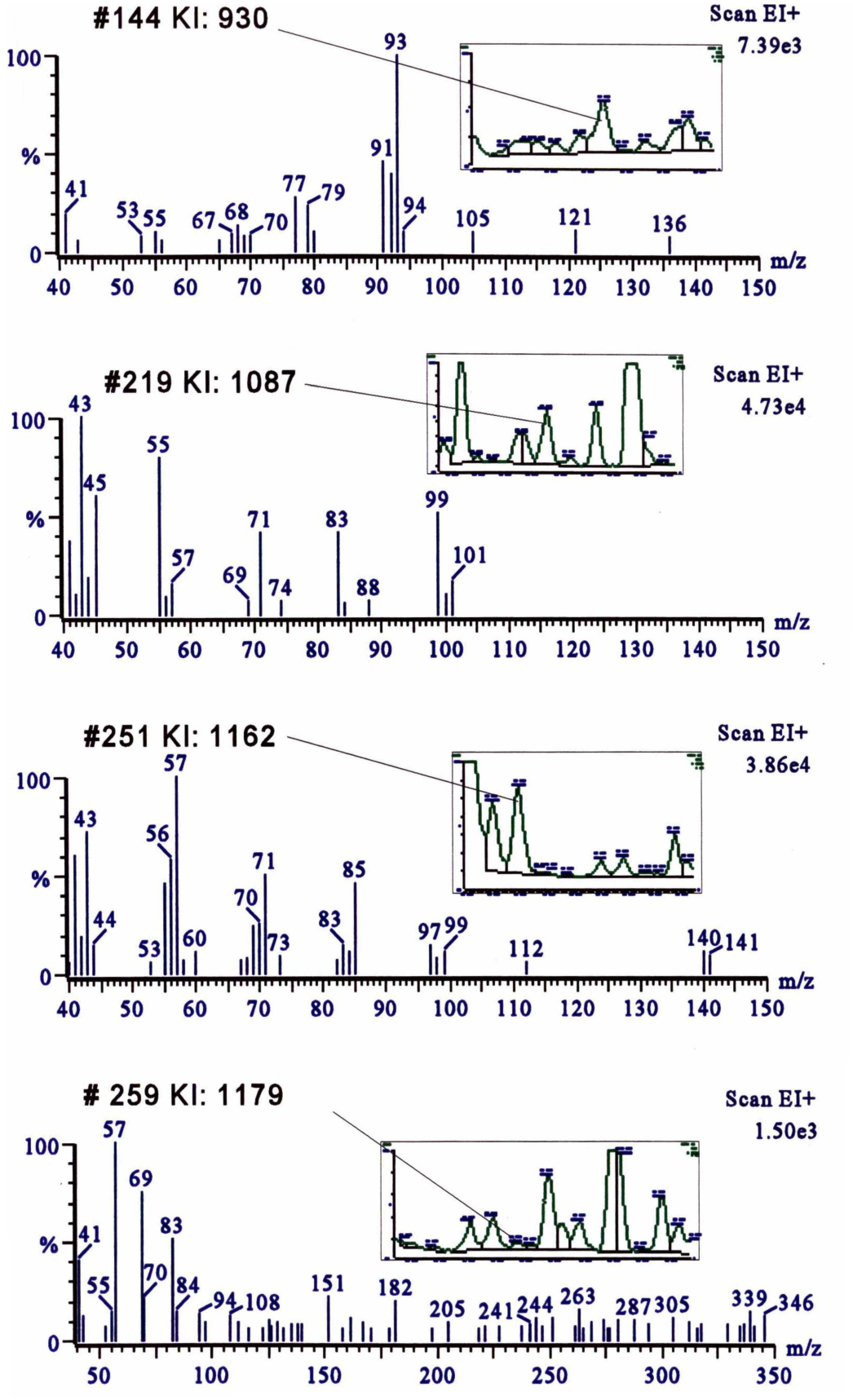


Figure A-5.
Mass spectra of unknown compounds found inthe headspace of cooked sheepmeat that changed significantly between the low-, medium- and high pH groups after titration with HCl (chapter 5).

Appendix B.

Calculation of Free Fatty Acid Content

A series of fatty acid methyl ester external standards spanning 0.05 to 2 mg ml^{-1} were used to determine individual fatty acid methyl esters (FAMES) concentrations in each sample. Calculations were done using Maxima Integration software (Water, Inc,).

For standards:

$$\text{Response factor (Rs)} = \frac{\text{peak area of standard}}{\text{peak area of internal standard}}$$

The Maxima calibration curve plots the Response factor (R*s*) * Internal standard amount vs standard concentration (mg). Using *Least Squares*, the software calculated the curve which fitted the data best.

The concentration of FAME (mg g^{-1}) for each sample was calculated as follows:

FAMES (mg mL^{-1}) = conversion factor from curve fit * (R * sample internal standard amount).

Where:

$$\text{R (Response factor)} = \frac{\text{peak area of sample}}{\text{peak area of internal standard}}$$

$$\text{Sample concentration (mg } g^{-1}) = \text{FAME (mg } mL^{-1}) * \frac{\text{Dilution factor}}{\text{sample weight}}$$

Conversion of fatty acid methyl esters to free fatty acids:

$$\text{Free fatty acid } (\mu g\ g^{-1}) \quad \frac{\text{MW fatty acid}}{\text{MW FAME}} \times \text{FAME } (mg\ g^{-1} \quad 1000)$$

where: MW = molecular weight of individual fatty acid or FAME

An example chromatogram, standard curve, set of calculations, and full set of free fatty acid results are presented on the following pages (figures B-1, B-2, B-3, B-4, respectively).

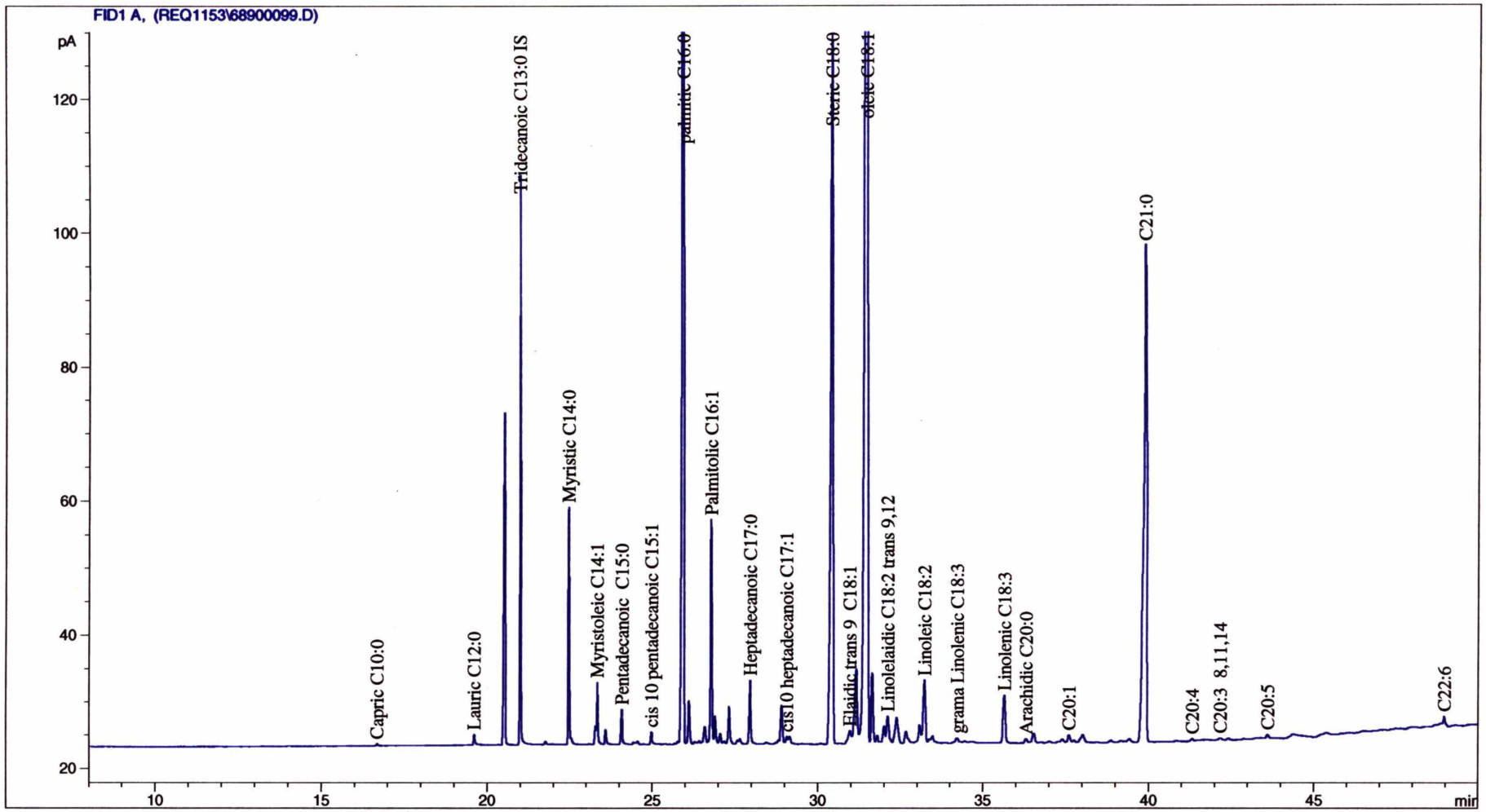


Figure B-1.
Chromarographic profile of methyl esters of free fatty acids extracted from minced *semimembranosus* muscles.

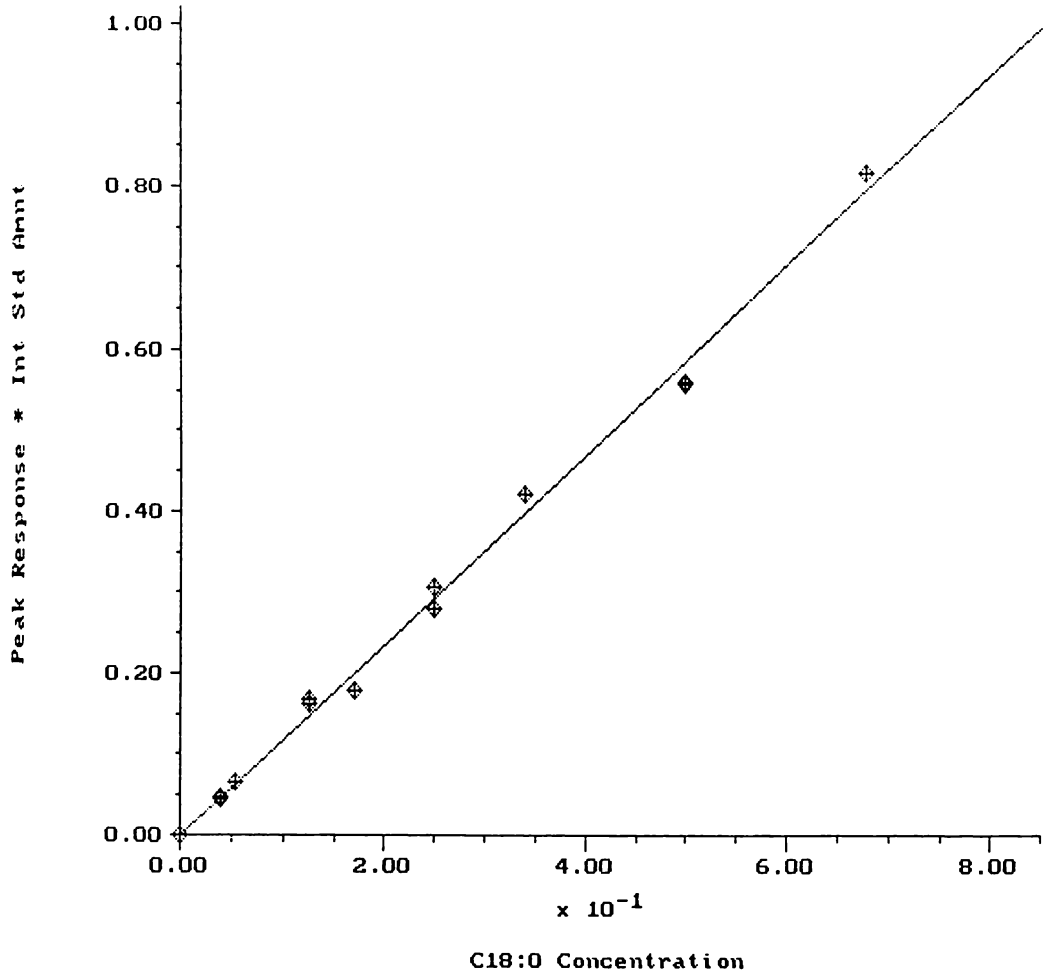


Figure B-2.
Standard curve plot of methyl ester.

	B	C	D	E	F	G	H	I	J	K	M
15		Fatty acid methyl esters									FFA
16	Sample				Sample	Internal	Standard	Solution	Sample	Conversion	
17	11M	Peak area	Sample	Dilution	peak	standard	curve	conc.	conc.	factor	ug per g fat
18	NAME		wt (g)	factor	response (R)	Conc. (mg)	equation	(mg)	ug per g fat		
19	C8:0	1196	4.044	10	0.001043441	0.5	1.239012	0.00065	1.60	0.91139	1.46
20	C9:0	1693	4.044	10	0.001477044	0.5	1.162006	0.00086	2.12	0.91860	1.95
21	C10:0	2499	4.044	10	0.002180233	0.5	1.086035	0.00118	2.93	0.92473	2.71
22	C11:0		4.044	10		0.5	1.016913			0.93000	
23	C12:0	5516	4.044	10	0.00481239	0.5	0.9906487	0.00238	5.89	0.93458	5.51
24	C14:0	37986	4.044	10	0.033140582	0.5	0.9614454	0.01593	39.40	0.93860	36.98
25	C14:1	550	4.044	10	0.000479843	0.5	0.9484066	0.00023	0.56	0.94215	0.53
26	C15:0	10555	4.044	10	0.009208625	0.5	0.9423529	0.00434	10.73	0.94531	10.14
27	C16:0	282624	4.044	10	0.246573048	0.5	0.9087874	0.11204	277.06	0.94815	262.69
28	C16:1	18235	4.044	10	0.01590898	0.5	0.9924803	0.00789	19.52	0.94776	18.50
29	C17:0	17342	4.044	10	0.015129889	0.5	0.905809	0.00685	16.94	0.95070	16.11
30	C18:0	262371	4.044	10	0.22890348	0.5	0.8553772	0.09790	242.09	0.95302	230.71
31	C18:1	442716	4.044	10	0.386244032	0.5	0.901251	0.17405	430.39	0.95270	410.04
32	C18:2	60220	4.044	10	0.052538457	0.5	0.9741482	0.02559	63.28	0.95238	60.27
33	C19:0	5916	4.044	10	0.005161367	0.5	0.8351398	0.00216	5.33	0.95513	5.09
34	C18:3	35210	4.044	10	0.030718683	0.5	1.106603	0.01700	42.03	0.95205	40.01
35	C18:4	15424	4.044	10	0.013456545	0.5	0.9436266	0.00635	15.70	0.95172	14.94
36	C20:0	23473	4.044	10	0.020478831	0.5	0.7796786	0.00798	19.74	0.95706	18.89
37	C20:1		4.044	10		0.5	0.7636635			0.95679	
38	C20:2		4.044	10		0.5	0.9826826			0.95652	
39	C21:0	22413	4.044	10	0.019554043	0.5	0.759757	0.00743	18.37	0.95882	17.61
40	C20:4	16073	4.044	10	0.01402276	0.5	0.9640492	0.00676	16.71	0.95597	15.98
41	C20:3		4.044	10		0.5	0.9620272			0.95625	
42	C22:0	53401	4.044	10	0.046589275	0.5	0.7329574	0.01707	42.22	0.96045	40.55
43	C20:5	15843	4.044	10	0.013822099	0.5	0.9902511	0.00684	16.92	0.95570	16.17
44	C22:1	18664	4.044	10	0.016283257	0.5	0.3904912	0.00318	7.86	0.96000	7.55
45	C24:0	35786	4.044	10	0.031221209	0.5	0.3345	0.00522	12.91	0.96335	12.44
46	C24:1	42643	4.044	10	0.037203544	0.5	0.3708552	0.00690	17.06	0.96316	16.43
47	C22:6	7126	4.044	10	0.006217022	0.5	0.9541368	0.00297	7.33	0.95977	7.04
48	(IS) C13:0	1146208									

Figure B-3.

Example of calculations used to convert FAME peak areas to free fatty acids (ug g^{-1} fat).

Free fatty acid raw data: concentrations in ug per g sample.
Example data from chapter 5.

Sample ID	pH	C8:0	C9:0	C10:0	C11:0	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C19:0	C18:3	C18:4	C20:0	C20:1	C20:2	C21:0	C20:4	C20:3	C22:0	C20:5	C22:1	C24:0	C24:1	C22:6	Total ug per g	mg g	% Lipid
37/1	5.54	0.57	1.49	2.61	0.30	19.76	24.87	0.42	7.58	235.55	15.98	15.15	219.94	405.06	79.74	1.58	70.63	10.72	2.17	1.10	2.65	0.98	16.10	0.97	5.26	22.65	0.23	0.99	6.73	9.05	1181	1.18	17.88
39/1	5.61	0.56	1.26	2.39	0.17	23.98	29.10	0.67	7.35	251.93	15.83	14.89	216.16	408.93	83.25	1.21	54.98	15.94	2.34	1.17	2.59	0.77	21.17	0.63	5.10	22.73	0.00	1.03	7.16	9.09	1202	1.20	16.41
48/1	5.64	1.02	21.60	4.55	0.58	43.71	31.04	0.60	9.62	253.84	16.85	16.41	214.69	376.81	87.60	1.49	63.10	15.40	3.39	1.15	3.74	1.04	20.84	0.63	10.52	28.00	0.72	1.45	7.80	11.41	1250	1.25	16.52
18/1	5.65	0.54	1.39	1.19	0.17	15.48	16.80	1.02	5.55	173.24	9.99	12.03	217.89	285.67	65.33	0.00	40.80	10.85	2.76	1.03	2.97	0.95	18.82	0.56	5.77	17.68	0.17	0.95	5.58	6.27	921	0.92	18.35
19/1	5.66	0.73	1.42	3.01	0.00	16.63	26.35	1.85	6.58	248.24	19.72	16.01	230.54	454.63	58.16	1.36	38.60	17.55	3.31	1.19	3.67	0.96	19.55	0.00	8.09	17.75	0.32	3.56	6.19	8.81	1215	1.21	19.60
29/1	5.7	0.66	1.59	2.34	0.34	16.32	22.22	0.68	5.73	174.32	12.22	11.96	174.01	297.90	49.56	1.25	30.84	13.41	2.08	0.80	2.29	0.79	15.38	0.37	4.42	13.32	0.14	0.89	4.29	5.82	866	0.87	18.58
38/1	5.7	0.87	1.94	2.40	0.00	10.42	35.55	1.70	9.53	292.30	22.98	16.51	255.77	453.13	102.27	0.93	62.80	21.58	2.38	1.22	3.28	0.77	28.48	0.80	5.44	23.15	0.00	0.93	6.05	11.83	1375	1.38	19.45
40/1	5.72	0.52	0.93	2.64	0.12	20.29	23.91	0.71	6.88	199.24	12.35	11.92	187.08	309.13	76.38	1.21	62.80	10.49	2.16	1.05	2.41	0.81	15.61	0.84	5.19	21.55	0.18	0.98	6.50	9.55	993	0.99	14.97
49/1	5.72	0.98	13.22	4.10	0.60	38.96	36.24	0.70	10.70	249.73	17.36	13.88	230.44	383.78	80.48	1.64	56.92	17.72	4.02	1.43	3.27	1.49	21.24	1.12	11.26	22.51	0.49	1.85	6.92	10.03	1243	1.24	15.06
20/1	5.78	0.74	1.43	2.44	0.43	16.48	22.76	0.60	6.45	200.67	14.64	13.85	202.00	378.17	64.25	1.58	38.39	15.74	2.15	1.12	3.21	0.95	19.18	0.61	4.92	17.71	0.09	0.88	6.51	8.95	1047	1.05	16.38
3/1	6.14	0.57	1.16	1.83	0.00	20.10	18.84	1.02	1.00	178.51	14.42	13.03	202.81	332.78	59.38	1.68	46.25	15.62	2.65	1.48	2.05	0.00	13.59	0.00	7.43	15.82	0.00	4.24	4.98	7.00	968	0.97	18.52
34/1	6.14	0.75	2.56	2.26	0.29	21.37	23.06	1.40	6.73	186.28	13.43	13.18	205.80	315.33	57.00	1.50	37.26	12.75	2.91	1.14	2.42	0.69	14.81	0.00	8.50	13.41	0.00	5.21	5.16	4.62	960	0.96	19.89
21/1	6.16	0.85	1.43	2.39	0.27	20.65	28.53	0.98	7.47	192.29	14.43	13.62	206.61	314.42	75.18	1.60	47.56	18.24	2.64	1.22	3.04	0.94	23.73	0.84	5.16	21.50	0.27	1.14	7.97	12.30	1027	1.03	14.26
33/1	6.25	1.05	1.64	1.95	0.43	20.53	24.77	0.49	9.35	187.33	14.08	15.38	243.43	321.32	60.53	2.39	45.23	11.98	4.12	1.28	2.51	1.71	12.70	0.81	8.70	11.99	0.17	1.61	5.59	8.03	1021	1.02	18.01
36/1	6.25	0.85	17.39	2.56	0.27	41.88	21.48	0.24	7.78	213.33	13.20	13.98	206.67	332.61	72.22	1.75	51.10	17.07	3.94	1.57	3.30	1.41	18.09	1.28	11.19	18.38	0.56	1.79	5.34	7.41	1089	1.09	16.99
1/1	6.28	0.40	0.90	1.81	0.00	19.50	21.20	0.79	5.95	180.08	14.11	12.37	188.62	303.41	41.98	1.32	27.73	11.46	2.38	1.33	2.55	0.92	9.42	0.35	5.77	11.70	0.16	4.15	3.47	4.92	879	0.88	17.16
4/1	6.28	0.69	1.17	2.46	0.00	21.92	45.20	1.68	7.92	259.59	22.85	14.21	250.30	469.63	65.32	1.97	50.20	22.32	3.20	1.71	4.56	1.10	11.83	0.87	8.69	14.85	0.19	5.30	6.87	7.51	1304	1.30	19.20
5/1	6.29	0.86	18.69	4.09	0.41	40.53	41.67	0.87	11.79	290.92	22.39	15.12	259.86	500.32	68.63	2.03	51.28	21.41	4.13	2.20	4.82	1.65	19.77	1.09	11.38	21.99	0.59	1.77	6.28	11.77	1438	1.44	18.08
2/1	6.33	0.39	1.05	1.26	0.00	17.98	24.33	1.08	5.56	156.78	12.27	9.82	153.25	322.32	52.93	1.25	40.60	18.87	2.02	1.14	2.73	0.75	14.51	0.55	4.43	17.60	0.15	0.77	5.30	7.89	878	0.88	13.84
32/1	6.33	1.38	4.59	3.61	0.00	10.70	34.95	1.32	12.10	261.40	22.33	16.91	306.04	494.22	80.72	2.56	70.42	15.52	2.78	0.00	4.07	0.00	16.90	1.13	5.22	19.00	0.00	1.00	7.61	7.06	1404	1.40	18.79
11/1	6.36	1.43	5.81	2.38	0.00	5.66	29.49	0.00	8.78	214.49	16.85	14.48	210.87	415.09	46.58	0.00	38.86	15.69	2.68	38.13	4.25	0.00	13.33	0.00	6.19	14.16	0.00	0.00	5.34	7.44	1118	1.12	15.23
24/1	6.44	1.75	2.07	3.60	0.38	24.01	31.41	0.60	10.18	252.27	21.04	17.03	249.59	426.09	78.81	2.01	71.69	17.09	3.43	1.72	4.35	1.22	18.33	1.70	8.94	24.53	0.38	1.31	7.10	9.78	1292	1.29	15.34
14/1	6.68	0.42	1.14	1.82	0.00	19.31	32.42	1.09	7.32	203.34	17.11	13.10	213.24	438.84	53.08	1.66	40.44	19.17	2.55	1.63	3.51	0.99	11.93	0.67	4.19	13.49	0.18	0.84	5.61	5.50	1115	1.11	20.31
35/1	6.69	1.49	2.22	3.36	0.00	9.01	27.79	0.80	7.91	243.34	24.02	15.77	251.92	418.08	54.56	1.92	45.84	14.04	2.37	4.83	3.92	0.00	12.22	0.00	3.84	11.98	0.00	0.94	3.97	5.02	1171	1.17	21.11
26/1	6.77	0.73	5.59	2.00	0.00	9.55	34.43	1.37	7.50	213.24	21.23	12.45	210.94	462.32	60.91	1.35	41.15	24.86	2.31	2.08	4.66	0.00	18.30	0.00	3.32	16.84	0.00	0.78	4.90	7.53	1170	1.17	15.29
16/1	6.84	0.40	0.62	2.23	0.18	21.32	31.73	0.82	7.99	215.20	18.35	13.83	226.54	449.52	83.39	1.44	56.38	23.91	2.60	2.05	5.18	0.98	17.62	0.82	4.57	24.03	0.20	0.75	5.87	3.83	1222	1.22	16.66
45/1	6.86	0.58	1.25	1.98	0.28	21.29	27.92	0.81	8.54	236.11	19.19	17.74	267.98	428.23	94.38	1.83	67.56	16.42	2.93	1.73	4.92	0.77	19.76	0.86	4.65	26.09	0.45	1.01	6.11	8.31	1290	1.29	15.00
46/1	6.9	1.07	2.27	3.20	0.45	24.66	34.33	0.90	9.14	225.15	18.69	14.81	249.24	476.78	79.77	2.30	58.32	26.57	3.80	2.14	4.76	1.41	21.65	1.25	8.58	23.06	0.76	1.34	5.75	6.65	1309	1.31	16.42
17/1	6.92	0.37	0.69	2.26	0.00	24.67	32.23	0.81	7.23	203.71	16.64	12.59	213.00	393.31	87.28	1.67	52.59	20.87	2.60	2.83	4.83	0.98	19.62	1.27	4.16	23.25	0.24	0.77	6.44	4.81	1142	1.14	16.92
47/1	6.98	0.86	1.58	3.42	0.00	12.10	31.55	1.13	7.80	211.40	17.10	11.71	187.46	350.73	83.88	1.77	53.77	14.61	2.94	2.46	2.42	1.29	23.89	0.00	5.25	19.51	0.00	1.00	6.31	12.19	1068	1.07	15.11
																															0	0.00	
3/2	6.14	0.83	1.29	2.29	0.27	20.47	26.89	0.53	7.23	224.30	16.82	15.89	237.19	349.06	79.85	1.93	54.57	14.19	2.92	1.08	3.00	0.96	18.38	0.62	6.35	19.10	0.24	1.00	6.29	7.44	1121	1.12	17.14
34/2	6.14	1.21	2.02	2.84	0.41	20.76	28.01	2.04	8.21	224.72	15.22	15.56	229.70	367.25	77.39	1.68	49.37	15.73	2.98	1.07	3.47	1.97	18.63	0.43	6.83	17.58	0.07	1.22	6.39	7.20	1130	1.13	20.11
21/2	6.16	0.79	1.01	2.15	0.33	21.11	33.70	1.21	9.48	221.69	16.85	14.74	213.34	343.86	90.26	1.72	56.26	20.17	2.84	1.28	3.36	1.23	26.84	0.79	6.40	25.13	0.89	1.03	8.30	13.21	1140	1.14	14.80
33/2	6.25	0.85	1.55	2.15	0.30	20.56	27.67	0.56	10.20	212.14	16.66	16.19	256.80	383.62	75.63	1.92	59.61	14.20	2.91	1.48	2.66	0.88	17.67	0.82	4.89	16.80	0.45	0.89	6.34	11.33	1168	1.17	17.39
36/2	6.25	0.87	7.30	2.08	0.00	7.19	19.35	1.28	7.26	220.13	14.95	13.13	205.74	384.60	85.85	1.12	65.71	18.21	2.18	1.31	3.18	0.79	25.36	0.00	4.55	25.29	0.00	0.98	7.00	9.61	1135	1.14	14.62
1/2	6.28	0.64	1.10	1.95	0.00	16.94	23.89	1.01	6.29	207.37	15.34	13.81	209.33	344.68	51.43	1.53	34.62	12.17	2.27	1.36	2.93	0.83	11.82	0.54	5.22	14.30	0.22	0.98	4.30	5.22	992	0.99	17.08
4/2	6.28	0.54	1.08	2.82	0.00	23.03	48.19	1.99	8.54	277.17	24.05	14.37	249.45	495.36	73.80	1.81	59.89	24.27	2.65	1.56	4.49	1.13	14.84	0.95	8.17	19.13	0.19	3.30	6.34	9.39	1379	1.38	17.96

Figure B-4.
Free fatty acid results, expressed as ug g^{-1} sample, from chapter 5.

Sample ID	pH	C8:0	C9:0	C10:0	C11:0	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C19:0	C18:3	C18:4	C20:0	C20:1	C20:2	C21:0	C20:4	C20:3	C22:0	C20:5	C22:1	C24:0	C24:1	C22:6	Total ug per g	mg g	% Lipid
5/2	6.29	1.12	18.94	4.43	0.65	40.29	41.04	0.88	12.13	302.73	24.82	16.44	250.19	509.87	78.08	1.93	57.34	22.28	4.54	2.09	4.33	1.24	23.68	1.22	13.77	26.61	0.53	2.16	7.07	13.15	1484	1.48	18.69
2/2	6.33	0.69	1.05	2.05	0.00	18.77	32.79	0.55	7.12	203.74	15.02	12.33	187.01	372.07	71.17	1.45	53.20	20.37	2.14	1.01	2.67	1.04	19.30	0.62	8.55	24.26	0.26	2.77	6.92	13.08	1082	1.08	14.90
32/2	6.33	0.85	1.23	3.84	0.00	24.47	34.78	0.65	11.98	276.52	22.03	18.45	299.79	496.05	96.30	2.25	84.63	20.70	3.93	1.95	3.90	1.30	21.54	1.10	9.71	26.14	0.51	1.45	9.40	10.87	1486	1.49	16.36
11/2	6.36	1.60	2.07	2.52	0.00	5.69	33.83	0.00	8.66	247.87	19.24	13.76	214.82	421.98	63.82	0.00	47.42	17.26	2.89	0.00	0.00	0.00	17.26	0.00	6.92	19.97	0.00	4.91	7.02	6.98	1167	1.17	15.12
24/2	6.44	0.97	15.44	4.46	0.41	42.48	37.27	0.69	11.25	305.82	23.46	19.04	290.04	482.63	100.65	2.18	87.50	19.40	4.44	1.93	4.55	1.03	23.50	2.02	11.56	30.74	0.46	1.77	8.45	12.42	1547	1.55	18.68
14/2	6.68	1.32	1.50	1.93	0.00	20.16	36.06	1.58	8.16	223.81	19.83	13.57	207.41	455.72	67.24	1.56	49.65	20.03	2.46	1.51	3.46	1.08	16.62	1.15	10.38	22.90	0.41	1.35	7.36	8.23	1206	1.21	18.30
35/2	6.69	0.78	1.06	3.00	0.20	19.01	28.42	0.84	7.62	253.80	20.13	17.55	246.32	425.72	71.00	1.52	57.31	15.76	2.63	1.64	3.41	0.94	16.34	0.75	5.70	18.23	0.15	0.99	6.31	8.95	1236	1.24	18.43
26/2	6.77	0.93	1.25	3.79	0.30	23.76	45.06	1.56	9.86	282.97	25.48	15.65	232.09	570.75	84.47	1.51	61.27	32.11	2.75	1.94	5.40	0.91	31.07	0.94	7.59	29.08	0.83	1.08	7.92	15.73	1498	1.50	13.61
16/2	6.84	1.22	3.33	2.68	0.30	25.92	41.47	1.04	9.59	260.72	21.86	15.11	234.77	489.76	78.84	1.65	60.61	30.60	3.64	2.19	4.48	1.11	20.30	1.37	9.46	24.01	0.78	1.35	8.19	7.42	1364	1.36	15.67
45/2	6.86	1.29	7.31	4.44	0.39	20.84	33.69	0.68	9.81	288.64	24.68	19.64	281.46	574.77	100.94	1.96	83.48	22.06	3.35	2.21	5.12	1.12	23.81	1.49	8.99	29.08	0.48	1.25	7.48	13.71	1574	1.57	15.20
46/2	6.9	1.49	7.89	2.87	0.00	12.99	39.33	1.38	9.04	257.70	22.74	13.55	246.98	517.20	87.75	0.75	64.67	24.72	2.72	3.51	3.86	0.80	26.33	0.00	5.29	23.77	0.43	1.03	7.73	11.25	1398	1.40	15.66
17/2	6.92	1.21	1.78	3.79	0.88	20.66	41.91	1.16	9.85	263.38	20.47	16.44	251.47	500.42	97.54	2.10	64.30	27.99	3.13	3.05	5.26	1.55	24.39	1.31	5.48	27.32	0.23	1.03	9.33	8.97	1416	1.42	19.26
47/2	6.98	0.84	1.52	2.97	0.72	23.07	37.30	0.96	9.24	255.49	16.70	15.88	212.90	402.45	99.33	1.68	66.43	18.02	2.69	1.30	2.82	1.19	26.48	1.03	5.63	23.76	0.19	1.15	8.30	15.23	1255	1.26	14.60

Figure B-4.

Free fatty acid results, expressed as ug g^{-1} sample, from chapter 5.

Appendix C.

Free Amino Acid Data

All free amino acid data, expressed as μmole $100g^{-1}$ sample, is presented on the following pages (figure C-1).

The method of calculation is presented in Chapter 5.

Amino acid raw data: concentrations in umole per 100g sample.

Example data from chapter 5.

		1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	27	28	29	30	
Sample		Asp	Glu	OH	Ser	Asn	Gly	Gln	bAla	Tau	His	Thr	Ala	Carn.	Arg	Pro	1Me	3Me	ABA	Tyr	Val	Met	Cyst	Ile	Leu	Phe	Try	Orn	Lys	Total
ID	**pH**			pro													His	His												
37/1	**5.54**	5.5	298	3.0	31.7	199.6	108.0	2543	14.9	312.9	12.1	28.0	290	795	34.5	25.8	1.0	612.6	11.3	19.3	34.5	16.8	1.5	22.9	48.8	23.0	6.0	4.9	29.6	5535
39/1	**5.61**	0.9	239	2.4	15.5	64.9	72.3	900	6.2	123.9	4.0	13.5	60	384	12.4	17.0	0.8	255.1	4.3	9.0	14.4	5.9	0.4	10.4	19.9	9.7	1.3	0.7	7.8	2255
48/1	**5.64**	4.7	308	3.6	27.0	244.9	95.9	2838	19.3	235.9	15.4	35.2	275	939	36.2	35.2	4.5	561.3	8.6	18.5	36.7	49.6	1.1	23.5	48.4	25.4	6.7	2.7	25.2	5925
18/1	**5.65**	2.2	313	1.9	25.3	103.6	89.9	1199	9.8	97.4	7.0	21.7	135	509	20.1	22.8	0.8	360.3	13.2	13.6	21.6	10.3	0.4	13.0	26.8	14.3	2.6	1.2	5.8	3042
19/1	**5.66**	0.7	286	1.0	10.1	30.2	67.3	345	10.0	130.6	1.0	9.1	26	281	5.3	15.1	0.4	237.1	4.2	5.5	9.4	3.0	0.4	7.8	13.0	5.1	1.0	0.4	5.2	1510
29/1	**5.7**	1.3	205	2.4	21.0	73.2	97.0	1455	7.8	105.5	5.9	18.3	112	354	18.8	20.1	0.9	365.6	10.7	9.8	16.4	6.7	0.4	10.0	22.1	11.2	1.8	1.1	3.4	2958
38/1	**5.7**	6.0	228	2.2	30.6	302.4	114.6	2867	19.6	154.3	19.2	37.9	296	643	46.3	29.3	4.8	505.1	22.5	20.6	41.4	22.0	1.5	27.6	54.3	26.4	6.3	5.2	36.9	5570
40/1	**5.72**	0.0	216	1.5	8.1	20.7	55.2	509	6.7	119.0	0.7	9.4	17	336	5.1	14.1	0.4	212.5	6.2	4.4	7.7	2.6	0.4	5.8	10.8	4.1	1.0	0.8	2.3	1577
49/1	**5.72**	4.1	305	2.6	23.9	219.3	96.3	2885	12.5	177.6	13.4	30.0	226	749	33.8	32.6	4.7	442.6	17.9	18.3	34.3	16.4	1.5	22.8	48.3	25.4	6.8	3.9	21.1	5475
20/1	**5.78**	2.0	266	1.6	19.4	84.0	68.1	936	9.7	120.1	5.9	16.1	131	367	16.3	18.4	0.4	327.5	18.3	10.5	17.6	6.9	0.4	11.0	22.9	11.2	2.1	0.8	1.8	2494
3/1	**6.14**	1.2	321	2.6	19.4	58.7	96.0	1001	9.4	171.9	1.5	14.9	71	436	14.0	19.4	0.4	272.9	13.5	6.5	12.2	3.7	0.0	5.6	12.8	3.6	0.4	0.0	0.0	2570
34/1	**6.14**	0.0	265	2.3	12.1	27.2	80.3	670	5.0	74.1	0.9	10.3	20	305	6.1	19.9	0.7	225.7	7.5	5.5	9.0	3.0	0.4	7.8	12.1	4.9	2.4	1.0	4.7	1784
21/1	**6.16**	3.5	333	4.0	37.8	195.3	139.3	2494	11.6	207.9	11.9	26.3	179	448	36.7	33.4	1.2	496.9	7.0	16.5	28.4	14.5	1.1	18.7	38.6	20.4	3.8	2.8	1.0	4812
33/1	**6.25**	3.3	214	2.5	33.8	165.5	94.2	1693	12.6	125.5	10.2	21.7	128	466	32.3	21.4	1.2	331.1	19.6	14.1	24.7	11.7	0.4	15.8	32.0	17.1	4.1	2.2	17.9	3515
36/1	**6.25**	6.1	417	3.9	62.6	417.2	191.3	4661	26.0	243.5	26.7	66.9	330	1044	75.6	45.3	2.1	735.0	32.5	31.9	65.9	27.9	3.3	36.5	75.5	34.0	8.2	5.4	59.0	8734
1/1	**6.28**	1.5	286	3.4	30.8	125.0	117.0	1894	6.0	211.2	2.4	22.0	138	496	26.4	24.9	1.0	383.1	11.1	11.5	23.7	5.5	0.0	12.8	25.5	10.9	0.0	1.1	3.2	3874
4/1	**6.28**	12.2	427	5.0	83.4	363.3	210.1	3231	10.5	258.1	13.8	57.6	236	673	60.6	56.9	2.0	571.0	16.2	28.6	56.8	18.3	0.8	29.1	55.1	28.6	5.6	3.5	41.1	6555
5/1	**6.29**	5.8	373	5.4	42.5	284.4	180.1	4236	17.4	245.0	19.3	47.6	275	1005	58.0	44.2	1.6	747.1	20.9	22.4	42.3	19.8	3.0	22.2	48.7	26.8	6.7	4.6	40.8	7846
2/1	**6.33**	2.7	365	2.6	34.9	142.0	80.7	1396	6.1	99.9	1.8	25.3	104	334	24.9	27.9	0.4	329.5	7.7	13.3	20.1	3.8	0.0	7.3	17.4	5.0	0.8	0.0	0.6	3054
32/1	**6.33**	5.7	353	5.0	36.1	260.2	128.9	2030	12.5	204.7	13.8	36.1	164	581	39.4	41.6	8.4	429.9	7.8	20.4	38.3	10.9	0.5	21.4	40.3	23.6	1.7	2.6	25.5	4542
11/1	**6.36**	9.6	405	3.8	58.2	379.6	180.2	3251	22.7	214.8	19.3	60.0	308	1035	80.8	51.5	1.7	628.3	51.4	26.5	59.2	14.6	2.8	32.2	63.7	28.7	5.8	5.4	44.7	7044
24/1	**6.44**	9.7	450	3.6	45.4	314.8	136.9	2809	16.1	204.5	21.7	56.5	256	843	53.9	57.9	1.6	560.9	22.2	21.9	52.8	14.0	1.4	27.7	50.2	25.5	4.8	4.2	36.9	6104

Figure C-1.

Free amino acid data, expressed as umole 100 g^{-1} sample, from chapter 5.

		1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	27	28	29	30	
Sample		Asp	Glu	OH	Ser	Asn	Gly	Gln	bAla	Tau	His	Thr	Ala	Carn.	Arg	Pro	1Me	3Me	ABA	Tyr	Val	Met	Cyst	Ile	Leu	Phe	Try	Orn	Lys	Total
ID	pH			pro													His	His												
14/1	**6.68**	12.0	431	3.4	66.2	300.5	151.7	2998	14.1	218.5	14.6	45.4	218	713	51.1	45.0	1.7	445.9	19.8	22.2	43.6	13.6	5.7	23.0	42.9	22.8	4.8	4.3	32.1	5965
35/1	**6.69**	16.2	386	3.6	64.0	405.4	189.5	3913	24.0	281.7	27.1	69.3	290	922	71.2	48.2	3.2	591.5	33.9	27.0	62.7	22.3	2.6	33.4	65.0	28.1	4.0	4.8	59.5	7649
26/1	**6.77**	18.0	428	5.0	64.5	436.0	197.6	4666	16.0	225.0	23.4	72.4	300	585	64.2	55.0	6.3	613.3	22.9	25.9	60.6	17.1	2.7	32.4	62.5	27.8	0.0	10.5	55.6	8094
16/1	**6.84**	3.4	450	2.2	21.6	55.1	78.1	762	8.6	107.8	1.7	16.6	38	334	11.3	29.6	0.4	203.8	8.4	6.7	13.6	3.4	2.4	8.7	14.1	5.3	0.8	0.7	6.9	2195
45/1	**6.86**	11.7	359	3.4	66.5	280.1	189.8	2934	25.5	163.1	19.6	47.8	257	633	54.0	49.0	1.1	483.2	15.3	20.8	46.8	15.5	1.2	25.9	49.7	20.3	3.6	8.4	47.3	5833
46/1	**6.9**	16.0	398	3.1	51.8	380.7	184.6	3063	19.6	171.2	24.4	58.6	284	836	63.1	60.8	7.0	608.5	11.3	20.2	47.6	13.9	2.4	25.6	48.5	21.4	3.0	5.0	46.3	6476
17/1	**6.92**	16.9	431	3.3	64.0	213.7	132.5	1770	10.6	147.4	14.2	44.6	139	468	39.7	58.2	1.0	347.1	15.0	21.6	38.8	12.1	4.5	20.3	37.5	20.3	3.2	2.5	31.1	4108
47/1	**6.98**	3.5	225	2.8	28.6	257.8	151.0	2530	17.3	106.9	16.7	31.5	223	637	37.2	24.1	7.3	531.5	9.8	16.4	29.9	12.4	1.2	20.3	43.0	23.1	4.0	2.4	25.5	5019
3/2	**6.14**	1.2	331	3.4	18.2	82.7	101.4	1639	11.3	205.3	1.4	12.9	98	511	16.7	21.2	0.4	318.7	13.8	6.7	7.2	1.0	0.4	1.7	0.0	1.3	0.8	0.0	1.1	3408
34/2	**6.14**	1.9	227	2.8	20.1	67.2	86.7	1640	6.2	82.6	5.2	14.2	116	374	20.7	21.0	0.9	278.2	15.7	8.9	14.7	6.7	0.4	9.1	20.6	11.0	2.5	1.2	3.0	3059
21/2	**6.16**	1.7	303	3.1	21.9	106.2	96.6	1635	6.7	138.1	6.0	16.3	112	294	19.8	24.4	0.4	306.8	3.0	10.1	16.1	7.7	0.4	10.8	23.6	13.1	2.3	1.6	10.3	3191
33/2	**6.25**	7.2	294	3.6	49.6	264.2	146.3	3162	20.5	189.1	16.7	31.6	254	870	54.3	27.2	2.5	596.7	18.5	23.1	36.1	1.8	1.0	26.2	55.6	28.1	7.9	5.1	39.6	6232
36/2	**6.25**	6.1	340	3.8	41.1	308.1	161.1	4048	19.5	226.0	19.9	42.0	280	974	57.7	30.4	3.9	689.4	26.8	24.6	39.7	23.6	2.5	26.8	58.9	29.9	6.2	4.6	40.1	7534
1/2	**6.28**	2.2	281	2.8	22.2	84.3	100.5	1605	4.7	173.7	4.7	15.9	112	434	20.3	19.1	1.1	327.7	10.6	8.8	15.9	6.6	0.4	9.0	20.2	10.0	1.3	0.6	0.8	3296
4/2	**6.28**	2.6	418	3.3	27.9	83.0	110.1	1192	4.5	155.5	4.3	20.2	86	405	20.0	26.1	0.8	334.9	7.0	10.1	14.7	6.5	0.0	6.1	15.4	5.1	0.0	0.0	0.0	2959
5/2	**6.29**	4.4	368	5.1	31.2	230.5	155.6	3833	16.7	230.8	15.7	35.2	239	957	47.7	37.3	1.8	721.5	18.9	20.6	31.5	20.6	2.5	19.8	46.3	28.5	6.4	4.2	30.2	7160
2/2	**6.33**	3.8	379	3.9	42.1	172.0	129.4	2424	12.3	175.5	7.4	30.6	226	686	44.4	26.6	2.0	674.1	20.3	20.4	35.4	16.8	1.3	21.4	48.5	23.7	3.5	2.3	19.2	5251
32/2	**6.33**	5.5	302	4.3	41.4	222.1	144.7	2781	11.7	219.9	12.6	24.2	215	703	39.1	25.9	1.4	525.7	10.0	18.7	31.0	15.3	1.2	20.5	44.1	22.5	4.9	5.5	31.6	5484
11/2	**6.36**	3.6	303	2.9	39.5	180.8	131.2	2247	15.7	150.6	12.2	25.0	185	728	46.5	28.3	1.6	459.3	12.1	19.0	27.6	10.1	0.7	17.9	37.2	19.6	2.5	3.9	23.3	4733
24/2	**6.44**	2.6	450	2.9	20.4	137.4	79.6	1516	8.2	123.4	9.3	21.3	115	490	21.5	36.8	0.9	315.8	11.2	12.9	22.6	11.4	0.5	13.4	26.1	16.1	2.9	2.5	13.3	3483
14/2	**6.68**	5.7	458	3.3	39.9	186.5	111.8	2294	12.5	182.1	11.1	29.1	167	585	32.6	35.2	1.0	349.0	14.4	17.7	26.9	11.8	4.4	15.2	32.6	21.2	4.0	3.0	18.6	4674
35/2	**6.69**	2.0	278	2.3	21.7	91.3	77.4	1447	5.2	122.9	6.0	15.3	81	315	17.1	17.0	0.5	218.5	8.2	9.0	15.6	6.4	0.0	9.3	20.0	10.9	1.9	1.2	10.1	2812
26/2	**6.77**	1.0	340	2.5	15.9	48.9	76.2	1046	3.2	90.7	1.9	12.5	33	188	9.3	15.3	0.5	214.6	3.2	6.1	10.5	3.8	0.5	6.8	13.9	6.2	0.9	1.4	5.5	2158
16/2	**6.84**	2.3	405	2.5	20.7	74.6	70.3	1278	6.9	111.9	4.3	14.4	60	366	13.7	21.7	0.4	222.9	6.2	7.3	12.2	4.4	0.0	6.9	15.3	7.4	1.0	0.9	3.0	2739
45/2	**6.86**	5.1	362	3.3	41.1	194.4	156.6	2497	21.0	159.9	13.0	27.8	168	573	37.7	31.1	1.3	453.1	9.2	15.4	28.6	14.3	1.4	18.1	36.6	20.2	2.7	5.7	24.2	4922
47/2	**6.89**	0.0	216	1.1	6.7	16.3	71.2	332	5.0	47.6	0.0	6.6	10	237	3.8	10.3	0.4	210.8	6.0	3.1	5.5	1.7	2.2	4.7	10.3	3.3	0.7	0.0	2.3	1215

Figure C-1.

Free amino acid data, expressed as umole 100 g^{-1} sample, from chapter 5.

		1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	27	28	29	30	
Sample		Asp	Glu	OH	Ser	Asn	Gly	Gln	bAla	Tau	His	Thr	Ala	Carn.	Arg	Pro	1Me	3Me	ABA	Tyr	Val	Met	Cyst	Ile	Leu	Phe	Try	Orn	Lys	Total
ID	**pH**			pro													His	His												
46/2	**6.9**	7.6	398	3.6	34.4	274.8	164.4	3066	17.9	170.0	17.1	36.4	234	834	42.7	36.5	5.7	613.4	12.3	18.7	31.1	19.9	2.2	19.4	42.6	25.5	3.0	5.5	28.4	6166
17/2	**6.92**	0.0	414	1.7	7.8	14.5	35.4	218	0.0	57.7	0.0	5.3	6	115	2.6	16.2	0.0	98.7	2.1	3.7	2.6	1.2	0.0	1.0	3.1	1.8	0.0	0.0	0.0	1009
37M	**5.54**	0.6	296	1.8	8.6	19.3	63.3	332	7.6	180.0	0.0	9.3	28	398	5.5	16.5	0.4	305.0	0.0	4.5	8.9	3.8	0.4	6.4	12.5	4.1	2.0	1.1	4.7	1721
39M	**5.61**	0.0	266	2.1	7.6	23.4	64.8	302	4.9	118.3	0.9	7.1	21	346	4.5	14.3	0.8	231.7	5.6	4.1	7.3	3.0	0.0	6.6	10.8	3.9	0.9	0.4	3.8	1463
48M	**5.64**	2.7	290	2.9	20.4	88.0	85.0	1255	12.8	170.9	5.8	19.5	91	655	13.5	27.3	1.6	392.7	10.8	9.6	17.0	8.9	0.4	12.5	21.4	12.0	2.2	1.6	10.0	3241
18M	**5.65**	0.0	318	1.1	2.5	7.2	18.8	103	0.9	25.1	0.4	2.7	3	76	0.4	10.9	0.0	62.5	0.9	1.7	1.7	0.4	0.0	1.4	2.6	1.6	0.4	0.0	0.0	644
19M	**5.66**	3.7	365	2.0	27.1	143.4	124.6	1359	18.1	236.8	12.8	23.0	199	565	22.9	27.6	1.7	471.4	11.1	13.9	25.6	12.5	0.4	17.3	31.6	17.7	4.5	2.8	18.2	3759
29M	**5.7**	2.5	271	3.6	25.6	102.7	132.2	1970	11.2	138.5	8.0	23.1	129	438	18.2	27.8	1.9	448.6	7.9	10.7	18.8	10.0	0.4	12.4	23.8	13.8	2.6	2.3	11.7	3867
38M	**5.7**	0.8	230	1.6	13.6	37.0	81.1	493	10.9	101.8	2.2	14.6	34	357	10.7	22.2	0.4	297.4	0.0	6.9	13.6	5.9	0.4	10.8	16.6	7.1	2.8	1.4	9.0	1783
40M	**5.72**	2.3	267	2.9	17.6	67.9	87.1	1266	10.9	189.0	4.2	18.4	71	495	11.8	26.9	1.6	314.1	9.7	8.6	15.1	7.6	0.0	10.3	17.7	10.3	1.9	2.6	6.6	2944
49M	**5.72**	4.7	315	3.1	32.3	187.8	128.8	2568	12.8	193.3	11.6	28.0	251	818	25.7	35.4	2.6	486.6	21.8	16.3	31.0	16.7	0.9	20.2	39.4	22.6	3.3	4.9	16.6	5299
20M	**5.78**	0.7	295	1.6	11.0	40.0	65.8	471	9.9	121.3	2.0	10.7	36	359	6.4	17.1	0.4	329.0	11.5	5.6	10.5	4.1	0.4	8.8	13.0	6.0	2.4	1.4	5.3	1847
3M	**6.14**	0.0	361	2.7	11.3	42.4	79.9	768	7.4	148.9	1.9	9.4	51	351	7.9	15.8	0.4	224.6	8.9	3.9	7.7	3.5	0.0	5.3	9.6	5.1	1.6	1.5	3.3	2134
34M	**6.14**	4.1	296	4.9	36.7	153.0	161.2	3558	12.9	152.8	12.0	26.3	238	698	34.9	35.5	3.6	516.4	26.2	14.1	26.7	15.2	0.5	17.0	34.4	20.3	6.4	5.3	19.3	6129
21M	**6.16**	1.3	373	4.2	24.7	80.2	128.7	1148	9.8	186.5	6.9	19.8	123	412	23.1	32.3	0.9	425.2	10.1	10.5	18.7	11.6	0.4	13.6	27.1	13.9	3.1	2.4	11.3	3123
33M	**6.25**	5.4	330	4.7	40.9	198.3	157.0	2383	21.5	221.2	15.3	28.6	204	816	39.2	31.7	3.1	564.8	1.1	19.0	29.1	19.9	0.6	19.9	39.8	26.6	6.6	3.8	20.1	5253
36M	**6.25**	1.0	339	2.3	11.8	30.5	89.6	567	7.6	109.1	0.9	10.0	25	418	6.5	16.5	1.0	305.9	6.5	5.3	8.9	4.6	0.0	7.3	13.2	5.2	1.2	0.6	4.6	1998
1M	**6.28**	2.0	332	4.6	31.4	154.0	154.4	2578	9.3	280.0	8.9	23.4	177	682	26.1	28.9	2.1	530.7	11.1	11.0	24.0	10.9	0.5	14.7	27.8	17.2	5.1	5.2	16.6	5169
4M	**6.28**	1.6	501	4.3	24.3	81.0	121.4	1067	3.4	173.6	5.6	20.4	47	374	14.2	35.2	1.8	321.8	4.4	10.3	14.0	8.1	0.0	8.9	17.0	10.8	1.8	0.8	6.0	2879
5M	**6.29**	2.9	334	4.2	22.6	91.5	119.7	1712	8.7	148.2	7.5	17.2	112	565	16.7	26.8	2.1	418.4	10.2	9.4	13.3	9.4	0.4	8.1	19.1	13.8	2.6	2.1	8.8	3705
2M	**6.33**	0.6	367	1.4	14.9	64.1	64.3	771	5.8	69.5	3.0	12.4	69	334	9.3	13.6	0.7	256.1	5.1	5.9	11.2	5.0	0.0	7.3	13.4	6.3	1.6	1.1	5.1	2117
32M	**6.33**	5.9	430	5.0	49.5	228.5	190.8	3023	20.9	318.6	18.6	37.8	245	932	42.1	33.8	3.0	636.4	28.0	20.3	38.7	21.1	0.6	22.4	46.9	27.8	6.1	4.1	29.2	6467
11M	**6.36**	1.1	376	2.7	21.9	51.3	108.1	608	10.2	119.9	4.2	16.6	68	552	19.0	26.9	1.1	334.4	11.7	8.8	15.0	8.1	0.0	10.1	19.8	8.9	1.6	2.1	9.8	2417
24M	**6.44**	3.0	434	3.5	30.8	147.1	114.1	1627	11.4	159.1	10.8	24.9	174	637	21.6	35.1	2.7	429.9	15.0	12.9	25.2	13.3	0.4	15.3	29.5	18.1	3.9	3.2	15.2	4018
14M	**6.68**	0.0	417	2.1	12.4	43.4	60.7	603	4.1	104.3	1.7	9.8	28	308	7.9	17.0	0.4	192.3	4.9	4.7	7.2	3.5	2.1	5.3	9.9	4.9	1.3	0.9	3.1	1860

Figure C-1.

Free amino acid data, expressed as umole 100 g^{-1} sample, from chapter 5.

		1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	27	28	29	30	
Sample		Asp	Glu	OH	Ser	Asn	Gly	Gln	bAla	Tau	His	Thr	Ala	Carn.	Arg	Pro	1Me	3Me	ABA	Tyr	Val	Met	Cyst	Ile	Leu	Phe	Try	Orn	Lys	Total
ID	**pH**			pro													His	His												
35M	**6.69**	0.0	354	3.1	11.1	27.3	87.6	404	6.1	133.1	1.1	8.4	17	361	5.3	18.0	0.4	274.6	7.1	5.5	8.6	4.2	0.4	7.7	11.7	5.7	1.2	0.5	5.2	1771
26M	**6.77**	0.6	420	3.5	15.5	32.6	100.0	647	6.6	121.6	1.5	14.4	29	283	9.1	20.8	0.4	320.3	5.0	6.0	12.3	5.8	0.4	8.5	15.4	6.2	1.1	1.8	6.3	2095
16M	**6.84**	6.0	588	5.5	49.3	208.3	159.0	3036	21.6	267.2	19.0	38.0	223	897	32.2	45.2	2.9	550.6	23.5	16.9	33.0	18.6	0.5	18.5	38.3	24.6	5.2	4.9	23.9	6357
45M	**6.86**	2.2	473	3.6	26.0	82.6	141.7	1168	12.4	139.0	6.5	21.5	68	459	17.8	36.2	1.6	359.8	10.0	10.4	17.4	9.9	0.4	12.2	20.9	12.6	4.9	3.3	11.1	3132
46M	**6.9**	4.1	476	3.9	33.0	144.3	155.8	1677	14.4	149.8	10.6	25.2	155	596	22.3	37.1	2.5	456.8	17.6	12.7	20.7	14.2	0.5	12.1	26.0	19.1	3.3	3.0	13.5	4105
17M	**6.92**	10.1	642	5.3	58.7	195.0	178.0	2670	17.9	241.2	18.8	40.5	176	804	37.1	49.0	2.9	590.3	21.1	20.1	33.5	21.2	0.5	19.3	41.5	28.1	5.5	5.4	24.1	5958
47M	**6.98**	1.0	256	2.4	22.7	81.3	134.7	1006	11.8	88.1	8.4	19.4	109	490	16.9	20.7	0.9	427.8	0.0	9.3	17.4	10.6	0.9	12.3	25.9	12.9	3.0	1.9	12.3	2804

Figure C-1.

Free amino acid data, expressed as umole 100 g^{-1} sample, from chapter 5.

Appendix D.

Overall flavour sensory data

Example data of sensory panellists' scores for overall sheepmeat flavour is presented in figure D-1. Note: * = missing data points.

Overall Flavour: Raw sensory data from chapter 5.

Sample	Sensory	Initial	Final	Panellist number											
#	Session	pH	pH	101	123	229	287	404	407	411	526	551	553	621	660
18	1	5.57	5.57	3	6	3	7	3	7	9	5	6	8	4	6
20	1	5.62	5.62	6	7	8	7	8	5	5	5	7	7	5	2
19	2	5.59	5.59	7	7	3	6	5	6	7	6	7	6	6	6
29	2	5.61	5.61	7	5	5	6	8	8	7	4	7	4	7	7
37	3	5.54	5.54	6	5	8	7	4	5	9	5	6	7	4	2
38	3	5.58	5.58	5	6	3	6	4	5	4	7	7	6	3	1
40	4	5.56	5.56	5	4	6	6	4	5	6	2	5	5	3	5
39	4	5.54	5.54	7	6	5	8	6	5	6	5	7	6	6	7
49	5	5.61	5.61	5	6	6	6	*	*	5	3	7	7	3	8
48	5	5.59	5.59	4	6	5	7	*	*	7	4	6	6	5	4
3	1	5.91	5.43	5	6	5	7	8	5	6	7	5	8	5	6
1	1	6.06	5.52	7	5	7	6	8	4	5	5	7	6	5	3
34	2	5.88	5.63	5	8	5	6	5	6	6	5	6	6	7	7
4	2	6.07	5.35	4	4	7	5	7	6	7	6	7	6	5	7
21	3	5.93	5.37	5	6	7	6	6	5	8	5	5	6	3	2
11	3	6.05	5.68	5	5	6	7	5	6	6	4	5	6	3	3
33	4	5.97	5.63	4	7	4	7	7	4	4	6	6	4	4	3
32	4	6.16	5.59	8	5	8	7	7	5	7	4	4	6	7	6
5	5	6.07	5.44	5	6	8	5	*	*	7	6	5	5	4	2
36	5	5.98	5.68	7	7	4	7	*	*	6	3	6	5	3	4
2	1	6.17	5.69	5	7	5	7	8	6	5	6	6	4	4	4
14	1	6.45	5.65	6	5	8	6	9	6	7	7	3	5	2	6
16	2	6.56	5.68	6	6	4	5	8	6	7	4	5	7	4	4
17	2	6.95	5.65	7	7	7	6	6	7	6	7	7	7	5	6
26	3	6.50	5.58	7	6	8	7	7	5	5	7	6	5	4	5
47	3	6.79	4.92	4	6	8	6	5	5	6	7	7	5	4	4
45	4	6.63	5.62	7	4	8	7	7	4	3	6	5	7	7	5
35	4	6.37	5.67	5	6	6	7	7	6	6	5	6	6	7	5
46	5	6.69	5.64	4	6	7	6	*	*	8	4	5	3	2	5
24	5	6.28	5.55	7	7	8	7	*	*	4	4	7	5	5	3

Figure D-1.
Example data of sensory panellists' scores for overall sheepmeat flavour. Note * = missing data point.

Appendix E.

References

Aalhus, J.L. and Price, M.A. (1991a) Endurance-exercised growing sheep: I. Post-mortem and histological changes in skeletal muscles. *Meat Sci.*, **29**, 43-56.

Aalhus, J.L., Gariepy, C., Murray, A.C., Jones, S.D.M. and Tong, A.J.W. (1991b) Stunning and shackling influences on quality of porcrine *lonissimus dorsi* and *semimembransosus* muscles. *Meat Sci.*, **29**, 323-334.

Acree, T. E., Barnard, J. and Cunningham, D. G. (1984) A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem.*, **14**, 273-286.

Asghar, A. and Bhatti, A.R. (1987) Endogenous proteolytic enzymes in skeletal muscle: their significance in muscle physiology and during postmortem aging events in carcasses. *Adv. Food Res.*, **31**, 343-449.

Ballance, P.E. (1961) Production of volatile compounds realted to the flavor of foods from Strecker degradation of dl-methionine. *J. Sci. Food. Agr.*, **12**, 532-536.

Bergmeyer, H.U. (1983) *In*: Methods of Enzymatic Analysis. Third Edition. (Ed: H.U. Bergmeyer) Verlag chemie, Florida.Vol. II, 86-95.

Bendall, J.R. (1973) Postmortem changes in muscle. *In:* the Structure and Function of Muscle. (Ed. G.H. Bourne) Academic Press, New York.Vol. II, (2) 244-309.

Bendall, J.R. (1978) Variability in rates of pH fall and of lactate production in the muscles on cooling beef carcasses. *Meat Sci.*, **2**, 91-104.

Bishop, R.W. and Valis, R.J. (1990) A laboratory evaluation of sorbent tubes for use with a thermal desorption gas chromatography-mass selective detection technique. *J. Chrom. Sci.*, **28**, 589-593.

Blackwell, G.J. and Flower, R.J. (1983) Inhibition of phospholipase. *Br. Med. Bull.*, **39**, 260-264.

Boakye, K. and Mittal, G.S. (1993) Changes in pH and water holding properties of *longissimus dorsi* muscle during beef ageing. *Meat Sci.*, **34**, 335-349.

Bodwell, C.E., Pearson, A.M. and Spooner, M.E. (1965) Post-mortem changes in muscle: I. Chemical changes in beef. *J. Food Sci.*, **30**, 766-772.

Bouton, R.E, Howard, A. and Lawrie, R.A. (1957) Studies on beef quality: part vi. *Spec. Rept. Fd. Invest. Bd.*, **66**, 1-12. London.

Brennand, C. P. and Lindsay, R. C. (1992a) Distribution of volatile branched-chain fatty acids in various lamb tissues. *Meat Sci.*, **31**, 411-421.

Brennand, C. P. and Lindsay, R. C. (1992b) Influence of cooking on concentrations of species-related flavour compounds in mutton. *Lebensm. Wiss. Technol.*, **25**, 357-364.

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

Brown, R.H. and Purnell, C.J. (1979) Collection and analysis of trace organic vapour pollutants in ambient atmospheres. *J. Chromatogr.*, **178**, 79-90.

Burford, M.D., Hawthorne, S.B. and Miller, D.J. (1992) Comparison of methods to prevent restrictor plugging during off-line supercritical extraction. *J. Chrom.*, **609**, 321-332.

Burford, M.D., Hawthorne, S.B. and Miller, D.J. (1994) Analysis of organics by supercritical fluid extraction coupled to gas chromatography: I. Optimization of chromatographic parameters. *J. Chromatogr. A.*, **685**, 79-94.

Buscailhon, S., Berdague, J. L., Gandemer, G., Touraille, C. and Monin, G. (1994) Effects of initial pH on compositional changes and sensory traits of french dry-cured hams. *J. Muscle Foods*. **5**, 257-269.

Buttery, R.B., Teranishi, R. and Ling, L.C. (1987) Fresh tomato aroma volatiles: A quantitative study. *J. Agric. Food Chem.*, **35**, 540-544.

Caporaso, F., Sink, J.F., Dimick, P.S., Mussinan, C.J. and Sanderson, A. (1977) Volatile flavor constituents of ovine adipose tissue. *J. Agric. Food Chem.*, **25**, 1230-1234.

Carse, W.A. (1973) Meat quality and the acceleration of post-mortem glycolysis by electrical stimulation. *J. Food Technol.*, **8**, 163-166.

Chan, H.W.-S. (1987) *In*: Autoxidation of Unsaturated Lipids. (Ed: H.W.-S. Chan) Academic Press. London, pp1-16.

Chrystall, B.B. and Hagyard, C.J. (1976) Electrical stimulation and lamb tenderness. *N.Z. J.Agric. Res.*, **19**, 7-11.

Contarini, G. and Leardi, R. (1994) Use of experimental design to optimize the analysis of volatile compounds by dynamic headspace extraction followed by cold trapping capillary GC. *J. High Res. Chrom.*, **17**, 91-94.

Coppock, B.M. and MacLeod, G. (1977) The effect of ageing on the sensory and chemical properties of boiled beef aroma. *J. Sci. Fd. Agric.*, **28**, 206-214.

Dahlmann, B., Kuehn, L., Rutschmann, M. and Reinauer, H. (1985) Purification and characterization of a multicatalytic high-molecular-mass proteinase from rat skeletal muscle. *J. Biochem.*, **228**, 161-170.

Daly, C.C. (1996) Unpublished data, MIRINZ, Hamilton, New Zealand.

Daum-Thunberg, D.L., Foregeding, E.A. and Ball, H.R. (1992) Rheological and water-holding properties of comminuted turkey breast and thigh: Effects of initial pH. *J. Food Sci.*, **57**, 333-337.

Davey, C.L. and Gilbert, K.V. (1966) Studies in meat tenderness: 2. Proteolysis and the aging of beef. *J. Food Sci.*, **31**, 135-140.

Davey, C.L., Gilbert, K.V. and Carse, W.A. (1976) Carcass electrical stimulation to prevent cold-shortening toughness in beef. *N.Z. J. Agr. Res.*, **19**, 13-18.

Devine, C.E. and Chrystall, B.B. (1989) High ultimate pH in sheep. *In*: Dark-Cutting in Cattle and Sheep. Proceedings of an Australian Workshop. (Eds: S.U. Fabiansson, W.R. Shorthose, R.D. Warner) Australian Meat and Live-stock Research and Development, Sydney, Australia, pp 55-65.

Devine, C.E., Graafhuis, A.E., Muir, P.D. and Chrystall, B.B. (1993) The effect of growth rate and ultimate pH on meat quality of lambs. *Meat Sci.*, **35**, 63-77.

Dezeure-Wallays, B., van Hoof, J. and Pensaert, R. (1984) Incidence and quality characteristics of dark cutting in Belgian beef. *Proc. 30th Europ. Mtg. Meat Res. Workers,* 152-153.

Dodd, G., Bartlett, P. and Gardner, J. (1991) Complex sensor systems: odour detection by the sense of smell and by electronic noses. *Biochem. Soc. Transactions.* **19**, 36-39.

Doherty, A.M., Sheridan, J.J., Allen, P. McDowell, D.A. and Blair, I.S. (1996) Physical charactertistics of lamb primals packaged under vacuum or modified atmospheres. *Meat Sci.*, **42**, 315-324.

Doty, D.M. and Pearce, J.C. (1961) Beef muscle characteristics as related to carcass grade, carcass weight, and degree of ageing. *U.S. Dept. Agr., AMS, Tech. Bull.*, 1231.

Dransfield, E. (1981) *In*: The Problem of Dark-Cutting Beef (Eds: D.E. Hood, P.V. Tarrant) Martinus Nijhoff Publishers: The Hague. pp 344-358.

Dransfield, E. (1993) Modelling post-mortem tenderisation-IV: role of calpains and calpastatin in conditioning. *Meat Sci.*, **34**, 217-234.

Dransfield, E. (1994) *In*: Quality Attributes and Their Measurement in Meat, Poultry and Fish Products. (Eds: T.R. Dutson, A.M. Pearson) Advances in Meat Research Series Vol. 9. Blackie Academic, pp 289-311.

Dransfield, E. (1981) Eating quality of DFD meat. *In*: the Problems of Dark-cutting in Beef. (Eds: D.E. Hood, P.V. Tarrant) Martinus Nijhoff, The Hague, Vol. 10, pp 344-358.

Drumm, T.D. and Spanier, A.M. (1991) Changes in the content of lipid autoxidation and sulfur-containing compounds in cooked beef during storage. *J. Agric. Food Chem.* **39**, 336-343.

Dupuy, H.P., Fore, S.P. and Goldglatt, L.A. (1973) Direct gas chromatographic examination of volatiles in salad oil and shortening. *J. Am. Oil Chem. Soc.*, **50**, 340.

Dutson, T.R., Smith, G.C. and Carpenter, Z.L. (1980) Lysosomal enzyme distribution in electrically stimulated ovine muscle. *J. Food. Sci.*, **45**, 1097-1098.

Dutson, T. R., Savell, J.W. and Smith, C.G. (1981) Electrical stimulation of *ante mortem* stressed beef. *In:* The Problem of Dark-cutting Beef (Eds: D.E. Hood, P.V. Tarrant) Martinus Nijhoff Publishers: The Hague, pp 253-268.

Farmer, L.J., Mottram, D.S. and Whitfield, F.B. (1989) Volatile compounds produced in Maillard reactions inviolving cysteine, ribose and phospholipid. *J. Sci. Food. Agric.*, **49**, 347-368.

Farmer, L.J. and Mottram, D.S. (1990a) Recent studies on the formation of meat like aroma compounds. *In*: Flavour Science and Technology. (Eds: Y. Bessiere, A.F. Thomas) John Wiley and Sons, Chichester, pp113-116.

Farmer, L.J. and Mottram, D.S. (1990b) Interaction of lipid in the Maillard reaction between cysteine and ribose: the effect of a triglyceride and three phospholipids on the volatile products. *J. Sci. Food. Agric.*, **53**, 505-525.

Farmer, L.J. and Mottram, D.S. (1990) Lipid interaction in Maillard reactions of cysteine and ribose. *J. Sci. Food Agric.*, **53**, 505-525.

Farmer, L.J. (1992) Meat Flavour. *In*: the Chemistry of Muscle-based Foods. (Eds: D.A. Ledward, D.E. Johnston, M.K. Knight) The Royal Society of Chemistry, Cambridge, pp168-182.

Farmer, L.J. and Mottram, D.S. (1992) Effect of cysteine and ribose on the volatile thermal degradation products of a triglyceride and three phospholipids. *J. Sci. Food. Agric.*, **60**, 489-497.

Fjelkner-Modig, S. and Ruderus, H. (1983) The influence of exhaustion and electrical stimulation on the meat quality of young bulls: Part 2 - physical and sensory properties. *Meat Sci.*, **8**, 203-220.

Folch, J., Lees, M. and Stanley, G.H.S. (1957) A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.*, **226**, 497-509.

Ford, A. and Park, R.J. (1980) Odours and flavours in Meat. *In:* Developments in meat science, Vol. 1. (Eds: R. Lawrie) *Applied Science*. London. Ch.9, 219-248.

Forss, D.A. (1972) Odour and flavour compounds from lipids. *Proc. Chem. Fats other Lipids*. **13**, 181-258.

Gandemer, G., Morvan-Mahi, B., Meynier, A. and Lepercq, M. (1991) Quantitative and qualitative analysis of free fatty acids in meat and meat products using ion exchange resin.. *37th Int. Cong. Meat Sci. Technol.*, Kulmback. pp1139-1142.

Gasser, U. and Grosch, W. Z. (1990) Primary odourants of chicken broth: comparative study with meat broths from cow and ox. *Lebens. Unters. Forsch.*, **190**, 3-8.

Giddings, C.J., Myers, M.N. and King, J.W. (1969) Dense gas chromatography at pressures to 2000 atmospheres. *J Chromatogr. Sci.*, **7**, 276-283.

Gill, C.O. (1988a) CO_2 packaging -the technical background. *Proc. 25th Meat Ind. Res. Confr.*, Meat Ind. Ind. Inst. N.Z., Hamilton, N.Z., 181-185.

Gill, C.O. (1988b) The solubility of carbon dioxide in meat. *Meat Sci.*, **22**, 65-71.

Gill, C.O. (1990) Controlled atmosphere packaging of chilled meat. *Food Control.* **1**(2), 74-78.

Gill, C. O. and Newton, K. G. (1981) *In:* Current Topics in Veterinary Medicine and Animal Science. (Eds: D.E. Hood, and P.V. Tarrant) Microbiology of DFD Beef, Martinus Nijhoff Publishers: The Hague, Vol. 10, pp 305-321.

Gill, C.O. and Penny, N. (1986) Packaging conditions for extended storage of chilled dark, firm and dry beef. *Meat Sci.*, **8**, 41-53.

Gill, C.O. and Penny, N. (1989) Consumer trials of Captech-packaged consumer cuts of meat. *Meat Ind. Res. Inst. N.Z.* Rep. No. RM183.

Gill, C.O., Harrison, J.C.L. and Penny, N. (1990) The storage life of chicken carcasses packaged under carbon dioxide. *Int. J. Food Microbiol.*, **10**, 151-157.

Gopel, W. and Schierbaum, K.D. (1995) SnO_2 sensors: current status and future prospects. *Sensors and Acuators B.* **26-27**, 1-12.

Graafhuis, A.E. and Devine, C.E. (1994) Incidence of high pH beef and lamb II: results of an ultimate pH survey of beef and sheep plants in New Zealand. *28th Meat Ind. Res. Conf.*, MIRINZ Publication No. 942.

Grosch, W. (1982) Lipid degradation products and flavour. *In:* Food Flavours. Developments in Food Science. 3A. (Eds: I.D. Morton, A.J. MacLeod) Elsevier, Oxford. Ch. VI, pp325-398.

Greaser, M.L. (1986) Conversion of muscle to meat. *In:* Muscle as Food. (Eds: P.J. Bechtel) Academic Press, Florida, pp37-102.

Grob, K., Grob, G. and Grob, K. Jr. (1979) Deactivation of glass capillary columns by silylation. Part 1: Principles and basic technique. *J. High Resolut. Chromatogr. Chromatogr. Commun.*, **2**, 31-35.

Gunston, F.D. (1992) *In*: Lipid Analysis: A Practical Approach (Eds: R.J. Hamilton, S.E. Hamilton) Oxford University Press, Oxford, Chapter 2. pp13-64.

Ha, J.K. and Lindsay, R.C. (1990) Distribution of volatile branched-chain fatty acids in perinephric fats of various red meat species. *Lebensm. Wiss. Technol.* **23**, 433-440.

Ha, J.K. and Lindsay, R.C. (1991) Volatile alkylphenols and thiophenol in species-related characterizing flavours of red meat. *J. Food Sci.*, **56**(5), 1197-1202.

Hamm, R. and Deatherage, F.E. (1960) Changes in proteins during heating of meat. *Food Res.*, **25**, 587-610.

Hamm, R. (1977) Postmortem breakdown of ATP and glycogen in ground muscle: a review. *Meat Sci.*, **1**, 15-39.

Hallund, O. and Bendall, J.R. (1965) The long-term effect of electrical stimulation on the postmortem fall of pH in the muscles of Landrace pigs. *J. Food Sci.*, **30**, 296-299.

Hawthorne, S.B. and Miller, D.J. (1986) Extraction and recovery of organic pollutants from environmental solids and tenax-GC using supercritical CO_2. *J. Chromatogr. Sci.*, **24**, 258-264.

Hawthorne, S.B. (1990) Analytical-scale supercritical fluid extraction. *Anal. Chem.*, **62**, 633A-642A.

Hawthorne, S.B., Miller, D.J. and Langenfeld, J.J. (1990) Qualitative analysis using directly coupled supercritical fluid extraction-capillary gas chromatography (SFE-GC) with a conventional split/splitless injection port. *J. Chrom. Sci.*, **28**, 2-8.

Hedrick, H.B., Boillot, J.B., Dyer, A.J. and Naumann, H.D. (1961) Effect of antemortem administration of adrenaline on postmortem lamb carcass characteristics. *J. Anim. Sci.*, **20**, 558-560.

Ho, Chi-Tang and Chen, Q. (1994) Lipid in food flavours - an overview. *In:* Lipids in Food Flavours, ACS Symposium Series (Ed: T.G. Chi-Tang Ho Hartman) American Chemical Society, Washington, D.C., pp 558.

Honikel, K.O., Roncales, P. and Hamm, R. (1983) The influence of temperature on shrtening and rigor onset in beef muscle. *Meat Sci.*, **8**, 221-241.

Howard, A. and Lawrie, R.A. (1956) CSIRO Division Food Preservation and Transport Technical Paper, Sydney, Australia, Vol. 2 pp52-72.

Hurrell, R.F. (1982) Maillard reaction in flavour. *In:* Food Flavours. Developments in Food Science. 3A. (Eds: I.D. Morton, A.J. MacLeod) Elsevier, Oxford. Ch. VI, pp 399-437.

Jeon, I.J., Reineccius, G.A. and Thomas, E.L (1976) Artifacts in flavour isolates produced by steam distillation and solvent extraction of the distillate. *J. Agric. Food Chem.*, **24**, 433-434.

Jeremiah, L.E. (1978) A review of factors affecting meat quality. Agriculture Canada. *Tech. Bull.*, **1**, 84.

Jeremiah, L.E., Greer, G.G. and Gibson, L.L. (1991) Effects of lactic acid and postmortem aging on sensory and cooking properties of bovine *longissimus* muscle. *J. Muscle Foods.*, **2**, 119-131.

Jeremiah, L.E., Gill, C.O. and Penny, N. (1992) The effects on pork storage life of oxygen contamination in normally anoxic packagings. *J. Muscle Foods* **3**, 263-281.

Johnson, A.R. and Vickery, J.R. (1964) Factors influencing the production of hydrogen sulphide from meat during heating. *J. Sci. Food Agric.*, **15**, 695-701.

Joseph, R.L. and Connolly, J. (1977) The effects of suspension method, chilling rate and postmortem ageing period on beef quality. *J. Fd. Technol.*, **12**, 231-247.

Josephson, D.B., Lindsay, R.C. and Stuiber, D.A. (1984) Variations in the occurance of enzymatically derived volatile aroma compounds in salt and fresh water fish. *J. Agric. Food. Chem.*, **32**, 1344-1347.

Kazeniac, S.J. (1961) Chicken flavor. Proceedings Flavour Chemistry Symposium, Campbell Soup Company, Camden, N.J. 37-56.

Koohmaraie, M., Babiker, A.S., Merkel, R.A. and Dutson, T.R. (1988) Role of Ca^{++}-dependent proteases and lysomal enzymes in postmortem changes in bonine skeletal muscle. *J. Food Sci.*, **53**, 1253-1257.

Kovats, E. and Keulemans, A.I.M. (1964) The Kovats retention index system. *Analyt. Chem.*, **36**, 31A-40A.

Lacourt, A. and Tarrant, P.V. (1985) Glycogen depletion patterns in myofibres of cattle during stress. *Meat Sci.*, **15**, 85-100.

Laing, D.G. (1994) Perceptual odour interactions and objective mixture analysis. *Food Qual. and Pref.*, **5**, 75-80.

Laser-Reutersward, A., Johansson, G., Kullmar, B. and Ruderus, H. (1981) Influence of low-volatge stimulation on post mortem biochemistry in normal and DFD beef. *27th European Meeting of Meat Research Workers*, Wein, Austria. pp 157-160.

Lawrie, R.A. (1985) The eating quality of meat. Chapter 10. *In:* Meat Science, 4th ed., Pergamon Press: Oxford, U.K., pp 189-207.

Lawrie, R.A. (1992) Conversion of muscle into meat: Biochemistry. *In:* The chemistry of muscle-based foods. (Eds: D.A. Ledward, D.E. Johnston, M.K. Knight) Royal Society of Chemistry, Cambridge. pp 43-61.

Leahy, M.M. and Reineccius, G.A. (1989) Kinetics of the formation of alkylpyrazines: Effect of pH and water activity. *In:* Thermal Generation of Aromas. (Eds: T.H. Parliament, R.J. McGorrin, C-T. Ho) ACS Symopsium Series, 409. ACS. pp 196-208.

Ledward, D.A. (1970) Metmyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres. *J. Food Sci.*, **35**, 33-37.

Lewis, P.K., Brown, C.J., and Heck, M.C. (1967) The effect of ante-mortem stress on the internal temperature of beef during cooking. *Food Tech.*, **21**, 393-396.

Lewis, M.J. and Williams, A.A. (1980) Potential artifacts from using porous polymers for collecting aroma components. *J. Sci. Food Agric.*, **31**, 1017-1026.

Lister, D. (1988) Muscle metabolism and animal physiology in the dark cutting condition. *In:* Dark-cutting in Cattle and Sheep. (Eds: S.U. Fabiansson, W.R. Shorthose, R.D. Warner) AMLRDC, Sydney, Australia. pp 19-25.

Likens, S.T. and Nickerson, G.B. (1964) Determination of certain hop constituents in brewing products. *Amer. Soc. Brew. Chem. Proc.*, Vol: 5.

Lingnert, H. and Eriksson, C.E. (1981) Antioxidant effect of Maillard reaction products. *In:* Maillard Reaction in Food: Chemical, Physiological and Technological Aspects. (Ed: C.E. Eriksson) *Prog. Food. Nutr. Sci.,* Vol. 5, Pergamon Press. New York, pp 453-466.

Lineweaver, H. and Pippen, E.L. (1961) Chicken flavor. Proceedings Flavor Chemistry Symposium-1961. Campbell Soup Company. pp 21-34.

Locker, R.H and Hagyard, C.J. (1963) A cold shortening effect in beef muscle. *J. Sci. Food. Agr.*, **14**, 787-793.

Macku, C., Kallii, H., Takeoka, G and Flath, R. (1988) Aspects on the use of headspace GC on-column injections in flavour research. *J. Chromatogr. Sci.*, **26**, 557-560.

MacLeod, G., and Ames, J.M. (1986a) Comparative assessment of the artifact background on thermal desorption of Tenax GC and Tenax TA. *J. Chromatogr.*, **355**, 393-398.

MacLeod, G. and Ames, J.M. (1986b) The effect of heat on beef aroma: comparisons of chemical composition and sensory properties. *Flavour Fragrance. J.*, **1**, 91-104.

MacLeod, G. and Ames, J.M. (1987) Effect of water on the production of cooked beef aroma compounds. *J. Food. Sci.*, **52**. 42-45.

MacLeod, G. and Ames, J.M. (1988) Soy flavor and its improvement. *CRC Ctit. Rev. in Food Sci and Nutr.*, **27**, 219-400.

Macy, R.L., Naumann, H.D. and Bailey, M.E. (1964) Water-soluble flavor and odor precursors of meat. II. Effects of heating on amino nitrogen constituents and carbohydrates in lyophilized diffusates from aqueous extracts of beef, pork, and lamb. *J. Food Sci.*, **29**, 142-148.

Macy, R.L., Naumann, H.D. and Bailey, M.E. (1970) Water-soluble flavor and odor precursors of meat. 5. Influence of heating on acid-extractable non-nucleotide chemical constituents in beef, lamb and pork. *J. Food Sci.*, **35**, 83-87.

Madruga, S. and Mottram, D.S. (1995) The effect of pH on the formation of maillard-derived aroma volatiles using a cooked meat system. *J. Sci. Food Agric.*, **68**, 305-310.

Maier, I., and Fieber, M. (1988) Retention characteristics of volatile compounds on Tenax TA. *J. High Res. Chrom. and Chrom. Communications*, **111**, 566-576.

Manly, B.F.J. (1994) Multivariate Statistical Methods, 2nd ed., Chapman and Hall: London.

McGill, A.S., Hardy, R. and Gunstone, F.D. (1977) Further analysis of the volatile components of frozen cold stored cod and the influence of these on flavour. *J. Sci. Food Agric.*, **28**, 200-205.

McIntyre, B.L. (1989) The relationship between muscle pH and meat colour in yealing kimberley shorthorn cattle. *In:* Dark-cutting in Cattle and Sheep. (Eds: S.U. Fabiansson, W.R. Shorthose, R.D. Warner) AMLRDC, Sydney, Australia. p 88.

Melton, S.L., Black, J.M., Davis, G.W. and Backus, W.R. (1982) Flavor and selected chemical compounts of ground beef from steers backgrounded on pacture and fed corn up to 140 days. *J. Food. Sci.*, **47**, 699-704.

Miller, D.J., Hawthorne, S.B. and McNally M.E.P. (1993) Solventless collection of analytes by rapid depressurization after static supercritical fluid extraction. *Analyt. Chem.*, **65**, 1038-1042.

Monin, G. (1981) Muscle metabolic type and the DFD condition. *In:* the Problems of Dark-cutting in Beef. (Eds: D.E. Hood, P.V. Tarrant) Martinus Nijhoff, The Hague, 63-81.

Moore, V.J. and Gill, C.O. (1987) The pH and display life of chilled lamb after prolonged storage under vacuum or CO_2. *N.Z. J. Ag. Res.*, **30**, 449-452.

Mottram, D.S., Edwards, R.A. and MacFie, H.J.H. (1982) A comparison of the flavour volatiles from cooked beef and pork meat systems. *J. Sci. Food Agric.*, **33**, 934-944.

Mottram, D.S. and Edwards, R.A. (1983) The role of triglycerides and phospholipids in the aroma of cooked beef. *J. Sci. Food Agric.*, **34**, 517-522.

Mottram, D.S. (1985) The effect of cooking conditions on the formation of volatile heterocyclic compounds in pork. *J. Sci. Food. Agric.*, **36**, 377-382.

Mottram, D.S. (1991) Meat. *In:* Volatile Compounds in Foods and Beverages. (Ed: H. Maarse) Marcel Dekker, Inc. New York. Ch. 5, pp 107-177.

Mulcahey, L.J, and Taylor, L.T. (1992) Collection efficiency of solid surface and sorbent traps in supercritical fluid extraction with modified carbon dioxide. *Analyt. Chem.*, **64**, 2352-2358.

Nielen, M.W.F., Sanderson, J.T., Frei, R.W. and Brinkman, U.A. (1989) On-line system for supercritical fluid extraction and capillary gas chromatography with electron-capture detection. *J. Chrom.*, **474**, 388-395.

Nishimura, T., Rhue, M.R., Okitani, A. and Kato, H. (1988) Components contributing the improvement of meat taste during storage. *Agric. Biol. Chem.*, **52**, 2323-2330.

Offer, G. (1991) Modeling of the formation of pale, soft and exudative meat: effects of chilling regime and rate and extent of glycolysis. *Meat Sci.*, **30**, 157-184.

Offer, G., Knight, P., Jeacocke, R., Almond, R., Cousins, T., Elsey, J., Parsons, N., Sharp, A., Starr, R. and Purslow, P. (1989) The structural basis of the water-holding, appearance and toughness of meat and meat products. *Food Microstruct.*, **8**, 151-170.

Offer, G. and Trinick, J. (1983) On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. *Meat Sci.*, **8**, 245-281.

Official Methods of Analysis of AOAC International (1994) Method 39.1.19. 16th Edition. Arlington, USA.

Olafsdottir, G., Steinke, J.A. and Lindsay, R.C. (1985) Quantitative performance of a simple Tenax-GC adsorption method for use in the analysis of aroma volatiles. *J. Food. Sci.*, **50**, 1431-1436.

Ondarza, M. and Sanchez, A. (1990) Steam distillation and supercritical fluid extraction of some mexican spices. *Chromatographia.* **30**, 16-18.

Ouali, A. (1984) Sensitivity to inoic strength of Mg-Ca-enhanced ATPase activity as an index of myofibrillar ageing in beef. *Meat Sci.*, **11**, 79-88.

Ouali, A. (1990) Meat tenderization: possible causes and mechanisms: A review. *J. Musc. Foods.* **1**, 129-165.

Park, R. J. and Murray, K. E. (1975) Meat Research in *CSIRO*, Sydney, Australia, p 22.

Parrish, F.C., Goll, D.E., Newcomb, W.J., de Lumen, B.O., Chaudhry, H.M. and Kline, E.A. (1969) Molecular properties of post-mortem muscle. 7. Changes in nonprotein nitrogen and free amino acids of bovine muscle. *J. Food Sci.*, **34**, 196-202.

Patterson, H.D and Thompson, R. (1971) Recovery of interblock information when block sizes are unequal. *Biomettika* **58**, 545-554.

Pearson, A.M. and Young, R.B. (1989) Muscle and Meat Biochemistry. Academic Press. London. **13**, 391-444.

Peter, J.B., Barnard, R.J., Edgerton, V.R., Gillespie, C.A. and Stempez, K.E. (1972) Metabolic profiles of three fibre types of skeletal muscle in guinea pigs and rabbits. *Biochemistry.* **11**, 2627-2633.

Polesello, S., Lovati, F., Rizzolo, A. and Rovida, C. (1993) Supercritical fluid extraction as a preparative tool for strawberry aroma analysis. *J. High Res. Chrom.*, **16**, 555-559.

Prusa, K.J. and Bowers, J.A. (1984) Protein extraction from frozen, thawed turkey muscle with sodium nitrite, sodium chloride, and selected sodium phosphate salts. *J. Food. Sci.*, **49**, 709-713,720.

Purchas, R.W., Johnson, C.B., Birch, E.J., Winger, R.J., Hagyard, C.J. and Keogh, R.G. (1986) Flavour studies with beef and lamb. Department of Animal Science, Massey University, Palmerston North, New Zealand.

Purchas, R.W. and Aungsupakorn, R. (1993) Further investigations into the relationship between untimate pH and tenderness for beef samples from bulls and steers. *Meat Sci.*, **34**, 163-178.

Rao, M.V. and Gault, N.F.S. (1989) The influence of fibre-type compositionnabnd asociated biochemical characteristics on the acid buffering capacities of several beef muscles. *Meat Sci.*, **26**, 5-18.

Raymer, J.H. and Velez, G.R. (1991) Development of a flexible, on-line supercritical fluid extraction-gas chromatograhic (SFE-GC) system. **29**, 467-475.

Reineccius, G. (1994) *In:* Quality Attrubutes and Their Measurement in Meat, Poultry and Fish Products. (Eds: A.M. Pearson, T.R. Duston) Advances in Research Series. Blackie Academic and Professional Quality: Glasgow, Vol 9. pp 184-199.

Saffle, R.L. and Galgreath, J.W. (1964) Quantitative determination of salt-soluble protein in various types of meat. *Food Tech.*, **18**, 1943-1944.

Savell, J.W. (1979) Industry acceptance of electrical stimulation. *Proc. 32nd Recip. Meat Confr.*, pp 113-117.

Sayre, R.N., Kiernat, B. and Briskey, E.J. (1964) Processing characteristics of porcine muscle related to pH and temperature during rigor mortis development and to gross morphology 24 hr post-mortem. *J. Food Sci.*, **29**, 175-181.

Seiber, J.N. (1991) Determination of volatile and semivolatile mutagens in air using solid adsorbants and supercritical fluid extraction. *Anal. Chem.*, **63**, 1644-1650.

Shahidi, F., Rubin, L.J. and D'Souza, L.A. (1986) Meat flavour volatiles: a review of the composition, techniques of analysis, and sensory evaluation. *CRC Crit. Rev. Food Sci. Nutr.*, **24**, 141-243.

Shorthose, W.R. (1989) Dark-cutting meat in beef and sheep carcasses under different environments of Australia. *In:* Dark-cutting in Cattle and Sheep. (Eds: S.U. Fabiansson, W.R. Shorthose, R.D. Warner) AMLRDC, Sydney, Australia. pp 68-74.

Singh, B. and Rizvi, S.H.S. (1994) Design and economic analysis for continueous counter corrent processing of milk fat with supercritical carbon dioxide. *J. Dairy. Sci.*, **77**, 1731-1745.

Sinha, N.K., Guyer, D.E., Gage, D.A. and Lira, C.T. (1992) Supercritical carbon dioxide extraction of onion flavours and their analysis by gas chromatography-mass spectrometry. *J. Agric. Food Chem.*, **40**, 842-845.

Sink, J.D. and Smith, P.W. (1972) Changes in the lipid soluble carbonyls of beef muscle during ageing. *J. Food Sci.*, **37**, 181-182.

Skaln, D. and Tenne, Z. (1984) Changes in the lipid fractions and bacteriological counts in chilled broiler meat. *Poult. Sci.*, **63**, 76-81.

Smulders, F.J.M. and Eikelenboom, G. (1986) Electrical stimulation, cooling intensity and meat tenderness. *Fleischwirtschaft* **66**, 883-885.

Snyder, J.M. and Mounts, T.L. (1990) Analysis of vegetable oil volatiles by multiple headspace extraction. *J.A.O.C.S.*, **67**, 800-803.

Snyder, J.M. and King, J.W. (1994) Oilseed volatile analysis by supercritical fluid and thermal desorption methods. *J.A.O.C.S.*, **71**, 261-265.

Sorinmade, S.O., Cross, H.R., Ono, K. and Wergin, W.P. (1982) Mechanisms of ultrastructural changes in electrically stimulated beef longissimus muscle. *Meat Sci.*, **6**, 71-77.

Spanier, A.M., McMillin, K.W. and Miller, J. A. (1990) Enzyme activity levels in beef: effect of post-mortem aging and end-point temperature. *J. Food Sci.*, **55**, 318-322, 326.

Spanier, A.M., St. Angelo, A.J., Grimm, C.C. and Miller, J.A. (1994) Relationship of temperature to the production of lipid volatiles from beef. *In:* Lipids in Food Flavors. (Eds: C-T. Ho, T.G. Hartman) ACS Symopsium Series 558. ACS, Washington. p 78-97.

St. Angelo, A.J., Vercellotti, J.R., Legendre, M.G., Vinnett, C.H., Kuan, J.W., James, C. and Dupuy, H.P. (1987) Chemical and instrumental analyses of warmed-over flavour in beef. *J. Food Sci.*, **52**, 1163-1168.

Sugisawa, H. (1984) Prerequisites for sample preparation in flavour analysis. *In:* Analysis of Volatiles: Methods and Applications (Ed: P. Schreier) New York: Walter de Gruyter. pp 3-18.

Suzuki, J. and Bailey, M.E. (1985) Direct sampling capillary GLC analysis of flavor volatiles from ovine fat. *J. Agric. Food. Chem.*, **33**, 343-347.

Tarrant, P.V. (1989) Animal behaviour and environment in the dark-cutting condition. *In:* Dark-cutting in Sheep and Cattle, Proceedings of an Australian Workshop. (Eds: S.U. Fabiansson, W.R. Shorthose, R.D. Warner) AMLRDC, Sydney, Australia. pp 8-18.

Talmant, A., Monin, G., Briand, M., Dadet, M. and Briand, Y. (1986) Activities of metabolic and contractile enzymes in 18 bovine muscles. *Meat Sci.*, **18**, 23-40.

Ullrich, F. and Grosch, W. (1987) Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Lebens. Unters. Forsch.*, **184**, 277-282.

van den Ouweland, G.A.M., Peer, H.G. and Tjan, S.B. (1978) Occurance on Amadori and Heyns rearrangement products in processed foods and their role in flavor formation. *In:* Flavour in Foods and Beverages. (Eds: C. Charalambous, G.E. Inglett) Academic, New York. pp 131-143.

van den Ouweland, G.A.M., Demole, E.P. and Enggist, P. (1989) Process meat flavor development and the Maillard reaction. *In:* Thermal Generation of Aromas (Eds: T.H. Parliament, R.J. McGorrin, C-T, Ho) American Chemical Society, Washington, D.C., USA. pp 433-441.

Vercellotti, J.R., Mills, O.E., Bett, K.L. and Sullen, D.L. (1992) Gas chromatographic analyses of lipid oxidation volatiles in foods. *In:* Lipid Oxidation in Food, ACS Symposium Series (Eds: A.J. St Angelo) American Chemical Society, Washington. Ch. 13, 232-265.

Wasserman, A.E. and Spinelli, A.M. (1972) Effects of some water soluble components on aroma of heated adipose tissue. *J. Agric. Food. Chem.*, **20**, 171-174.

Watanabe, K. and Sato, Y. (1971) Lactones produced through thermal oxidation of higher fatty acids. *Agric. Biol. Chem.*, **35**, 278-284.

Watanabe, A., Daly, C.C. and Devine, C.E. (1996) The effects of the ultimate pH of meat on the tenderness changes during ageing. *Meat Sci.*, **42**, 67-78.

Wick, E.L., Koshika, M. and Mizutani, J. (1965) Effect of storage at ambient temperature on the volatile components of irradiated beef. *J. Food Sci.*, **30**, 433-440.

Winger, R.J. and Pope, C.G. (1981) Selection and training of panellists for sensory evaluation of meat flavours. *J. Food Technol.*, **16**, 661-669.

Wong, E., Johnson, C.B. and Nixon, L.N. (1975a) The contribution of 4-methyloctanoic (hircinoic) acid to mutton and goat meat flavour. *N.Z. J. Agric., Res.*, **18**, 261-266.

Wong, E., Nixon, L.N. and Johnson, C.B. (1975b) Volatile medium chain fatty acids and mutton flavour. *J. Agric. Food Chem.*, **23**, 495-498.

Wong, J.M., Kado, N.Y., Kuzmicky, P.A., Ning, H.-S., Woodrow, J.E., Hsieh, D.P.H. and Seiber, J.N. (1991) Determination of volatile and semi-volatile absorbants and supercritical fluid extraction. *Analyt. Chem.*, **63**, 1644-1650.

Wu, F.Y. and Smith, S.B. (1987) Ionic strength and myofibrillar protein solubilization. *J. Anim. Sci.*, **65**, 597-608.

Wyllie, S.G., Alves, S., Filsoof, M. and Jennings, W.G. (1978) Headspace sampling: use and abuse. *In:* Analysis of Foods and Beverages (Ed: G. Charalambous) Academic Press. pp 1-15.

Yaylayan, V. (1990) In search of alternative mechanisms for the Maillard reaction. *Trends in Food Sci. Technol.*, **1**, 20-22.

Young, O.A., Torley, P.J. and Reid, D.H. (1992) Thermal scanning rheology of myofibrillar proteins from muscles of defined fibre type. *Meat Sci.*, **32**, 45-63.

Young, O.A., Reid, D.H., Smith, M.E. and Braggins, T.J. (1994) Sheepmeat odour and flavour. *In:* Flavour of Meat and Meat Products (Ed: F. Shahidi) Blackie Academic and Professional: London, Chapter 5. pp 71-97.

Young, O.A., Reid, D.H. and Scales, G.H. (1993) Effect of breed and ultimate pH on the odour and flavour of sheepmeat. *N.Z. J. Agric., Res.*, **36**, 363-370.

Young, O.A., Berdague, J.L., Vialhon,C., Rousset-Akrim, S. and Theriez, M. (1996) Fat-borne volatiles and sheepmeat odour. Accepted for publication. *Meat Sci.*,

Zang, Y., Ritter, W.J., Barker, C.C., Traci, P.A. and Ho, C-T. (1994) Volatile formation by lipid-mediated Maillard reaction in model systems. *In:* Lipids in Food Flavors. (Eds: C-T. Ho, T.G. Hartman) ACS Symposium Series No. 558. ACS, Washington. Ch. 4., pp 49-60.

Zeece, M.G., Katoh, K., Robson, R.M. and Parrish, F.C. (1986) Effect of cathepsin D on bovine myofibrils under different conditions of pH and temperature.I., **51**, 769-773.

Ziegler, G.R. and Liaw, Y-J. (1993) Deodorization and deacidification of edible oils with dense carbon dioxide. *JAOCS*, **70**, 947-953.

Zervos, C. and Albert, R.H. (1992) Chemometrics: the use of multivariate methods for the determination and characterization of off-flavours. *In:* Off-Flavours in Foods and Beverages (Ed: G. Charalambous) Elsevier Science: New York, pp 669-742.

Appendix F.

Copies of Publications Arising From This Thesis

Two publications arising in whole, and one in part, from this thesis are presented in this appendix:

- Braggins, T.J. (1996) Effects of stress-related changes in sheepmeat ultimate pH on cooked odor and flavor. *J. Ag. Food. Chem.* (In press).
- Young, O.A. and Braggins, T.J. (1996) Variation in sheepmeat odour and flavour. *Proc. N.Z. Anim. Prod. Soc.*, **56**, 167-172.
- Young, O.A., Reid, D.H., Smith, M.E. and Braggins, T.J. (1994) Sheepmeat odour and flavour. *In:* Flavour of Meat and Meat Products (Ed: F. Shahidi) Blackie Academic & Professional: London, Chapter 5. pp71-97.